**Phenotypic responses to incubation conditions in ecologically distinct
populations of the lizard *Psammodromus algirus*:
a tale of two phylogeographic lineages**

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Short running title: Phenotypic responses to incubation conditions in a lizard

ABSTRACT.- We experimentally studied the effects of genetic legacy (eastern vs. western phylogeographic lineage) and population of origin (lowland vs. highland) on the sensitivity of lizard embryos and juveniles to incubation temperature and moisture among four populations of the lacertid *Psammodromus algirus*. Incubation time was longer at lower temperature, increased slightly at higher moisture, and was shorter for highland than for lowland females. Eggs incubated at 24 °C produced larger, heavier, and shorter-tailed hatchlings than those incubated at 32 °C. Western juveniles survived better during their first month of life than eastern ones, and juveniles incubated at 32 °C survived better than those incubated at 24 °C; survivorship was lowest for 24 °C hatchlings from the eastern, lowland population. Because juveniles incubated at 32 °C grew more rapidly, after one month they had compensated their initial size disadvantage. Juveniles incubated at 80% moisture were larger and/or heavier than those incubated at 10% moisture both at hatching and after one month. Our results show that although incubation temperature was the main source of phenotypic variation, not all its effects were evident at hatching. Because western juveniles were more tolerant to incubation at low temperature than eastern ones, we suggest that such differences may have limited the westward expansion of the eastern lineage.

KEY WORDS: body size, growth rate, incubation time, mtDNA lineage, phylogeography, tail length, hatchling survivorship.

1 INTRODUCTION

2
3 Reptiles are excellent models for studying the effects of temperature on phenotypic
4 variation due to the evolutionary effects of low energy requirements and behavioural
5 thermoregulation (Shine, 2005). Females can manipulate incubation conditions either by
6 selecting appropriate nest sites (Shine & Harlow, 1996; Iraeta, Díaz & Bauwens, 2007)
7 or by retaining developing eggs (Rodríguez-Díaz & Braña, 2012). Freedom from heat-
8 conserving constraints on body size allows the production of small propagules in large
9 numbers, promoting a dynamic matching of life-histories to local environments
10 (Sinervo & Adolph, 1989; Iraeta *et al.*, 2006) via local adaptation or phenotypic
11 plasticity. This flexibility has fostered intraspecific variation, leading to a large number
12 of studies focusing on the population level (e.g. Niewiarowski & Angilletta, 2008; Díaz
13 *et al.*, 2012).

14 Because early ontogenetic events can have more impact on the adult phenotype
15 than later ones (Qualls & Shine, 1998), environmentally-induced effects on embryonic
16 development are expected to be especially important. The role of factors such as
17 temperature and moisture (Ji & Braña, 1999; Angilletta, Winters & Dunham, 2000) in
18 shaping phenotypic plasticity seems most influential in oviparous species (Elphick &
19 Shine, 1998), where embryonic development occurs mainly outside the mother's body.
20 Temperature controls the velocity of metabolic reactions, thus determining incubation
21 times (Shine & Harlow, 1996; Parker & Andrews, 2007; Monasterio *et al.*, 2011). Wet
22 substrates allow soft-shelled reptilian eggs to increase their mass by water absorption
23 (Packard, 1991). Thus, thermal and moisture effects have been widely studied in
24 squamates, in which developmental rate, hatching success, and hatchling size, shape,
25 performance, behaviour, and sex can be modified by incubation conditions (Van

Damme *et al.*, 1992; Elphick & Shine, 1998; Qualls & Shine, 1998; Warner & Shine, 2008).

