

## Article

# Contributions of source population and incubation temperature to phenotypic variation of hatchling Chinese skinks

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## Abstract

Phenotypic plasticity and local adaptation are viewed as the main factors that result in between-population variation in phenotypic traits, but contributions of these factors to phenotypic variation vary between traits and between species and have only been explored in a few species of reptiles. Here, we incubated eggs of the Chinese skink (*Plestiodon chinensis*) from 7 geographically separated populations in Southeast China at 3 constant temperatures (24, 28, and 32 °C) to evaluate the combined effects of clutch origin, source population, and incubation temperature on hatchling traits. The relative importance of these factors varied between traits. Nearly all examined hatchling traits, including body mass, snout–vent length (SVL), tail length, head size, limb length, tympanum diameter, and locomotor speed, varied among populations and were affected by incubation temperature. Measures for hatchling size (body mass and SVL) varied considerably among clutches. Source population explained much of the variation in hatchling body mass, whereas incubation temperature explained much of the variation in other examined traits. Our results indicate that between-population variation in hatchling traits of *P. chinensis* likely reflects the difference in natural incubation conditions and genetic divergence.

**Key words:** egg incubation, hatchling phenotype, incubation temperature, local adaptation, *Plestiodon chinensis*, source population

Widespread species often display considerable geographic variation in phenotypes. Phenotypic variation may be caused by genetic differentiation due to local adaptation and by phenotypic plasticity in response to variation in local environmental factors (Mathies and Andrews 1995; Badyaev and Ghalambor 2001; Seigel and Ford 2001; Morrison and Hero 2003; Marshall 2005; Conover et al. 2009; Proćków et al. 2017; Schneiderová et al. 2020). In reptiles, for example, variation in hatchling phenotypes such as body size and shape, locomotor performance, and behavior can be induced by environmental conditions during embryonic development, and also

can be population-specific (Qualls and Shine 1998; Du et al. 2010; Lu et al. 2014). Identifying the relative contributions of environment-induced and population-specific (or genetic) influences to trait variation is important for our understanding of the adaptive significance of phenotypic variation in particular environments. However, previous studies have mainly addressed the environment-induced influences (Qualls and Shine 1998, 2000; Buckley et al. 2007, 2010; Du et al. 2010, 2014; Lin et al. 2012; Verdú-Ricoy et al. 2014; Ma et al. 2019), paying much less attention to the relative contributions of the main factors inducing trait variation.

It has been well documented in reptiles that incubation temperature affects hatchling phenotypes (Birchard and Deeming 2004; Deeming 2004). Within the range of temperatures producing viable hatchlings, eggs at moderate to relatively low temperatures are more likely to hatch and produce high-quality hatchlings than those at extremely low or high temperatures (e.g., Allsteadt and Lang 1995; Warner and Andrews 2002; Booth 2006; Brown and Shine 2006; Verdú-Ricoy et al. 2014). For example, within the range (24–33 °C) of temperatures, eggs of the northern grass lizard *Takydromus septentrionalis* at temperatures <30 °C are more likely to hatch and produce larger and faster-running hatchlings than those at higher temperatures (Lin and Ji 1998; Du and Ji 2006). Thermal environments vary geographically. Reproductive females have the potential to adjust seasonal timing of oviposition and nesting behavior in response to thermal differences, but behavioral adjustments cannot counteract completely for the thermal variation in different localities (Kolbe and Janzen 2002; Telemeco et al. 2009; Refsnider et al. 2013). Phenotypic responses of reptilian embryos and hatchlings to incubation temperature often vary among populations in widespread species (Sears and Angilletta 2003; Verdú-Ricoy et al. 2014). However, the magnitude of phenotypic variation in a species varies between traits. In *Sceloporus occidentalis*, for example, body mass, forelimb length, and inter-limb length at hatching are more highly variable in response to temperature variation, whereas other hatchling traits such as snout–vent length (SVL), hindlimb, and head length are less so (Buckley et al. 2010).

Diverse phenotypic responses to incubation temperatures have been reported in a number of oviparous reptiles (e.g., Ji et al. 2002a; Du et al. 2010; Sun et al. 2013; Zeng et al. 2013; Verdú-Ricoy et al. 2014). However, only a few of these studies were designed to identify the relative contribution of temperature-induced and population-specific (genetic) influences to geographic variation in hatchling phenotypes (but see also Qualls and Shine 1998; Buckley et al. 2007, 2010). To better illustrate this issue, we need to compare various phenotypes of hatchlings from different populations and incubated under multiple thermal regimes in a wide variety of taxa.

