

Body Size and Life History Traits in Native and Introduced Populations of Coqui Frogs

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Introduced populations often exhibit rapid phenotypic changes following colonization of new environments. These changes, which often contribute to the successful establishment and spread of introduced species, may result from evolution or phenotypic plasticity. We studied variation in adult body size across elevational gradients in native (Puerto Rico) and introduced (Hawaii) populations of Coquis (*Eleutherodactylus coqui*). To explore the possible mechanisms underlying variation in adult body size, we conducted a laboratory common-environment experiment to study the effects of temperature (19°C and 25°C), elevation (<300 m and >700 m), and area (two in Puerto Rico and one in Hawaii) on five size-related life history traits: clutch size, egg size, hatching size, size at 30 days, and growth rate. In the field in both Puerto Rico and Hawaii, body size was positively correlated with elevation, which is negatively correlated with temperature, but the magnitude of the slope was greater in Puerto Rico than in Hawaii. In the laboratory, egg size, hatching size, and body size at 30 days were positively correlated with elevation for populations from Puerto Rico and Hawaii. Egg size, hatching size, and body size at 30 days were negatively correlated with temperature for all populations. Clutch size and growth rate were positively correlated with elevation for populations from Puerto Rico but not for populations from Hawaii. Furthermore, both low and high elevation populations from Hawaii had life history traits more similar to low elevation populations than high elevation populations from Puerto Rico. Temperature effects in the laboratory suggest that plasticity in response to temperature contributes to the variation in adult body size with elevation in both Puerto Rico and Hawaii. However, temperature-induced plasticity cannot explain the difference in slopes between Puerto Rico and Hawaii, because temperature varies across elevations to the same degree in both regions. Variation in growth rate paralleled that for adult body size and, if heritable, provides a possible mechanism for the observed differences in adult body size across elevations between Puerto Rico and Hawaii.

INTRODUCED species often exhibit rapid phenotypic changes following colonization of new environments. These changes may be genetic (the result of evolution) or non-genetic (due to phenotypic plasticity), or a combination of both (Moran and Alexander, 2014; Colautti and Lau, 2015). Plasticity can cause phenotypic changes within just one or two generations, whereas measurable evolutionary change typically takes longer, but can still be quite rapid (Carroll et al., 2007), with the rate depending on the magnitude of selection and the underlying genetic variation present in the original colonizers (Lande, 1980; Barton and Charlesworth, 1984). The ability of a species to respond to environmental variation through either phenotypic plasticity or evolution is likely an important determinant of successful establishment and further range expansion in introduced species (Antonovics, 1976; Bossdorf et al., 2005; Holt et al., 2005; Dlugosch and Parker, 2008).

Introduced populations are expected to have less genetic variation than populations of the same species in the native range because of bottlenecks and founder effects (Nei et al., 1975; Barrett and Husband, 1990). Empirical studies confirm that allelic diversity and heterozygosity are frequently lower in introduced populations than in native populations (Novak and Mack, 1993; Bossdorf et al., 2005; Wares et al., 2005; Dlugosch and Parker, 2008); however, the opposite relationships has also been found (Kolbe et al., 2004; Roman and Darling, 2007; Kajita et al., 2012). Alternatively broad-sense variation in quantitative traits can remain very high in small populations (Wood et al., 2016; Hoffman et al., 2017), rarely declines with introductions (Dlugosch and Parker, 2008), and

can even increase when a bottleneck uncovers masked genetic variation (Dlugosch et al., 2015).

Despite the potential for founder effects, many introduced species exhibit rapid adaptation to their new environments, especially in quantitative traits (Huey et al., 2000; Bossdorf et al., 2005; Dlugosch and Parker, 2008). This is particularly evident in species that exhibit rapid range expansion across diverse environments following colonization of a new geographic area. For example, genetically-based clines in body size and life history traits, concordant with those found in the native range, have quickly evolved following introduction and expansion in new geographic areas (Huey et al., 2000; Gilchrist et al., 2004; Szűcs et al., 2012). Whether and how quickly introduced populations evolve clines, and the structure of the evolved cline, may depend on how quantitative trait variation is structured in the native range and which populations are the sources of the introduction (Lee, 2002; Keller and Taylor, 2008). Alternatively, clines in phenotype can also be caused by phenotypic plasticity. These two mechanisms are not mutually exclusive because both genetic and environmental effects may be important for the formation of clines or even similarities in body size (Conover and Schultz, 1995).

Coquis (*Eleutherodactylus coqui* Thomas, 1966) are endemic to Puerto Rico and were accidentally introduced to the island of Hawaii in the late 1980s (Kraus et al., 1999). In Puerto Rico, Coquis occur in a variety of habitats from sea level to 1180 m (Schwartz and Henderson, 1991). In Hawaii, Coquis have spread mainly at low elevations (<500 m), but a few populations occur at higher elevations (Beard and Pitt, 2005; Beard et al., 2009; O'Neill and Beard, 2011). Two major

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phylogeographic clades (eastern and western) have been identified for Coquis in Puerto Rico (Velo-Antón et al., 2007). The source of the introduced Hawaiian populations has been identified from the eastern clade, and more specifically a low elevation region in northeastern Puerto Rico (Velo-Antón et al., 2007; Peacock et al., 2009), suggesting that the genetic variation present in the original colonists was limited to lowland genotypes. Indeed, Hawaiian populations of Coquis from all elevations have greatly reduced variation in mtDNA (Velo-Antón et al., 2007; Peacock et al., 2009), microsatellites (Velo-Antón et al., 2007; Peacock et al., 2009; Everman and Klawinski, 2013), and genetically determined color patterns (Peacock et al., 2009; O'Neill and Beard, 2010; O'Neill et al., 2012) compared with native populations, indicating that they experienced a population bottleneck and represent a biased sample of available genotypes in the native populations. Whether or not these source population effects have influenced quantitative trait variation, and subsequent adaptation to higher elevations, remains unclear.