Although high temperatures generally decrease incubation times, their impact on hatching success is less predictable. Depending on the species, hot incubation environments may raise (Telemeco *et al.*, 2010), have no effect on (Parker & Andrews, 2007), or –perhaps more commonly– hamper hatching success (Ji & Braña, 1999; Angilletta *et al.*, 2000). Hatchling size may also exhibit species-specific responses to temperature (Shine & Harlow, 1996; Telemeco *et al.*, 2010). Variable responses to incubation conditions have also been reported at the intraspecific level for hatching success (Niewiarowski & Angilletta, 2008), incubation period (Qualls & Shine, 1998; Du *et al.*, 2010), hatchling size (Sears & Angilletta, 2003) or growth rate (Niewiarowski & Angilletta, 2008). Thus, intraspecific studies provide excellent opportunities to evaluate phenotypic and environmental covariation, particularly when populations of a single species occur along environmental gradients and exhibit some degree of genetic divergence as a result of past differentiation events (Hewitt, 2000).

Within this framework, we designed an experiment with a widespread Iberian lacertid, the Large Psammmodromus *Psammmodromus algirus*, to address the effects of incubation temperature and moisture, phylogeographic lineage (eastern vs. western, see below), and population of origin (with two replicates per lineage) on several phenotypic traits of eggs and hatchlings that may be related to individual fitness (incubation time, hatching success, hatchling and juvenile morphology, growth rates, and survivorship). To set our experiment in its appropriate phylogeographic context, it should be noted that the Iberian Peninsula shows wide climatic variation, with more humid and temperate areas in the northwest and warmer and drier regions in the southeast. Previous research on this species has revealed an ancient phylogeographic history involving two distinct

mitochondrial DNA (mtDNA) lineages in western and eastern areas of the Iberian Peninsula (Carranza *et al.*, 2006; Verdú-Ricoy *et al.*, 2010). Our specific questions were: 1) Do temperature and moisture levels during incubation affect the hatching success, size or condition of hatchling *P. algirus*, and how long do these effects last? 2) Do clutches from different lineages or populations differ in their responses to incubation conditions? 3) To what extent might these responses explain the current distribution of phylogeographic lineages?

MATERIALS AND METHODS

Study species

Psammodromus algirus (Linnaeus, 1758) is a medium sized (adult snout-vent length [SVL] 60-90 mm, mass 6-16 g) lacertid that inhabits shrub and woodland habitats of the western Mediterranean. Courtship and egg-laying occur between April and June and hatchlings are born in August-September. Phylogeographic structure is evident for this species (Carranza *et al.*, 2006), which has eastern and western Iberian lineages separated by a relatively narrow contact zone (Verdú-Ricoy *et al.*, 2010).

Husbandry of adults

Adult females were captured in May 2011 at four different locations (Table 1): El Pardo (lowland, western lineage), Navacerrada (highland, western lineage), Aranjuez (lowland, eastern lineage), and Torrejoncillo (highland, eastern lineage). Our four populations encompass nearly all of the altitudinal range of this species in central Spain, which is approximately 450-1,400 m above sea level.

We transported all lizards on the same day of capture to the Universidad

Complutense de Madrid and we placed them in white opaque terraria (40×60×30 cm; two females from the same population per cage) filled with moistened earth covered by leaf litter. A 60 W lamp suspended over one end of each terrarium created a photothermal gradient for thermoregulation within the preferred temperature range. UV light was also provided. We fed lizards with house crickets dusted with a commercial vitamin and calcium supplement. All cages were fed and watered *ad libitum*. Upon arrival at the lab, we measured (SVL), weighed and toe-clipped all lizards.

Husbandry of eggs and juveniles

Gravid females were daily monitored to detect egg-laying. Correct identification of clutches was achieved by checking females daily to identify the laying female as the one showing evident signs of mass loss and, in the only occasion in which both females laid on the same day, by weighing both clutches and determining mass loss for each female. Oviposition occurred between 19 May and 1 July (one clutch per female, 3-9 eggs per clutch). Overall, 36 females laid clutches, of which three (all from Navacerrada) were entirely composed of eggs that failed to hatch. Once detected, eggs were removed, counted, and weighed to the nearest mg. We placed each egg (n = 198) in a 150-ml closed plastic cup filled with ca. 35 g of moistened vermiculite. We assigned each egg to one of four possible treatments in a 2x2 factorial design with temperature and moisture as factors. We set the two moisture treatments by adding either 1 g or 8 g of water per 10 g vermiculite (hereafter 10% and 80% moisture). To set the temperature treatments, we distributed eggs over two incubators (Friocell, Königswinter, Germany) set at constant temperatures of either 24 or 32 °C. We tried to achieve a balanced split-clutch design by distributing eggs from the same clutch as equally as possible among treatments. We selected experimental temperatures of 24 and 32 °C assuming that these