The Chinese skink *Plestiodon chinensis* is a medium-sized (up to 134 mm SVL) ground-dwelling oviparous scincid lizard that is widely distributed in Southeast China (Zhao and Adler 1993). The skink shows considerable geographic variation in life-history traits such as size at sexual maturity, the number of eggs produced per season, egg size, and thus hatchling size (Lin and Ji 2000; Ji et al. 2002a; Lu et al. 2014; Ma et al. 2018a). Eggs of this species can be successfully incubated at temperatures from 24 °C to 32 °C (hatching success >82%, Ji and Zhang 2001; Qu et al. 2014; Shen et al. 2017; Ma et al. 2018a). However, temperatures optimal for embryonic development differ among populations. The mean size of eggs and hatchlings from colder localities tends to be larger than that from warmer localities, probably reflecting the adaptation to local thermal environments (Ji et al. 2002a; Lu et al. 2014). Here, we incubated *P. chinensis* eggs from 7 geographically distinct populations at 3 constant temperatures (24, 28, and 32 °C) to identify the sources of between-population variation in hatchling phenotypes. Our aims were to show phenotypic variation in hatchlings from the 7 sampled populations, and to evaluate the relative contribution of temperature-induced plasticity and population-specific effects to geographic variation in hatchling phenotypes. From previous studies on *P. chinensis* (Ji et al. 2002a; Lu et al. 2014) and other lizards such as *S. occidentalis* (Buckley et al. 2007, 2010) and *Psammodromus algirus* (Verdú-Ricoy et al. 2014), we predict that contributions of source population and incubation temperature should vary between

hatchling traits. More specifically, we predict that highly plastic traits such as SVL, tail, and limb length should be more likely to be affected by incubation temperature than by source population and/or clutch origin.

## Materials and Methods

### Egg collection, incubation and hatchling phenotype measurement

In early May of 2004 and 2006, we captured gravid skinks by hand from 7 populations (localities) in mainland China (Shaoguan [SG], Quanzhou [QZ], Rongjiang [RJ], Ningde [ND], Nanfeng [NF], Lishui [LS], and Jiande [JD]; Table 1). Females are rarely found to ovulate earlier than mid-May (Hu et al. 2004; Lu et al. 2014). Accordingly, we believed that females collected in early May had not yet ovulated, and thus the impact of local environments on phenotypic plasticity at early stages of embryonic development would be rather limited if it existed. The 7 localities differ environmentally, with altitudes ranging from 50 m (ND) to 750 m (RJ), annual mean air temperatures from 17.4 °C (JD) to 22.9 °C (SG), and annual precipitation from 1016 mm (RJ) to 1696 mm (QZ) (Table 1). The climate is generally warmer at lower-latitude than at higher-latitude localities (repeated-measures analysis of variance [ANOVA] on monthly mean air temperature,  $F_{6, 66} = 42.11$ ,  $P < 0.001$ , SG<sup>a</sup>, QZ<sup>b</sup>, RJ<sup>cd</sup>, NF<sup>cd</sup>, ND<sup>c</sup>, LS<sup>cd</sup>, JD<sup>d</sup>, a > b > c > d). All gravid females were transported to our laboratory in Hangzhou, where they were housed in outdoor enclosures (length × width × height: 1.5 × 1.5 × 0.6 m) with a substrate consisting of moist soil at a depth of 150 mm. Skinks had the opportunity to regulate body temperature using natural sunlight, and the natural photoperiod in May and June is close to 13 h light:11 h dark. Mealworms (larvae of *Tenebrio molitor*), house crickets (*Achetus domesticus*), and water enriched with vitamins and minerals were provided daily. We checked the enclosures at least twice daily for eggs after the first female laid eggs. Eggs were counted and weighed individually to the nearest milligram on an analytical balance (Mettler PB303-N, Shanghai, China).

A total of 181 clutches (SG: 28, QZ: 21, RJ: 10, ND: 22, NF: 12, LS: 74, and JD: 14) were collected. The size of reproductive females varied among populations (SVL:  $F_{6, 174} = 7.75$ ,  $P < 0.001$ ; post-oviposition mass:  $F_{6, 174} = 7.00$ ,  $P < 0.001$ , Table 1). Three fertilized eggs randomly selected from each clutch were incubated in covered plastic jars (50 mL) with known amounts of vermiculite and water at –12 kPa water potential (1 g dried vermiculite/2 g water; Ji and Zhang 2001). One-third of each egg was buried lengthwise in the substrate, with the surface near the embryo exposed to air inside the jar. Jars were assigned to 1 of 3 incubators (Binder KB115, Tuttlingen, Germany) that were set at the temperatures of 24, 28, and 32 °C (±0.2), respectively. The lowest (24 °C) and highest (32 °C) temperatures are, respectively, close to the lower and upper limits within which *P. chinensis* eggs can be incubated, and 28 °C is optimal for egg incubation of the species (Ji and Zhang 2001; Shen et al. 2017). Eggs from individual clutches were assigned equally to different treatments to minimize the family effect, and mean values for egg mass did not differ among the 3 temperature treatments ( $F_{2, 348} = 1.62$ ,  $P = 0.199$ ). Water was added into the substrate every other day to compensate for loss due to evaporation and the absorption caused by the embryonic metabolism. Jars were moved among the shelves daily following a predetermined schedule to minimize any influence of thermal gradients inside the incubator. One fertilized egg randomly selected from each clutch was dissected to

**Table 1.** Altitudes, mean values for annual mean temperature and precipitation over a 20-year period (1993–2013), and descriptive statistics, expressed as mean  $\pm$  SE (range), for maternal size of the 7 localities where skinks were collected