In native populations in northeastern Puerto Rico, body size of Coquis is positively correlated with elevation (i.e., Coquis are larger at higher elevations; Narins and Smith, 1986; O'Neill and Beard, 2011). A study of Hawaiian Coquis conducted shortly post-introduction and including recently established populations failed to detect evidence of a cline in body size (Beard et al., 2008). In contrast, a subsequent study of Hawaiian populations indicated that body size of calling male Coquis (established near the earliest known introduction point) is larger at higher elevations than at lower elevations (>700 m versus <300 m; O'Neill and Beard, 2011), suggesting that post-introduction evolution may be occurring. Alternatively, because frogs in general exhibit substantial plasticity in body size in response to temperature (Atkinson, 1994; Berven, 1982a, 1982b), the observed clines in Puerto Rico, and now in Hawaii, may reflect a plastic effect of temperature on body size or on other life history traits, such as egg size, that influence adult body size.

Our goal was to study phenotypic variation in natural populations and the laboratory to better understand the potential sources (genetics and phenotypic plasticity) of this variation. First, we compared body size variation of Coquis collected at high (>700 m) and low (<300 m) elevations at multiple areas in both regions to determine if introduced populations had converged on the same patterns as those found in the native range. Then, we conducted a laboratory common-environment experiment in which we manipulated temperature separately for both the maternal and offspring stages. We tested whether temperature-induced plasticity affected growth rate and four size-related life history traits (egg size, clutch size, hatchling size, and size at 30 days) in Coquis from different elevations within Puerto Rico and Hawaii. We also tested for population-level differences in growth rate and all four size-related life history traits. Because the parents were collected from the wild as adults, these differences may reflect genetic effects or maternal effects, where the phenotypes of the offspring reflect the environment of the mother.

MATERIALS AND METHODS

Field study of body size.—To determine existing body size variation in the field, we measured Coquis at five areas in Puerto Rico (Cayey, El Yunque, Guilarte, Rio Abajo, and Toro Negro) in May 2006 and two areas in Hawaii (Hilo and Kona) in June 2006 (Table 1; Fig. 1). Within each area, we measured

individuals from both high (>700 m) and low elevations (<300 m), for a total of ten populations in Puerto Rico and four populations measured in Hawaii. Areas and elevations were chosen to maximize the geographic and elevation coverage across each island. At each high and low elevation population within an area, we established 20 m × 20 m plots divided into four 5 m wide transects, following Woolbright (2005). Plots were located in closed canopy forests with moderate to heavy understory of herbaceous and/or woody vegetation. Beginning at dusk, around 1900 h, after Coquis had sufficient time to move to nocturnal perch sites, two people surveyed each transect for Coquis. We surveyed each transect for 15 minutes, not including handling time, for a minimum total of 60 minutes per plot with two individuals searching. We hand-captured, sexed (i.e., by the occurrence of calling, vocal sacs, or visible ova), and measured (to 0.1 mm with dial calipers) snout-vent length (SVL) of adult Coquis. Because we could not determine *a priori* at what size Coquis attained sexual maturity for all 14 populations, we used field observations during collection. Similar to previous studies, we did not observe any males calling or with vocal sacs, or females with ova that were <25 mm SVL (Woolbright, 1989, 2005; Woolbright et al., 2006). When possible, we sampled at least 20 adult males and females at each site.

Laboratory study.—To estimate the influence of environmental effects (i.e., temperature) and population effects (differences among populations due to genetic and/or maternal effects from the field) on life-history traits in a controlled setting, we collected a sub-sample of Coquis and sent them to a laboratory at Utah State University to be used in a common garden experiment. We collected at least 20 adult male and 20 adult female Coquis from each of two areas (including high and low elevation populations) in Puerto Rico (El Yunque and Rio Abajo) in May 2006 and one area in Hawaii (Hilo) in June 2006 (for a total of 260 Coquis). These two areas in Puerto Rico, El Yunque and Rio Abajo, are representative of the two major phylogeographic clades (eastern and western, respectively) of this species (Velo-Antón et al., 2007). Haplotypes from Hilo are nested within the eastern clade, and Coquis from this area are most closely related to low elevation populations in northeastern Puerto Rico (Velo-Antón et al., 2007; Peacock et al., 2009). Hilo was the only area in Hawaii for which genetic origin had previously been determined for low and high elevation populations. The populations studied in Hilo were some of the oldest known in Hawaii (Kraus et al., 1999; Beard, 2007).

In the laboratory, we housed each male with a female from the same population in half of a 37.85 L terrarium, which was divided using corrugated plastic. We randomly assigned pairs to one of two maternal temperatures (19°C and 25°C) with a 12:12 (L:D) cycle and humidity at greater than 90%. These temperatures were within the range of temperatures normally encountered in the field (O'Neill and Beard, 2011). For each pair, we provided two retreat areas (15 cm long, 2.5 cm diameter plastic pipe), one small potted plant (*Pothos* spp.), and moist sphagnum moss as substrate. We provided Coquis with vitamin-dusted crickets and water.