values represent opposite ends of the thermal range below and above the optimal incubation temperature described for *P. algirus*, with 24 °C acting as a threshold under which reproduction is constrained due to overextended incubation (Iraeta *et al.*, 2007; Monasterio *et al.*, 2011).

When incubation was about to end, we searched daily for newborns. Hatchlings were weighed to the nearest mg, measured (SVL and tail length, to the nearest mm) and individually placed in plastic boxes (225 mm length × 140 mm width × 150 mm height) with 60 W and UV lamps and a tile providing refuge. We gave all hatchlings equal feeding opportunities (3 small crickets per day and water *ad libitum*) during their first month of life. To estimate post-natal growth rates, we measured all juveniles again at an age of 30 days. We estimated food intake by counting the number of crickets consumed each day and calculating a 30-days average. After the 30-days period of controlled food supply, juveniles were transported to another room where they could feed *ad libitum* under natural conditions of illumination and temperature but with no additional sources of light or heat. All juveniles were measured again before being released at an age of 37-47 days (mean ± SE = 40.4 ± 0.1). At the end of the experiment, all lizards were released at their (or their mother's) site of capture.

Statistical analyses

Data are given as mean ± 1 SE. Growth rates, estimated both for SVL and weight, were calculated using the expression $\ln(\text{measure at 30 days} / \text{measure at hatching}) / 30$, which reflects the proportionate increase in size or mass on a per-day basis (Sinervo & Adolph, 1989). Statistical analyses were performed after log-transforming, when necessary, the corresponding variables. Because clutch (nested within population and lineage) and population (nested within lineage) must be regarded as random factors, we

employed the mixed-model approach, that requires finding the linear combinations of sources of random variation that serve as error terms for each effect. As a consequence, degrees of freedom can be fractional.

For the analyses of dam traits (SVL, clutch size, clutch mass, and mean egg mass), we used mtDNA lineage as a fixed factor and population (nested within lineage) as a random factor. For the analyses of egg and juvenile traits, main effects were clutch (nested within population and lineage) and population (nested within lineage) as random factors, and mtDNA lineage, temperature and moisture as fixed factors. When appropriate, we also included continuous predictors as covariates. We did not estimate two-factor interactions involving random effects or three-way interactions to avoid overparameterization of the models. The use of population as a random factor left us few degrees of freedom to test for the effects of lineage, thus making our analyses conservative. However, mixed-models allowed us to generalize our conclusions concerning incubation effects and differences between populations. Because in most cases these latter differences were consistent for both lineages, we assume they are associated with a gradient opposing lower-altitude and more xeric conditions, to higher-altitude and more productive ones.

RESULTS

Reproductive traits of females

In our sample, females from the western lineage were larger than those from the eastern lineage, with no significant differences between populations from the same lineage (Table 1). Clutch size was slightly higher for western than for eastern females, a difference that was mainly attributable to their larger size. There were no differences

1 between lineages in mean egg mass. However, for a given SVL and clutch size, western
2 females laid heavier eggs than eastern ones, so that total clutch mass was higher for
3 females of the western lineage, even after controlling for their larger size (Table 1).

4 5 *Incubation time and hatching success*

6 Data and tests for this and the following sections are provided in Tables 2 and 3.

7 Incubation time was longer in the low temperature treatment, and it increased slightly at
8 higher moisture. Lineages did not differ in their average incubation times, but in both
9 lineages eggs from highland populations hatched sooner than those from lowland sites.