Population (locality)	Geographic coordinate	Altitude (m)	Annual mean temperature ( $^{\circ}$ C)	Annual precipitation (mm)	Maternal size	
					SVL (mm)	Mass (g)
SG	24 $^{\circ}$ 48'N, 113 $^{\circ}$ 35'E	280	22.9	1540	111.0 $\pm$ 1.3 (95.8–120.5)	25.9 $\pm$ 1.0 (17.3–35.5)
QZ	25 $^{\circ}$ 56'N, 111 $^{\circ}$ 04'E	200	21.2	1696	99.9 $\pm$ 1.4 (85.8–110.6)	18.1 $\pm$ 0.6 (15.0–24.2)
RJ	25 $^{\circ}$ 58'N, 108 $^{\circ}$ 32'E	750	18.1	1016	106.8 $\pm$ 2.1 (97.0–117.5)	21.6 $\pm$ 1.1 (16.2–26.3)
ND	26 $^{\circ}$ 40'N, 119 $^{\circ}$ 31'E	50	19.0	1176	107.8 $\pm$ 1.4 (96.4–121.8)	23.2 $\pm$ 0.9 (19.1–36.0)
NF	27 $^{\circ}$ 13'N, 116 $^{\circ}$ 32'E	106	18.3	1303	109.6 $\pm$ 1.9 (98.5–117.6)	23.9 $\pm$ 1.1 (14.7–29.6)
LS	28 $^{\circ}$ 27'N, 119 $^{\circ}$ 55'E	70	17.8	1196	104.0 $\pm$ 0.8 (89.4–118.8)	22.4 $\pm$ 0.6 (12.9–40.6)
JD	29 $^{\circ}$ 29'N, 119 $^{\circ}$ 16'E	130	17.4	1262	104.7 $\pm$ 1.5 (93.8–112.1)	20.9 $\pm$ 0.9 (15.3–26.9)

All data other than maternal size were collected from the China meteorological data sharing service system.

determine its [Dufaure and Hubert's \(1961\)](#) embryonic stage and egg composition at oviposition ([Lu et al. 2014](#)). The remaining eggs were incubated under other thermal regimes or dissected to determine changes in egg composition during incubation, and data were reported elsewhere.

A total of 481 eggs hatched ([Table 2](#)), and hatchlings were individually weighed and measured for locomotor performance and morphological phenotype. We conducted locomotor trials on the second day after hatching and at the body temperature of 30  $^{\circ}$ C, which was achieved by placing hatchlings in an incubator (Sheldon MFG Inc., USA) at 30  $^{\circ}$ C for 30 min prior to testing. The temperature was chosen simply because *P. chinensis* hatchlings often attain the fastest running speed at body temperatures around 30  $^{\circ}$ C ([Qu et al. 2014](#)). Locomotor performance was assessed for each hatchling by chasing it along a 2 m racetrack with 1 transparent side. Hatchlings were filmed with a Panasonic NV-DS77 digital video camera. The racetrack was kept in a room set at 30 ( $\pm$ 0.2)  $^{\circ}$ C, and it was always the first author (H.L.L.) who tapped on the tail of hatchlings with a paintbrush to encourage them to run. Each hatchling was run twice with a rest of 30 min between 2 successive trials and, during the interval, it was placed back to the incubator. Hatchlings that refused to run were excluded from analysis. The tapes were later examined with a computer using MGI VideoWave III software (MGI Software Co., Toronto, Canada) for sprint speed in the fastest 25-cm interval. After examination of locomotor performance, the hatchlings were individually measured with Mitutoyo digital calipers for SVL (from the tip of the snout to the anterior edge of the vent), tail length (from the posterior edge of the vent to the tip of the tail), head length (from the tip of the snout to the anterior edge of the tympanum), head width (taken at the widest part of head), forelimb length (from axilla to the base of the longest finger), hindlimb length (from groin to the base of the longest toe), and tympanum diameter (taken at widest point of tympanum) ([Ji et al. 2002b](#)). The hatchlings were released in early August at the sites where their mothers were collected.

### Data analyses

All statistical analyses were performed using SPSS version 13.0. Prior to parametric analyses, data were tested for normality using Kolmogorov–Smirnov test and for homogeneity of variances using Levene's test. We did not find asymmetries in the examined bilateral hatchling traits (fore- and hind-limb lengths, and tympanum diameter) (all  $P > 0.05$ ); therefore, we used mean values of the measurements from both sides for further analyses. We used mixed-model ANOVA or analysis of covariance (ANCOVA, with egg mass as the covariate for hatchling SVL and body mass, and hatchling SVL as