For about ten months, we checked each terrarium daily for eggs and, when found, removed clutches and counted the number of eggs in each clutch (clutch size). We also measured egg size along two orthogonal directions (to 0.01 mm using an optical micrometer) for ten eggs per clutch. We divided each clutch in half and placed each half in a separate Petri dish (95 mm diameter) lined with a moist paper towel.

Table 1. Locality data for field study populations in Hawaii and Puerto Rico and mean adult body size (snout–vent length, SVL, in mm) for male and female *Eleutherodactylus coqui* from each population.

Range	Area	Symbol	Elevation category	Elevation (m)	Latitude (N)	Longitude (W)	Temperature (°C)	Sample size (n) (M, F)	Mean male SVL (SD)*	Mean female SVL (SD)*	Min, Max Male SVL	Min, Max Female SVL
Hawaii	Hilo	HL	High	766	19°28'49"	155°09'29"	20.5	50, 38	29.0 (1.7) ^{ab}	36.4 (2.3) ^{abc}	25.0, 32.5	32.3, 40.2
			Low	192	19°28'58"	154°54'10"	23.0	54, 35	27.7 (1.3) ^c	33.2 (2.5) ^e	25.5, 31.0	27.8, 38.5
Kona		KN	High	952	19°42'25"	155°56'57"	18.5	35, 16	30.8 (1.9) ^h	36.0 (2.3) ^{bcd}	27.4, 34.6	31.9, 41.0
			Low	265	19°38'01"	155°57'38"	22.0	34, 11	27.6 (1.3) ^c	34.4 (1.9) ^{de}	25.9, 30.8	31.7, 37.1
Puerto Rico	Cayey	CY	High	865	18°06'53"	66°04'47"	21.0	24, 4	35.7 (1.9) ^e	47.7 (3.4) ^{fg}	32.3, 39.3	42.8, 49.9
			Low	232	18°04'20"	66°04'20"	24.5	27, 3	28.7 (1.2) ^b	41.8 (5.2) ^{abghij}	26.8, 31.3	36.1, 46.5
	El Yunque	EY	High	714	18°17'54"	65°47'15"	21.5	31, 23	38.5 (1.9) ^d	48.7 (4.4) ^f	33.5, 41.1	39.6, 56.3
			Low	198	18°20'01"	65°45'38"	24.5	49, 35	31.3 (1.8) ^h	39.2 (3.1) ⁱ	25.4, 35.2	32.8, 45.8
	Guilarte	GU	High	995	18°08'36"	66°44'00"	20.5	29, 30	32.9 (1.4) ^g	41.0 (3.2) ^h	30.4, 35.7	35.9, 48.2
			Low	150	18°04'24"	66°48'05"	26.0	12, 4	29.0 (0.9) ^{ab}	33.7 (5.0) ^{cde}	27.6, 30.8	28.4, 38.2
	Rio Abajo	RA	High	714	18°12'59"	66°44'51"	24.0	25, 27	32.2 (1.6) ^g	41.4 (3.0) ^h	28.8, 34.9	35.6, 47.9
			Low	80	18°21'28"	66°41'02"	25.5	35, 31	29.6 (1.0) ^a	37.8 (2.6) ^{aj}	27.9, 31.2	32.8, 43.8
	Toro Negro	TN	High	978	18°11'12"	66°29'36"	19.5	26, 4	34.2 (1.8) ^f	40.8 (1.9) ^{hij}	31.5, 37.5	39.4, 43.0
			Low	241	18°06'57"	66°34'29"	25.5	28, 0	27.1 (1.2) ^c	NA	25.6, 30.1	NA

* Numbers followed by the same lower case letter are not significantly different for SVL when using *post-hoc* means test comparisons at $P < 0.05$.

We randomly assigned half clutches to one of two rearing temperatures (19°C and 25°C). To prevent desiccation, we watered each Petri dish every two days. At this time, we also checked for infertile eggs, which we removed, or hatching eggs. If any eggs had hatched, we placed the open Petri dish with all the eggs inside a plastic container (10 cm × 10 cm) to allow hatchlings to leave the egg mass. Approximately seven days after hatching began, when all the hatchlings had left the egg mass, we moved each juvenile frog to a separate Petri dish (95 mm diameter) lined with moist paper towel and sphagnum moss, and fed them *Collembola ad libitum*. We changed these Petri dishes every 4–5 days. We measured hatching size (to 0.01 mm using an optical micrometer) seven to ten days after hatching and again approximately 30 to 40 days later to determine growth rates. We waited one week to measure hatching size because they were too fragile to handle for about one week. Because the amount of time between hatch and measurements was not biased by treatments (population source or room temperatures), we do not think these small differences in time influenced overall results. For statistical analyses, we standardized body size to 30 days using the following equation: hatch size + (growth rate × 30). Growth rate was estimated as: log (change in body size)/time.

Statistical analyses.—To characterize variation in adult body size across Puerto Rico and Hawaii, we used analysis of variance (ANOVA) to evaluate the effects of area (seven levels: five in Puerto Rico and two in Hawaii), elevation (high and low), and sex. To estimate source populations and temperature effects on life history traits in the common garden, we used an ANOVA in a completely randomized design to evaluate the effects of area (El Yunque and Rio Abajo, from Puerto Rico and Hilo from Hawaii), elevation (high: >700 m and low: <200 m), and maternal temperature (the temperature at which eggs were laid: 19°C and 25°C) on clutch size, egg size, hatching size, body size at 30 days, and growth rate over 30 days, and we added rearing temperature (the temperature at which eggs and hatchlings were reared: 19°C and 25°C) to the model for hatching size, body size at 30 days, and growth rate over 30 days.