10 Overall, hatching success was high (91.4%), with no significant differences between
11 lineages, populations, or moisture or temperature treatments.

12 13 *Morphological traits of hatchlings*

14 Hatchling size (SVL) was strongly dependent on incubation temperature, with eggs
15 incubated at 24 °C producing larger hatchlings. Tail length showed the opposite pattern:
16 lizards incubated at 32 °C hatched with longer tails than those incubated at 24 °C.

17 Within both lineages, lizards from lowland populations had longer tails, and tended to
18 be larger, than those from highland populations. All other effects and interactions were
19 not significant.

20 Hatchlings incubated at 80% moisture were heavier than those incubated at 10%
21 moisture, and hatchlings incubated at 24 °C were heavier than those incubated at 32 °C.

22 After controlling for the effects of temperature, moisture, and egg mass (as an index of
23 maternal investment), hatchlings from lowland clutches were heavier than those from
24 highland ones (ANCOVA with egg mass as the covariate; population nested within
25 lineage: $P = 0.0009$; egg mass: $P = 0.0015$).

Survivorship and growth

A log-linear analysis on the data set of hatchlings classified according to lineage, temperature, moisture, and survivorship to 30 days (Maximum likelihood $\chi^2 = 4.53$, $df = 10$, $P = 0.921$) showed that juveniles from the western lineage had a higher survival rate than those from the eastern lineage (Figure 1: 96.0% of western juveniles, vs. 79.7% of eastern ones, survived; partial association $\chi^2 = 11.32$, $df = 1$, $P = 0.0008$) and that survivorship was higher at 32 than at 24 °C (95.5% of juveniles incubated at 32 °C survived, vs. 82.6% of those incubated at 24 °C; partial association $\chi^2 = 7.14$, $df = 1$, $P = 0.0075$). Although survivorship at 24 °C was lower at Torrejoncillo (76.5%) and especially Aranjuez (57.9%) than at Navacerrada (90.1%) or El Pardo (96.4%), no triple interaction was significant (all P 's > 0.35), probably due to the overall low mortality rate in the rearing experiment (only 19 of 174 hatchlings, or 10.9%, died during their first month of life).

Hatchlings incubated at 32 °C grew on average 50% more rapidly during their first month of life than those incubated at 24 °C. These differences remained highly significant after controlling for the effects of the average number of crickets eaten per day (ANCOVA with food intake as the covariate; temperature: $P < 0.001$; food intake: $P < 0.001$). Western lizards had marginally higher food intake rates than eastern ones, especially when incubated at low temperature. Rates of body mass gain were higher for western than for eastern lizards, and hatchlings incubated at 32 °C gained mass marginally faster than those incubated at 24 °C. Moisture treatment had no significant effects on growth rates.

Because juveniles incubated at 32 °C grew more rapidly than those incubated at 24 °C (both in SVL and body mass), after one month they had compensated their initial

size disadvantage, and temperature treatment had no significant effects on SVL or body mass. However, some of the other effects that were present at hatching were still evident. Thus, one-month juveniles from lowland populations were larger than those from highland populations, and juveniles incubated at 80% moisture were larger than those incubated at 10% moisture. Tail length was the only variable for which temperature effects present at hatching were still apparent after one month: juveniles incubated at 32 °C had longer tails than those incubated at 24 °C. Again, juveniles from lowland populations had longer tails than those from highland populations. Interestingly, after the short period of growth under natural conditions of illumination and temperature, the larger growth rates of juveniles incubated at 32 °C allowed them to overcompensate their smaller hatchling size and to reach larger SVL and body mass ($P < 0.002$ in both cases, results not shown) than those incubated at 24 °C.