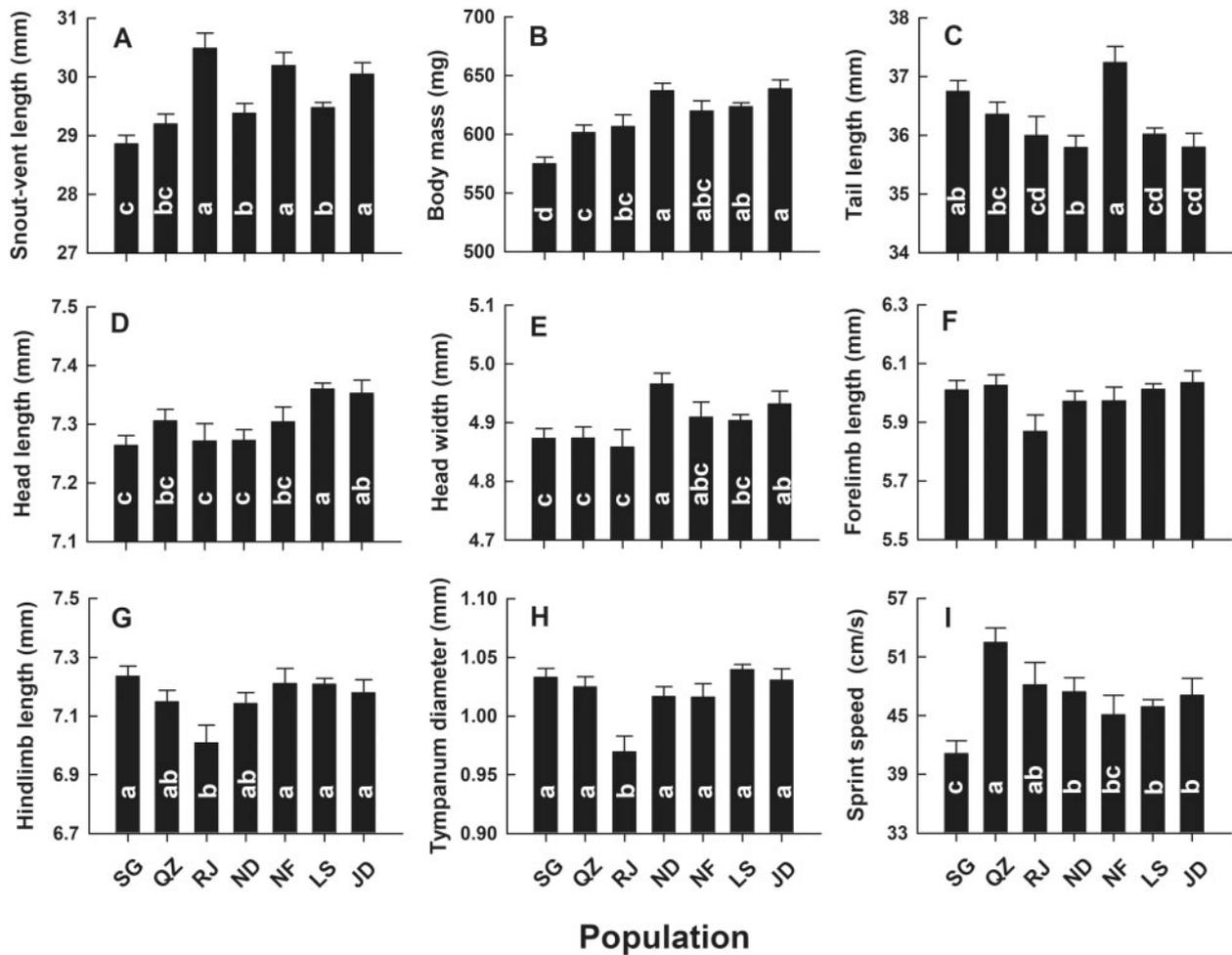
**Table 2.** The number of eggs incubated and hatched in each combination of source population and incubation temperature

Population (locality)	Incubation temperature ( $^{\circ}$ C)	Number of eggs incubated	Number of eggs hatched
SG	24	28	24
	28	28	19
	32	28	28
QZ	24	21	18
	28	21	14
	32	21	21
RJ	24	10	9
	28	10	5
	32	10	10
ND	24	22	18
	28	22	16
	32	22	22
NF	24	12	9
	28	12	12
	32	12	9
LS	24	74	72
	28	74	61
	32	74	74
JD	24	14	13
	28	14	13
	32	14	14

the covariate for all other examined hatchling morphological traits) with source population and incubation temperature as the fixed factors, and clutch origin as the random factor to examine differences in hatchling traits, and to estimate the relative magnitude of these factorial effects on hatchling traits. We used Kruskal–Wallis test to examine whether mean values for embryonic stage at oviposition differed among populations. Throughout this article, values are presented as mean  $\pm$  standard error (SE) and the significance level is set at  $\alpha = 0.05$ .

### Results

A mixed model ANOVA revealed that mean values for initial egg mass (mass at laying) varied among populations ( $F_{6, 174} = 5.51$ ,  $P < 0.0001$ ; SG<sup>c</sup>, QZ<sup>c</sup>, RJ<sup>ab</sup>, NF<sup>ab</sup>, ND<sup>ab</sup>, LS<sup>b</sup>, JD<sup>a</sup>,  $a > b > c$ ) and among clutches ( $F_{174, 348} = 110.36$ ,  $P < 0.0001$ ). Eggs from the colder localities were on average larger than those from the warmer localities. Embryonic stages at oviposition varied from [Dufaure and Hubert's \(1961\)](#) Stages 31–35, with a mean stage of 32.7. The mean



**Figure 1.** Mean (for SVL and sprint speed) and adjusted mean (for other 7 traits, with hatchling SVL set at 29.5 mm) values (+SE) for the 9 hatchling traits that were compared among 7 populations of *P. chinensis*. Populations labeled with different letters differ significantly (Tukey's *post hoc* test,  $\alpha = 0.05$ ;  $a > b > c > d$ ).