To meet assumptions of normality and homogeneity of variances and to avoid scaling effects, we used log transformations of all variables for the analyses. Mean trait values, averaged across eggs or offspring within a clutch, were used in analyses. Replicate clutches for a single female were treated as random effects. All statistical analyses were conducted using SAS 9.2 (SAS Institute). ANOVAs were conducted using PROC MIXED. Because our models range from three to four main effects with one random effect and multiple high level interactions, which can be difficult to interpret, we used AIC to choose the best fit ANOVA model. We constrained this model selection process to include all the main effects and the Area × Elevation interaction, because the primary interest was to determine whether or not elevation effects on traits differ among areas in Puerto Rico and Hawaii. Only the best fit models, based on AIC, are presented. *Post-hoc* means comparison tests were conducted using differences of least squares means. For all analyses, significance was considered as $P < 0.05$.

Because Coquis in this study were collected in the field as adults, the main effects of area and elevation might contain both genetic and residual maternal effects from the field. Isolating genetic differences is generally not possible with only first generation laboratory-reared animals because of

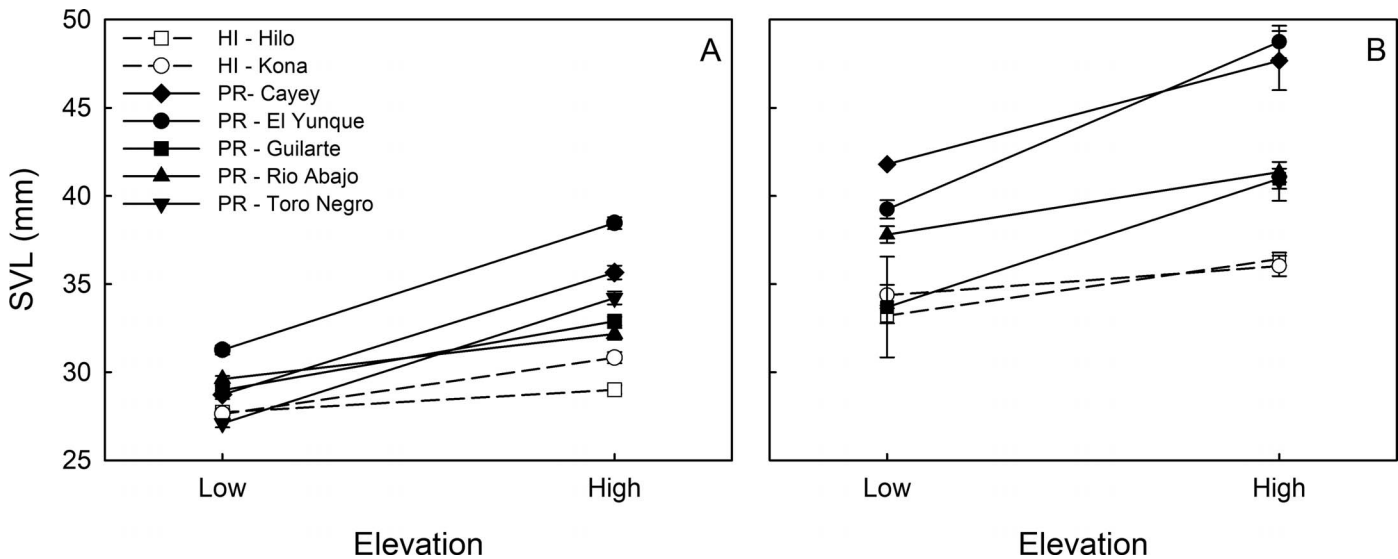


Fig. 1. Adult body size (SVL) of male (A) and female (B) *Eleutherodactylus coqui* from Hawaii and Puerto Rico in the field (means \pm 1 SE). See Table 1 for symbols.

possible environmentally-induced maternal effects of the field caught mothers on offspring. Maternal effects can often be statistically reduced by using analysis of covariance (e.g., controlling for maternal body size), but the strong correlation between maternal body size and elevation, an important factor in our models, resulted in no significant effects of maternal body size when it was included as a covariate in ANCOVAs along with the effect of elevation (results not shown). Therefore, we were not able to control statistically for maternal effects, and we acknowledge in our discussion that differences among areas and elevations may contain both genetic and maternal effects.

RESULTS

Field study

Female Coquis collected in the field were larger (26% averaged across all areas) than males. Coquis of both sexes were, on average, 16% larger in Puerto Rico than in Hawaii (Table 1; Fig. 2A, B). Averaged across both Puerto Rico and Hawaii, Coquis were on average 14% larger at higher elevations than at lower elevations (Tables 1, 2; Fig. 2A, B). The difference in body size between low and high elevations was generally smaller in Hawaii than in Puerto Rico (significant area \times elevation interaction); high elevation Coquis were, on average, 17% larger than low elevation Coquis in Puerto Rico, but only 9% larger in Hawaii. The magnitude of the elevation difference also varied among areas within Puerto Rico (Fig. 2A, B), with the greatest difference between high and low elevations (29%) in Toro Negro and the smallest difference (10%) in Rio Abajo. In contrast, the elevational difference in body size did not vary between the two Hawaii areas. The best-fit model (chosen using AIC) did not include interactions with sex, indicating that both sexes showed similar geographic (area and elevation) patterns.