DISCUSSION

Our data bear some relevance for the discussion of the so-called temperature-size rule (Atkinson, 1994), which proposes that ectotherms that develop at higher temperatures will be relatively small as adults, or in other words that the thermal reaction norm for final size has a negative slope. Although the temperature-size rule seems to apply to a wide variety of animal taxa, including fish, amphibians, aschelminthes, molluscs, and especially arthropods (Atkinson, 1994), our results support the opposite view, namely that growth efficiency increases with increasing temperature (Angilletta & Dunham, 2003), which is the expected result if temperature has a stronger effect on growth rate than on development rate (Van der Have & de Jong, 1996).

According to our results, females from more mesic and productive environments attain larger size and, as a consequence, lay more eggs than their conspecifics from

1 more xeric and less productive locations (Iraeta *et al.*, 2006; Díaz *et al.*, 2012; Iraeta,
2 Salvador & Díaz, 2013). However, in our study this effect was evident when comparing
3 phylogeographic lineages rather than populations of the same lineage living at different
4 elevations, which is in contrast with previous data (Iraeta *et al.*, 2013). We conclude that
5 clutch size is influenced, either directly or through differences in female body size, by
6 environmental differences in productivity that may operate at different spatial scales
7 (e.g. between populations or between lineages). Since western females laid larger eggs
8 (for a given value of female and clutch size) than eastern ones, reproductive investment
9 (clutch mass) was higher for western females, suggesting poorer environmental
10 opportunities for the eastern lineage. Adverse environmental conditions in fragmented
11 habitat have also been shown to have negative impacts on reproductive output in this
12 species (Díaz *et al.*, 2005).

13 As expected, incubation times decreased at higher temperatures. Moisture had a
14 smaller but still significant effect on incubation time; high moisture delayed hatching
15 approximately one day, especially at low temperatures. This implies that the shorter
16 incubation times of eggs from highland sites, where temperature is lower and soil
17 humidity is higher, could be due to faster developmental rates (Storm & Angilletta,
18 2007). Such a scenario would suggest that countergradient variation related to both
19 temperature and moisture has evolved independently in the two lineages (Díaz *et al.*,
20 2012). Alternatively, there could be differences in embryo stage at oviposition, with
21 high elevation females laying more developed embryos (Telemeco *et al.*, 2010;
22 Rodríguez-Díaz & Braña, 2012).

23 The lack of significant difference between lineages in incubation times, which is
24 in contrast with previous analyses based on a larger sample of populations (Díaz *et al.*,
25 2012), is probably related to low statistical power. It is also noteworthy that our data

1 failed to confirm that western eggs were worse adapted than eastern ones to drier
2 incubation conditions, perhaps because 10% water is still wetter than the threshold
3 value below which hatching is physiologically hampered. This is supported by the fact
4 that overall hatching success was high, with no differences between lineages,
5 populations, or treatments.

6 Incubation at 24 °C produced larger hatchlings than those incubated at 32 °C. A
7 negative correlation between incubation temperature and body size has been
8 documented for this and other species (Shine & Harlow, 1996; Elphick & Shine, 1998;
9 Monasterio *et al.*, 2011), which could be due to longer incubation times at lower
10 temperatures that would allow newborns to attain larger sizes. It is well known that
11 higher temperatures shorten incubation and fasten embryonic development, but the
12 balance between these two processes and their combined effect on hatchling size are
13 less clearly documented; newborns incubated at lower temperature may be smaller than
14 (Telemeco *et al.*, 2010), similar to (Qualls & Shine, 1998; Parker & Andrews, 2007), or
15 larger than (Monasterio *et al.*, 2011) those incubated at higher temperature. In our case,
16 the shortening of incubation seemed to override the effects of increased metabolism,
17 because size decreased at higher temperature.