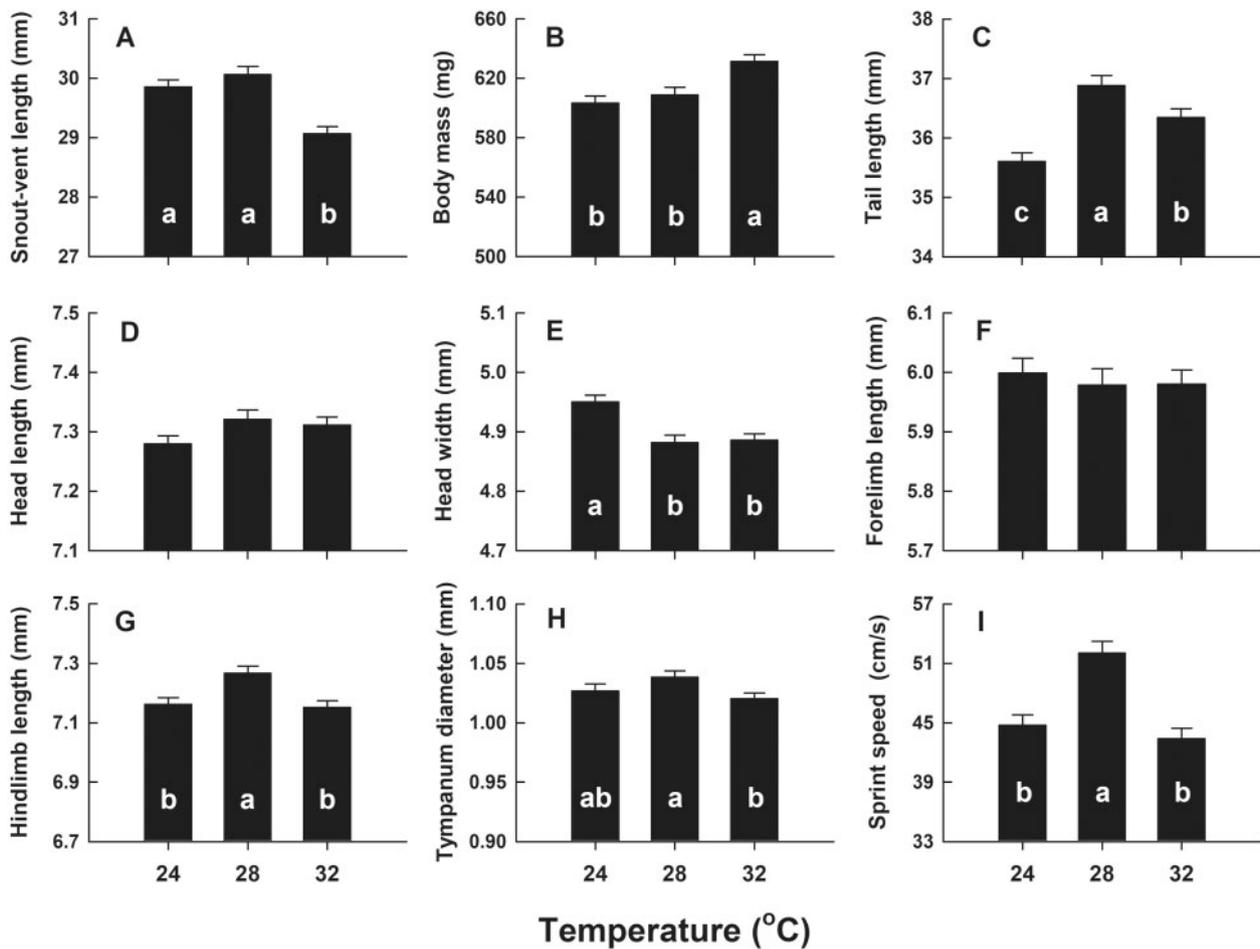
embryonic stage did not differ among populations (Kruskal–Wallis test;  $H_{6, N=118} = 6.81$ ,  $P = 0.339$ ).

From the results of mixed model ANCOVAs with egg mass as the covariate, we knew the following. First, hatchlings from the RJ and JD populations were longer ( $F_{6, 164} = 5.20$ ,  $P < 0.0001$ ) and heavier ( $F_{6, 165} = 4.88$ ,  $P < 0.0001$ ) than those from the QZ and SG populations after accounting for egg mass, with the other 3 populations in between (Figure 1). Second, hatchlings incubated at 32 °C were shorter and lighter than those incubated at 24 °C or 28 °C (SVL:  $F_{2, 311} = 48.07$ ,  $P < 0.0001$ ; body mass:  $F_{2, 311} = 11.43$ ,  $P < 0.0001$ ; Figure 2). Third, clutch origin (family effect) was a significant source of variation in hatchling SVL ( $F_{174, 286} = 2.53$ ,  $P < 0.001$ ), but the effect was absent after accounting for egg mass ( $F_{174, 285} = 1.04$ ,  $P = 0.389$ ).