Laboratory study

Temperature effects.—Clutch size was not significantly affected by the temperature at which the eggs were laid (maternal temperature; Fig. 2B). In contrast, most other traits were

affected by maternal temperature; eggs laid at 19°C were 6% larger and produced hatchlings that were 5% larger at hatching and after 30 days compared to eggs laid at 25°C (averaged across rearing temperatures; Table 3). Eggs reared in 19°C were 1% smaller at hatching and 2% smaller after 30 days (Table 4; Fig. 3). Growth rate for offspring was not significantly affected by either the temperature at which their egg was laid (maternal temperature) or the temperature at which they were reared (Table 4). The best fit models for each variable (chosen using AIC) did not include interactions with temperature effects, and none of these interactions were significant in the full models, suggesting that the temperature effects were similar among areas and between elevations.

Population effects.—Clutch size (the number of eggs laid per clutch in the laboratory under controlled environmental conditions) varied among source areas and between elevations (Table 3; Fig. 2A). Averaged across both elevations, clutch sizes for Coquis from El Yunque were 11% larger than for Coquis from Rio Abajo (difference of LS-means: $t_{106} = 3.85$; $P = 0.0002$) and 18% larger than for Coquis from Hilo ($t_{106} = 3.79$; $P = 0.0002$), but clutch size did not differ between Rio Abajo and Hilo ($t_{106} = 0.03$; $P = 0.98$; Fig. 2A). Averaged across areas, clutch size was 16% larger for Coquis from higher elevations than low elevations; however, there was a significant area \times elevation interaction (Table 3; Fig. 2A). Coquis from the two Puerto Rico areas had clutches that were 28% and 29% larger from high elevations than from low elevations (Fig. 2A; El Yunque $t_{106} = 3.32$; $P = 0.0012$ and Rio Abajo $t_{106} = 3.31$; $P = 0.0013$). In contrast, clutch sizes were not different between Coquis from high and low elevations in Hawaii (Hilo: $t_{106} = 3.31$; $P = 0.69$).

Egg size and hatching size both varied among source areas and between elevations, and there was no significant area \times elevation interaction for either trait (Table 3; Figs. 2C, 3A). Averaged across elevations, eggs from El Yunque were 4% and 7% larger in diameter than eggs from Rio Abajo ($t_{82} = 4.13$; $P < 0.0001$) and Hilo ($t_{82} = 5.90$; $P < 0.0001$), respectively, but did not differ in size between Rio Abajo and Hilo ($t_{82} = -1.74$; $P = 0.09$; Fig. 2C). As expected, hatching size exhibited this same general pattern (Table 4; Fig. 3A); hatchlings from El Yunque were 6% larger than those from Rio Abajo ($t_{92} = 5.49$;