18 However, this size difference disappeared after 30 days of post-hatching growth
19 under common garden conditions, because juveniles incubated at higher temperature
20 grew faster than those incubated at lower temperature (see Qualls & Andrews, 1999 and
21 Hare *et al.*, 2004, for similar results). Such result could not be explained by different
22 rates of food intake, because the number of crickets eaten did not differ between
23 treatments. Therefore, we must invoke physiological differences that would enable
24 juveniles to increase digestive efficiency or decrease metabolic expenditure (Sears,
25 2005). In fact, incubation temperature may affect the energy conversion coefficient

1 during embryonic development (Angilletta *et al.*, 2000), with longer incubation periods
2 resulting in higher metabolic expenditure due to greater maintenance costs (Booth,
3 Thompson & Herring, 2000). The advantage of juveniles incubated at 32 °C seemed to
4 be long-lasting, as shown by the fact that they managed to overpass their cooler-
5 incubated siblings shortly after the end of the growing experiment. This reinforces the
6 view that the large hatching size of cold-incubated lizards was more a by-product of
7 delayed hatching than a truly advantageous trait (see Elphick & Shine, 1998, and Qualls
8 & Andrews, 1999, for similar results). As a corollary, our data emphasize the need of
9 monitoring the fitness consequences of incubation treatments on post-natal phenotypes
10 for a sufficiently long period, because phenotypes at hatching may be delusive (Radder,
11 Warner & Shine, 2007). It should also be noted that hatchlings were heavier when
12 incubated at higher moisture, and that growth rates did not differ significantly between
13 moisture treatments. As a consequence, moisture had more lasting phenotypic effects on
14 body size than did temperature, and should not be discarded as unimportant.

15 Interestingly, in both lineages lizards incubated at higher temperature, and those
16 from lowland populations, hatched with longer tails than their conspecifics incubated at
17 lower temperature or from highland populations. Caudal autotomy, or the ability to shed
18 the tail in response to predation attack, is probably the most widespread function of
19 lizard tails, representing a major predator escape tactic (Bateman & Fleming, 2009).
20 Moreover, tail autotomy is especially important as an antipredatory mechanism for
21 newborns because their locomotor capacities are not fully developed. As a consequence,
22 having longer tails may help to increase survival. Our data are consistent with this
23 scenario, because hatchlings were longer-tailed in lowland Mediterranean habitats,
24 where the frequency of natural autotomy is higher (Iraeta, Salvador & Díaz, 2012). On
25 the other hand, hatchlings were longer-tailed when incubated at higher temperature, a

1 reliable environmental cue for the fact that activity after hatching and associated
2 predation risk are likely to be high.

3 Differences between populations were still evident after one month of postnatal
4 growth: in both lineages, lowland juveniles were larger, heavier and longer tailed. Such
5 differences, presumably of an adaptive nature, are consistent with the fast growing rates,
6 under common garden conditions, of western juveniles coming from Mediterranean
7 xeric lowlands (Iraeta *et al.*, 2006; 2013). The adaptive scenario is supported by the fact
8 that this biogeographic pattern appears convergently in eastern and western lizards.

9 Overall, at the end of the growing period western juveniles were in better
10 condition than eastern ones: they survived better, ate more crickets (especially when
11 incubated at low temperature), and gained mass faster. Combined, all these lines of
12 evidence suggest that embryos and juveniles of this species, especially those from the
13 eastern lineage, face developmental difficulties when incubated at low temperature
14 (Monasterio *et al.*, 2011). This differential response to environmental conditions during
15 incubation may also contribute to create a ‘physiological border’ for the expansion of
16 eastern females, which might help to explain the apparent isolation between both
17 lineages.

18 In summary, our results show that incubation temperature was the main source
19 of phenotypic variation for hatchlings and juveniles of *P. algirus*. However, not all the
20 effects of incubation temperature were evident at hatching, because juveniles incubated
21 at higher temperature, although smaller than those incubated at lower temperature, were
22 able to survive better and grow faster during their first month of life. In addition, we
23 also detected differences between lineages in the thermal sensitivity of incubating
24 embryos, with the western group being more tolerant to lower incubation temperature.
25 Since the eastern lineage occupies warmer and drier areas and it is more severely

1 affected by incubation at low temperatures, we speculate that such differences may have
2 limited the westward expansion of the eastern lineage. Although we lack data on
3 temperatures in natural nests to support this idea, we have previously shown that
4 incubation temperatures can limit the (altitudinal) distribution of this species
5 (Monasterio *et al.*, 2011).