Mixed model ANOVA or ANCOVAs with hatchling SVL as the covariate revealed that nearly all hatchling morphological traits were affected by source population and incubation temperature (Table 3). Hatchlings from the JD and ND populations had relatively stockier bodies (mass relative to SVL) than those from the SG and QZ populations (Figure 1B); hatchlings from the SG and NF populations had relatively longer tails than those from the RJ, JD, and ND populations (Figure 1C); hatchlings from the LS and JD populations had relatively longer head than those from the SG, RJ and ND populations (Figure 1D); hatchlings from the JD and ND populations had

relatively wider head than those from the SG, QZ, and RJ populations (Figure 1E); forelimb length differed from all other hatchling traits measured, as it did not vary among populations (Figure 1F); hatchlings from the SG, NF, LS, and JD populations had relatively longer hindlimbs than those from the RJ population (Figure 1G); hatchlings from the RJ population had relatively smaller tympanum diameters than those from the other 6 populations (Figure 1H). Hatchlings incubated at 32 °C had relatively stockier bodies than those at 24 and 28 °C (Figure 2B); hatchlings incubated at 28 °C had relatively longer tails and hindlimbs and larger tympanum diameters than those at 24 and 32 °C; hatchlings incubated at 24 °C had relatively wider (but not longer) heads than those at 28 and 32 °C (Figure 2). The effect of clutch origin was significant on hatchling body mass and head width, but not on other morphological traits (Table 3).

None of the potential covariates was found to be correlated with sprint speed of the hatchlings; we, therefore, used ANOVA to analyze data. Hatchling sprint speed varied among populations ( $F_{6, 165} = 5.68$ ,  $P < 0.001$ ) and was affected by incubation temperature ( $F_{2, 310} = 40.40$ ,  $P < 0.001$ ); clutch origin was not a significant source of variation in this trait ( $F_{174, 286} = 0.98$ ,  $P = 0.544$ ). Hatchlings from the QZ population were fastest and those from the SG population (~28% slower than the mean sprint speed of QZ hatchlings) were slowest, with hatchlings from other populations in



**Figure 2.** Mean (for SVL and sprint speed) and adjusted mean (for other 7 traits, with hatchling SVL set at 29.5 mm) values (+SE) for the 9 hatchling traits that were compared among 3 temperatures. Temperature treatments with different letters differ significantly (Tukey's *post hoc* test,  $\alpha = 0.05$ ;  $a > b > c$ ).

**Table 3.** Results of mixed model ANCOVAs (for hatchling SVL with egg mass as the covariate and for all other traits with hatchling SVL as the covariate) with source population and incubation temperature as the fixed factors, and clutch origin as the random factor

Hatchling variables	Covariate	Population	Temperature	Clutch	Population × temperature
SVL	$F_{1, 140} = 474.00^{***}$	$F_{6, 164} = 5.20^{***}$	$F_{2, 311} = 48.07^{***}$	$F_{174, 285} = 1.04^{NS}$	$F_{12, 285} = 0.84^{NS}$
Wet body mass	$F_{1, 233} = 633.70^{***}$	$F_{6, 172} = 7.95^{***}$	$F_{2, 428} = 44.00^{***}$	$F_{174, 285} = 3.91^{***}$	$F_{12, 285} = 1.45^{NS}$
Tail length	$F_{1, 363} = 901.97^{***}$	$F_{6, 167} = 6.29^{***}$	$F_{2, 336} = 42.03^{***}$	$F_{174, 285} = 1.11^{NS}$	$F_{12, 285} = 1.26^{NS}$
Head length	$F_{1, 355} = 758.73^{***}$	$F_{6, 167} = 6.89^{***}$	$F_{2, 339} = 2.97^{NS}$	$F_{174, 285} = 1.17^{NS}$	$F_{12, 285} = 0.49^{NS}$
Head width	$F_{1, 304} = 344.14^{***}$	$F_{6, 169} = 2.95^{**}$	$F_{2, 362} = 15.60^{***}$	$F_{174, 285} = 1.72^{***}$	$F_{12, 285} = 1.44^{NS}$
Forelimb length	$F_{1, 354} = 316.26^{***}$	$F_{6, 167} = 1.51^{NS}$	$F_{2, 339} = 0.44^{NS}$	$F_{174, 285} = 1.18^{NS}$	$F_{12, 285} = 1.75^{NS}$
Hindlimb length	$F_{1, 376} = 400.03^{***}$	$F_{6, 166} = 2.85^{*}$	$F_{2, 332} = 8.13^{***}$	$F_{174, 285} = 1.01^{NS}$	$F_{12, 285} = 0.50^{NS}$
Tympanum diameter	$F_{1, 361} = 40.23^{***}$	$F_{6, 167} = 6.90^{***}$	$F_{2, 337} = 4.74^{**}$	$F_{174, 285} = 1.13^{NS}$	$F_{12, 285} = 1.99^{*}$

NS (no significance):  $P > 0.05$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

between (Figure 11). Hatchlings incubated at 28 °C ran faster than those at 24 °C and 32 °C (Figure 21). The mean sprint speed of hatchlings incubated at 28 °C was ~16% faster than those incubated at 24 °C, and ~20% faster than those incubated at 32 °C.