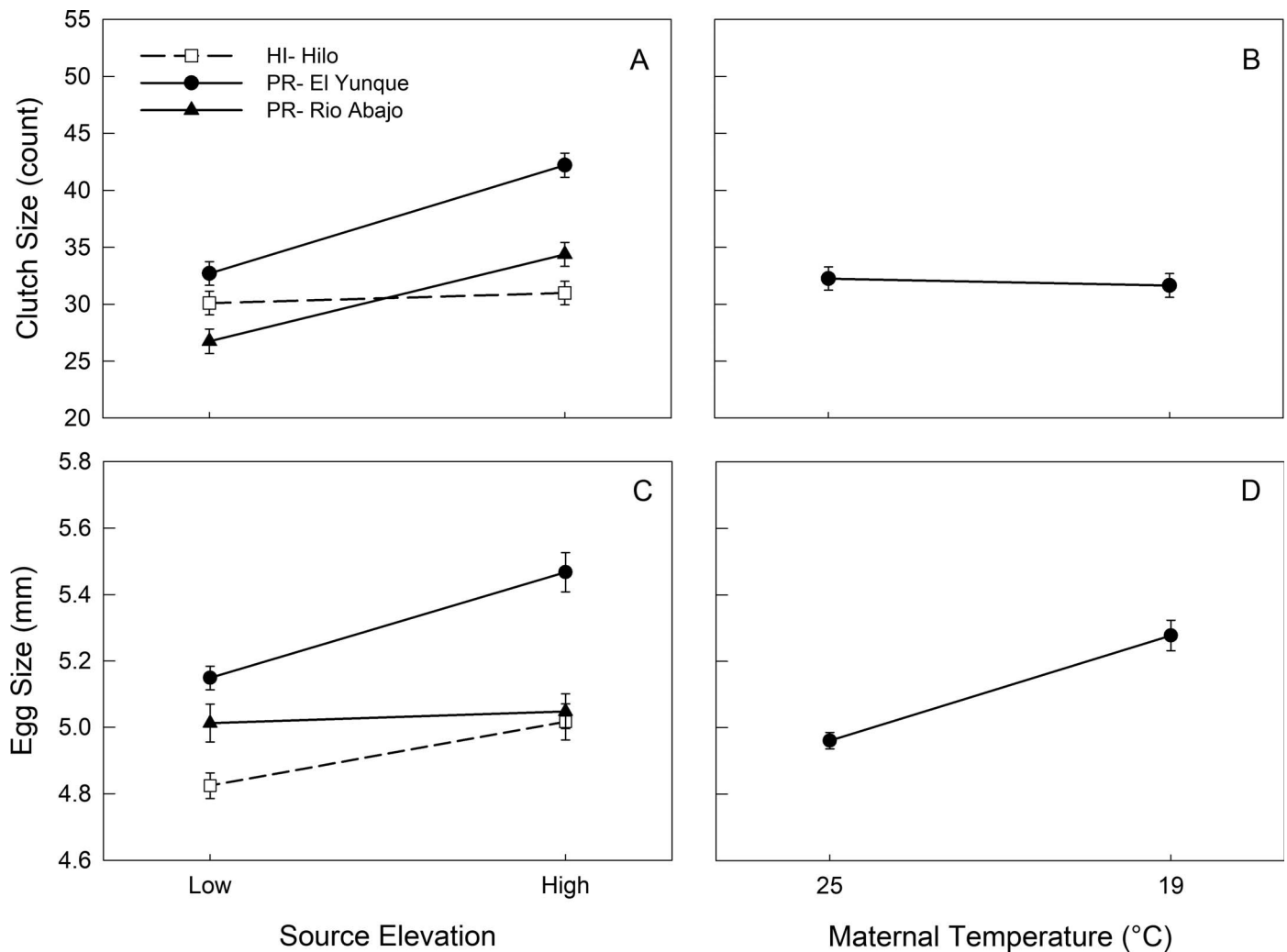


Fig. 2. The relationships between area and elevation (A, C), and maternal temperature (B, D) on clutch size (A, B) and egg size (C, D) in the laboratory (means \pm 1 SE).

$P < 0.0001$) and Hilo ($t_{116} = 4.72$; $P < 0.0001$), but hatchlings did not differ in size between Rio Abajo and Hilo ($t_{92} = 0.98$; $P = 0.33$; Fig. 3A). Eggs from high elevation populations were, on average, 2.7% larger, and hatchlings were 3.8% larger, compared to those from low elevation areas (Fig. 2C).

Both body size at 30 days and growth rate over the first 30 days after hatching also varied among source areas and between elevations. There was no area \times elevation interaction for body size at 30 days, but there was an area \times elevation interaction for growth rate (Table 4; Fig. 3C, E). Averaged across elevations, Coquis from El Yunque were 7% and 10% longer at 30 days than Coquis from Rio Abajo ($t_{75} = 6.22$; $P < 0.0001$) and Hilo ($t_{75} = 7.46$; $P < 0.0001$), respectively, but body size at 30 days did not differ between

Rio Abajo and Hilo ($t_{75} = -0.18$; $P < 0.86$). Similarly, growth rate for hatchlings of Coquis collected from El Yunque was 6% and 27% faster than those collected in Rio Abajo ($t_{75} = 3.55$; $P = 0.0007$) or Hilo ($t_{75} = 4.5$; $P < 0.0001$), respectively, but did not differ between Rio Abajo and Hilo ($t_{75} = -0.21$; $P = 0.83$). Averaged across areas, body size at 30 days was 7% greater, and growth rate was 30% greater, for Coquis whose parents were collected from higher elevations than low elevations (Fig. 3C). However, the difference in growth rate

Table 2. ANOVA for the relationships between area, elevation, and sex and adult body size (SVL) for *Eleutherodactylus coqui* in Hawaii and Puerto Rico. Best-fit model (chosen using AIC), which did not include higher-level interactions.

Source	df	F	P
Area	6,693	162.80	<0.0001
Elevation	1,693	656.74	<0.0001
Area \times Elevation	6,693	34.09	<0.0001
Sex	1,693	1702.66	<0.0001

Table 3. ANOVAs for the effects of area, elevation, and maternal temperature on clutch size and egg size in the laboratory. Best-fit model (chosen using AIC), which did not include higher-level interactions.

Source	df	F	P
Clutch size			
Area	2,106	9.44	0.0002
Elevation	1,106	16.97	<0.0001
Area \times Elevation	2,106	5.54	0.0454
Maternal temperature	1,106	6.55	0.1327
Egg size			
Area	2,82	18.32	<0.0001
Elevation	1,82	8.90	0.0037
Area \times Elevation	2,82	2.92	0.0594
Maternal temperature	1,82	21.42	<0.0001

Table 4. Best fit ANOVAs for hatching size, egg development period, and growth rate in the laboratory. Best fit models (chosen using AIC), which did not include higher-level interactions.

Source	Hatching size (mm)			Size at 30 days (mm)				Growth rate (mm day ⁻¹)			
	df	F	P	Source	df	F	P	Source	df	F	P
Area	2,92	19.01	<0.0001	Area	2,75	35.18	<0.0001	Area	2,75	12.37	<0.0001
Elevation	1,92	21.74	<0.0001	Elevation	1,75	40.31	<0.0001	Elevation	1,75	30.50	<0.0001
Area × Elevation	2,92	1.83	0.1660	Area × Elevation	2,75	1.94	0.1505	Area × Elevation	2,75	4.46	0.0148
Maternal temperature	1,92	10.65	0.0015	Maternal temperature	1,75	6.00	0.0166	Maternal temperature	1,75	0.03	0.8580
Rearing temperature	1,73	8.38	0.0050	Rearing temperature	1,54	8.25	0.0058	Rearing temperature	1,54	1.88	0.1765

between elevations for native areas was greater than that for the introduced area (area × elevation interaction). For the two native (Puerto Rico) areas, the growth rate was 43% and 79% faster for high elevation Coquis than for low elevation Coquis (El Yunque: $t_{75} = 5.09$; $P < 0.0001$ and Rio Abajo: $t_{75} = 3.94$; $P < 0.001$, respectively). In contrast, growth rate was not significantly different for high and low elevation Coquis for the introduced (Hawaii) populations (Hilo: $t_{75} = 0.89$; $P = 0.37$; Fig. 3E).

DISCUSSION

Following their introduction to Hawaii in the late 1980s and subsequent expansion into some high elevation areas, a difference in body size has emerged between high and low elevation populations of Coquis. Specifically, Coquis are larger at higher elevations than at lower elevations. This difference is similar, though of a lower magnitude, to the body size differences observed between high and low elevation populations in the native range of Puerto Rico, and is consistent with the observation that frogs typically mature at larger sizes at higher elevations or latitudes (Ashton, 2002; Morrison and Hero, 2003; Olalla-Tárraga and Rodríguez, 2007; but see Laugen et al., 2005a; Ma et al., 2009). Because ectotherms generally mature at larger sizes in lower temperatures (Ray, 1960; Atkinson, 1994; but see Walters and Hassall, 2006 for an exception in insects), and populations at higher elevations experience lower temperatures, temperature-induced plasticity may explain some of the variation in adult body size across elevations within both Puerto Rico and Hawaii. Our laboratory results support this hypothesis; for all populations, egg size, hatching size, and body size at 30 days were larger for frogs at lower temperatures in the laboratory. Variation in initial size has been shown to propagate through an individual's life (Chambers and Leggett, 1996; Laugen et al., 2003); therefore, we would expect larger eggs to have the potential to become larger adults. Indeed, variation in both hatching size and body size at 30 days largely mirrors variation in egg size in the laboratory.

Some of the variation in adult body size in both regions is therefore likely the result of plasticity in response to temperature, but this plasticity does not appear to explain all of the variation in adult body size in Puerto Rico or the differences between Puerto Rico and Hawaii. If plasticity in response to temperature was the only factor driving these patterns, we would expect to find larger Coquis in Hawaii because body size is negatively correlated with temperature and temperatures in Hawaii are lower than in Puerto Rico at any specific elevation (O'Neill and Beard, 2011). Instead, we found that Coquis were smaller at any specific elevation in Hawaii than in Puerto Rico. Furthermore, we would not

expect to find different relationships between body size and elevation in Puerto Rico and Hawaii because temperature varies across elevations to the same degree in both regions (O'Neill and Beard, 2011). However, variation in adult body size is smaller in Hawaii (i.e., the slope is shallower) than in Puerto Rico across the same elevation gradient (the area × elevation interaction). Combined, these results suggest that some factor besides temperature-induced plasticity is influencing body size of Coquis.

Like most quantitative traits, adult body size in Coquis is probably affected by both genetic and environmental effects. Indeed reciprocal transplant experiments using the temperate frog *Lithobates sylvaticus* (LeConte, 1825) indicate that body size is determined by a combination of genetic and environmental effects (Berven, 1982a, 1982b; Riha and Berven, 1991). Therefore, one possibility is that variation in adult body size is heritable to some degree and that, in Puerto Rico, Coquis from higher elevations are genetically larger than Coquis from lower elevations. Under this hypothesis, we would expect Coquis to be generally smaller in Hawaii than in Puerto Rico because all populations in Hawaii are descended from low elevation populations in Puerto Rico where Coquis are smaller (Velo-Antón et al., 2007; Peacock et al., 2009). Additionally, we would expect less variation in adult body size between elevations in Hawaii because the proposed genetic differences would likely be absent there and only the plastic effects of temperature would likely remain. Our results are consistent with both of these expectations.

In amphibians, growth rate is often a significant source of variance in body size (Halliday and Verrell, 1988), and multiple studies have found significant heritability for growth rate in frogs (Berven, 1987; Laugen et al., 2002, 2003, 2005b; Laurila et al., 2002; Palo et al., 2003; Lindgren and Laurila, 2010). Our laboratory results indicate that variation in growth rate in the laboratory largely mirrors variation in adult body size in the field. We observed this despite rearing our frogs at constant temperatures mimicking average differences observed between sites in the field, but not representing natural daily fluctuations. Multiple experiments have shown that fluctuating temperatures can influence growth and reproduction of ectotherms in ways not easily predicted from temperature averages (Kern et al., 2015; Kingsolver et al., 2015), including some frog species (Niehaus et al., 2012; Arrighi et al., 2013). Despite this, laboratory growth rates largely mirrored observed variation in adult body size in the field. If growth rate is also heritable in Coquis, then this trait may provide the heritable variation in adult body size proposed above.

Typically studies attempting to identify genetic effects will rear multiple generations in the laboratory to eliminate potential maternal effects. This is because environmental conditions experienced by mothers in the field can influence