The percentage of a trait's total variance explained by source population varied from 11.0% (for hindlimb length) to 75.0% (for body mass), with a mean of 27.6% (Table 4). The percentage of a trait's total variance explained by incubation temperature varied from 13.0% (for body mass) to 78.8% (for hindlimb length), with a mean of 60.0% (Table 4). The percentage of a trait's total variance explained by clutch origin varied from 3.6% (for sprint

speed) to 10.6% (for forelimb length), with a mean of 6.7% (Table 4).

## Discussion

As reported for other widespread reptiles (Qualls and Shine 1998; Buckley et al. 2007, 2010; Du et al. 2010; Verdú-Ricoy et al. 2014), hatchling Chinese skinks exhibited significant between-population (geographic) variation in phenotypic traits. Phenotypic variation in hatchling traits may reflect the combined effects of incubation

**Table 4.** Proportions of phenotypic variation in hatchling *Plestiodon chinensis* from 7 populations that can be attributable to source population, incubation temperature, and clutch origin

Hatchling variables	Population (%)	Temperature (%)	Clutch (%)	Population × temperature (%)
SVL	24.2	68.0	4.9	2.9
Wet body mass	75.0	13.0	8.9	3.1
Tail length	16.4	75.4	4.5	3.7
Head length	40.0	48.9	7.4	3.7
Head width	22.3	68.5	5.8	3.4
Forelimb length	19.0	60.3	10.6	10.1
Hindlimb length	11.0	78.8	5.7	4.5
Tympanum diameter	28.4	51.0	8.8	11.8
Sprint speed	12.1	76.1	3.6	8.2

condition, source population as well as clutch origin (Qualls and Shine 1998; Buckley et al. 2010). We found that incubation temperature-induced phenotypic changes explained a substantial proportion of variation in most of the hatchling traits examined. Earlier studies have shown that even a small thermal gradient between populations (localities) may generate substantial geographic variation in hatchling phenotypes. For example, *S. occidentalis* hatchlings from 4 localities between which the maximum difference in annual mean temperature is smaller than 5.2 °C exhibit significant geographic variation in SVL, limb length, and tail length (Buckley et al. 2010). For our sampling localities, their differences in climatic conditions are also evident, as revealed by the fact that the difference in annual mean temperature between localities can be up to ~5.5 °C (Table 1). Accordingly, we expect that thermal differences between populations may contribute substantially to geographic variation in hatchling traits. Unfortunately, we currently have no data on thermal regimes in natural nests to confirm this expectation. Future work could usefully study whether the relative contribution of thermal differences in natural nests to geographic variation in hatchling traits is equal or close to the magnitude level obtained here.

Of the examined hatchling traits, only 3 (SVL, body mass, and head width) were affected by clutch origin. The clutch (or family) effect explains a large amount of variation in hatchling traits in *P. algirus* (Diaz et al. 2012) and *P. chinensis* (Lu et al. 2014). However, our results showed a relatively weak clutch effect, as revealed by the fact that smaller proportions of variation in hatchling SVL (4.9%) and body mass (8.9%) were explained by clutch origin when compared with those reported previously for *P. chinensis* (17.3% for SVL and 12.8% for body mass, Lu et al. 2014). This weakened clutch effect might result from a relatively weak detection power in this study where only a few eggs from each clutch were used.

Consistent with the results reported previously for *P. chinensis* (Ji et al. 2002a; Lu et al. 2014), hatchlings from colder localities (e.g., LS and JD) were larger (longer SVL) and had relatively stockier bodies (mass relative to SVL) than those from warmer localities (e.g., SG and QZ). Larger individuals are thought to have better locomotor performance and hence greater chance to survive (Ferguson and Fox 1984; Sinervo and Adolph 1989; Garland et al. 1990; Sinervo 1990; Sinervo et al. 1992; Warner and Andrews 2002). The active season is shorter in cold-climate lizards than in warm-climate conspecifics. Therefore, larger hatchlings in colder localities are favored to compensate for the adverse effects of the shorter active season. Relatively stockier body shape reduces the surface-area-to-volume ratio of animals, and thus decreases heat loss in colder localities (Carrascal et al. 1992). However, our results unexpectedly showed that hatchlings from colder localities did not

necessarily perform better than those from warmer localities. For example, larger LS and JD hatchlings did not run faster than did smaller QZ hatchlings. Another interesting finding was that hatchlings from eggs incubated at 24 °C were larger but not heavier than those from the incubation temperature of 32 °C. The explanation, most probably, is that longer incubation lengths at lower temperatures allow hatchlings to attain larger sizes, but simultaneously increase metabolic consumption and therefore reduce energy conversion efficiency during embryonic development (Angilletta et al. 2000; Booth et al. 2000; Oufiero and Angilletta 2010). That hatchlings from eggs incubated at 32 °C had stockier bodies should be due to the fact that they retained more residual yolk (Ji and Zhang 2001; Ji et al. 2002a). Source population explained most (75%) of the variation in hatchling mass, confirming that offspring mass is a more canalized trait more likely to be determined genetically (Lu et al. 2014). Conversely, incubation temperature explained most (68.0%) of the variation in hatchling SVL, indicating that it is a more plastic trait. Initial egg mass is a proxy of maternal investment in each egg, which is a proximate determinant of the size of each hatchling (mass and SVL). This explains why most (>85%) of the variation in hatchling size can be explained by egg mass (Lu et al. 2014). Here, clutch origin was a significant source of variation in hatchling mass but explained a small proportion of variation in hatchling SVL and mass. It seems likely that this portion of variation also stems from among-clutch difference in egg size, as revealed by the fact that the clutch effect on hatchling SVL and mass disappeared after accounting for egg mass.