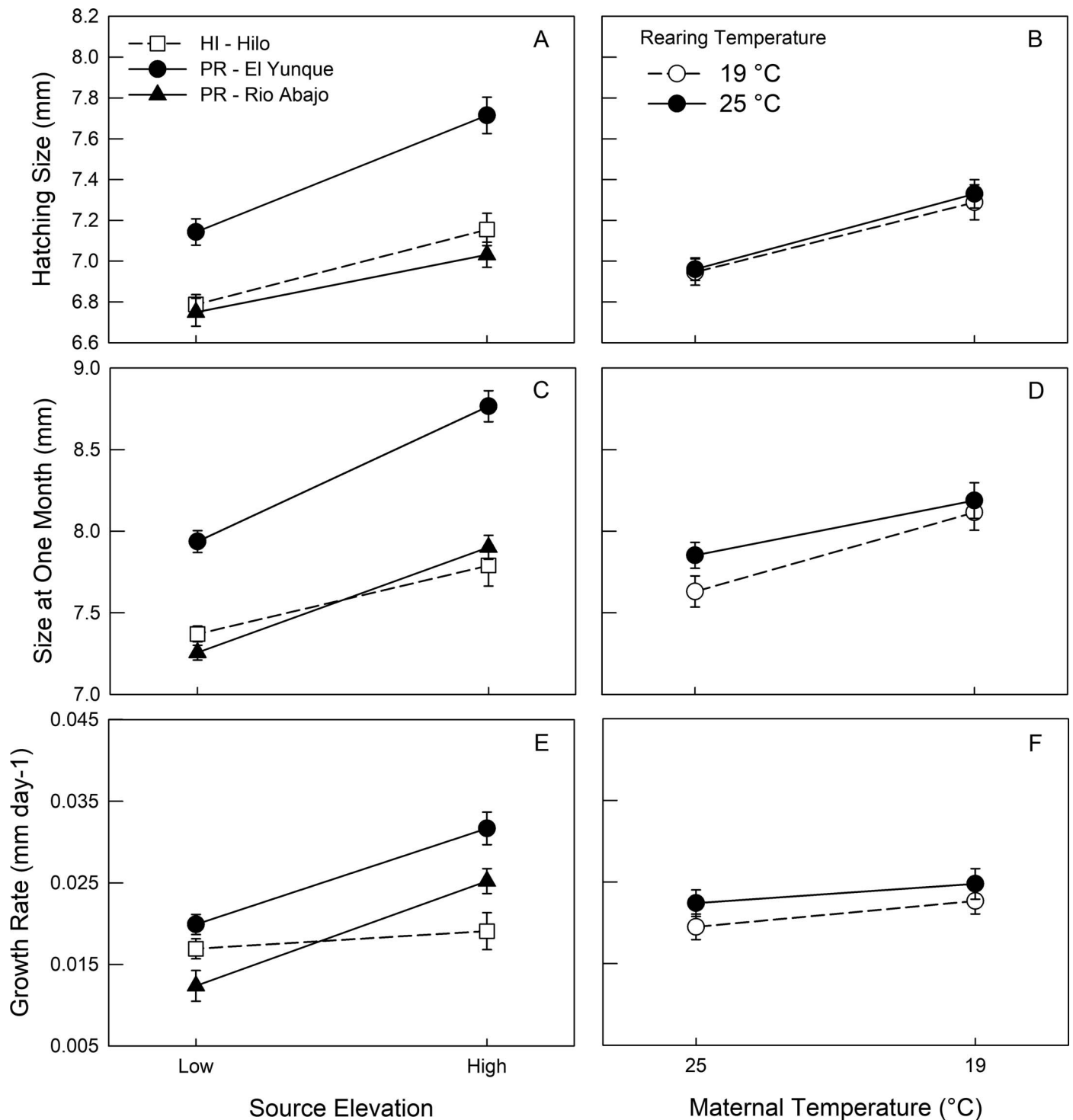


Fig. 3. The relationships between source area and elevation (A, C, E), and maternal and rearing temperature (B, D, F) on hatching size (A, B), body size at 30 days (C, D), and growth rate (E, F) in the laboratory (means \pm 1 SE).

egg quality and/or composition and thus influence growth and development of their offspring (Mousseau and Fox, 1998; Heath et al., 1999). Rearing field-collected populations in the laboratory for one or more generations before initiating an experiment can reduce the contribution of environmentally-based maternal effects to differences observed in a common garden. However, because Coquis can take eight months to a year to reach maturity (Townsend and Stewart, 1994), this was not possible for our experiment. Further, Coquis are hard to rear to maturity in a laboratory

setting, and to do that with enough individuals to test these hypotheses would have been extremely difficult and beyond our logistical constraints. We thus acknowledge that multi-generational studies that remove maternal effects, albeit impractical, are necessary to disentangle more conclusively the relative contributions of genetic and maternal effects in this species.

Plasticity is often considered as an important factor in the success of invasive species and multiple studies have confirmed this for plants and invertebrates (Daehler, 2003;

Davidson et al., 2011; Coccia et al., 2013; Niu et al., 2013). Phenotypic plasticity in response to temperature likely contributes to adult body size variation in both Puerto Rico and Hawaii and may have contributed to the establishment of Coquis in different elevations in Hawaii by allowing them to attain larger body sizes at higher elevations (in lower temperatures) and smaller body sizes in lower elevations (higher temperatures). At this point, Coquis have expanded their range in Hawaii at lower elevations much more than they have expanded to higher elevations. A positive outcome of this study is that source effects that limit adult body size variation in Coquis may also limit their ability to adapt to higher elevations in Hawaii where temperatures are lower. This is important from an ecological perspective because higher elevation areas in Hawaii host the remaining native forests, and most rare and endangered Hawaiian birds and invertebrates due to disease and habitat loss in the lowlands (Beard and Pitt, 2005).

Previous research in Hawaii has shown that Coquis reduce insect abundance (Choi and Beard, 2012), increase non-native birds, particularly birds that consume Coquis (Smith et al., 2017), and increase nutrient cycling rates (Sin et al., 2008) where they invade. Coquis could have a greater negative impact if they invade higher elevation areas because more native species are found there. While there have not been any studies investigating how frog body size influences these interactions, if Coqui frogs remain smaller at higher elevations in Hawaii than in Puerto Rico, there are likely ecological implications. Previous research in Puerto Rico (Stewart and Woolbright, 1996) and in Hawaii (Beard et al., 2008) have shown that where Coqui body size is smaller, Coqui density is higher, perhaps due to competition for resources. Smaller frogs also consume more but smaller prey items (Stewart and Woolbright, 1996; Beard, 2007), and they may also be more likely to serve as prey for non-native birds (Smith et al., 2017). So, we might expect greater reductions in invertebrates and increases in non-native birds where they invade with smaller body sizes. Future research could investigate these hypotheses.

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