Head length at hatching was significantly affected by source population, but marginally by incubation temperature, despite the fact that the 2 factors explained a similar proportion of variation in the trait. In contrast, head width at hatching seemed to be more sensitive to incubation temperature, although it also differed significantly among populations. Overall, head sizes were comparatively larger in *P. chinensis* hatchlings from colder localities and low incubation temperatures. The increased head size and thus the enhanced ability to use large food items might be adaptive for hatchlings in colder localities with relatively low food diversity and/or availability.

Hindlimb length and tail length were affected by both source population and incubation temperature, whereas forelimb length was not. Hindlimb length has functional links with sprint speed in lizards, with greater speeds generally recorded in individuals having longer hindlimbs (Mayr 1956; Vanhooydonck and Van Damme 2001). Short forelimbs and long tails are considered to be beneficial during terrestrial locomotion (Farley and Ko 1997). The finding that hatchlings from a moderate incubation temperature (28 °C) had

relatively longer hindlimbs and tails and ran faster than those from low or high temperatures adds further evidence for the positive correlation between sprint speed and hindlimb, or tail length. That high incubation temperatures produce slower hatchlings with more residual yolk has been documented in many lizard species (Du and Ji 2006; Dayananda et al. 2017). However, slower hatchlings are less commonly produced at low incubation temperatures. Compared with hatchlings from the 28°C treatment, those from the 24°C treatment had similar body sizes, but relatively shorter hindlimbs and tails. That stout bodied hatchlings are produced at colder temperatures has been reported for several species of lizards (Sinervo and Losos 1991; Qualls and Shine 1998; Buckley et al. 2007, 2010). Stout body shape might be adaptive in cold climates because of the increased thermal inertia, but potentially weaken functional performances such as locomotor speed (Qualls and Andrews 1999; Buckley et al. 2010). The relationship between hindlimb or tail length and sprint speed was not evident when compared across populations. For example, SG hatchlings had relatively longer hindlimbs and tails, but run slower than those from other populations. This is inconsistent with the findings of most interspecific studies (Sinervo and Losos 1991; Bauwens et al. 1995; Bonine and Garland 1999; Melville and Swain 2000; Gifford et al. 2008). In lizards, locomotor performance is influenced by the complex interactions among morphological, ecological, and physiological factors (Melville and Swain 2000; Vanhooydonck et al. 2002; Gifford et al. 2008). Here, we found that hindlimb length, tail length, and sprint speed were more strongly associated with incubation temperature than with source population. Interestingly, the proportion of variation explained by incubation temperature was similar between these 3 traits, possibly reflecting a potential link between morphological features and locomotor speed.

Taken together, most hatchling traits examined in this study were affected by incubation temperature and source population. The relative importance of these 2 factors varied between hatchling traits. Consistent with our prediction, most variation in highly plastic hatchling phenotypes (including SVL, tail length, limb length, and locomotor speed) came from incubation temperature rather than source population or clutch origin, indicating a greater relative contribution of developmental thermal conditions on variation in these traits. The pattern of variation in phenotypic traits seems to be somewhat inconsistent across studies on different lizard species, probably reflecting differential adaptive strategies to local environments (Buckley et al. 2010; Verdú-Ricoy et al. 2014). Plasticity in morphological and locomotor traits in response to varying thermal conditions may help ectotherms including lizards cope with future climate change (Ma et al. 2018b). Given that incubation-induced variation in phenotypic traits does not always persist for an animal's lifetime and can be swamped by the environment in which juvenile lizards live or by genetic effects (Qualls and Shine 2000; Buckley et al. 2007), future work could usefully perform a long-term follow-up measurement for the survival and growth of hatchlings from different thermal conditions. Body mass was more likely to be influenced by source population than by incubation temperature, probably reflecting greater genetic effects on the trait. The extent of temperature-induced plasticity in hatchling phenotypes is less likely to differ among populations, as revealed by the fact that the population  $\times$  temperature interaction was not a significant source of variation in nearly all examined traits. Summarily, geographic variation in hatchling traits within *P. chinensis* likely reflects the difference in natural incubation conditions and genetic divergence. The relative

importance of source population and incubation temperature varies between hatchling traits.

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## Author's contributions

H.L.L. and X.J. conceived and designed the experiments. X.J. supervised the study. H.L.L., Y.F.Q., and H.L. collected and analyzed the data. H.L.L. and X.J. wrote the article. All authors reviewed and contributed to the editing of the manuscript and approved its final publication.

## Conflict of interest

The authors declared that they had no conflicts of interest to this work.

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