6
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1 Figure legends

2

3 **Figure 1.** Survivorship to an age of 30 days of juvenile *P. algirus* lizards from different
4 populations and phylogeographic lineages as a function of incubation temperature.

5

1 Table 1. Geographic and climatic data for the locations used in this study (latitude,
2 longitude, altitude, and precipitation [P] and mean temperature [T] between June and
3 September, i.e. during the incubation period of *P. algirus*), mtDNA lineage of each
4 population, and reproductive traits (mean $\pm$ SE) of the females that laid viable eggs.
5 Results of mixed-model ANOVAs and ANCOVAs for reproductive traits are also
6 shown.
7

Population	mtDNA lineage	Latitude (° N)	Longitude (° E)	Altitude (m)	P (mm)	T (°C)	SVL females (mm)	Clutch size	Egg mass (g)	Clutch mass (g)	N
El Pardo	Western	40.511	-3.755	658	23.7	20.7	80.11 $\pm$ 1.36	6.7 $\pm$ 0.3	0.44 $\pm$ 0.01	2.95 $\pm$ 0.17	9
Navacerrada	Western	40.726	-4.023	1230	52.9	13.9	79.71 $\pm$ 1.74	7.7 $\pm$ 0.5	0.41 $\pm$ 0.02	3.14 $\pm$ 0.18	7
Aranjuez	Eastern	40.016	-3.586	594	20.3	21.7	71.22 $\pm$ 1.09	4.9 $\pm$ 0.4	0.40 $\pm$ 0.02	1.92 $\pm$ 0.13	9
Torrejoncillo	Eastern	40.035	-2.599	943	30.5	20.9	73.00 $\pm$ 1.85	5.0 $\pm$ 0.4	0.40 $\pm$ 0.03	1.96 $\pm$ 0.14	8
ANOVA											
Lineage							$F_{1,2.01} = 70.78$ $P = 0.013$	$F_{1,2.00} = 28.03$ $P = 0.034$	$F_{1,2.01} = 3.87$ $P = 0.187$	$F_{1,2.02} = 174.65$ $P = 0.005$	
Population nested within lineage							$F_{2,29} = 0.38$ $P = 0.689$	$F_{2,29} = 1.08$ $P = 0.352$	$F_{2,29} = 0.50$ $P = 0.609$	$F_{2,29} = 0.27$ $P = 0.766$	
ANCOVA											
Lineage							---	$F_{1,5.24} = 5.25$ $P = 0.068$	$F_{1,21.97} = 5.28$ $P = 0.031$	$F_{1,6.95} = 10.40$ $P = 0.015$	
Population nested within lineage							---	$F_{2,28} = 1.40$ $P = 0.262$	$F_{2,27} = 0.33$ $P = 0.723$	$F_{2,28} = 0.98$ $P = 0.388$	
SVL (covariate)							---	$F_{1,28} = 7.14$ $P = 0.012$	$F_{1,2.7} = 21.69$ $P < 0.001$	$F_{1,28} = 33.42$ $P < 0.001$	
Clutch size (covariate)							---	---	$F_{1,2.7} = 26.57$ $P < 0.001$	---	

Table 2. Incubation time of eggs, and phenotypic data of juvenile *P. algirus* (body mass, SVL, and tail length, both at hatching [t = 0] and after one month of postnatal development [t = 30]), as a function of mtDNA lineage, population, and incubation (temperature and moisture) treatment.

Data are presented as mean $\pm$ se, with sample size between brackets.

		El Pardo (western lineage)		Navacerrada (western lineage)		Aranjuez (eastern lineage)		Torrejoncillo (eastern lineage)	
		658 m		1230 m		594 m asl		943 m	
		24 °C	32 °C	24 °C	32 °C	24 °C	32 °C	24 °C	32 °C
Incubation (days)	10%	88.5 $\pm$ 0.9 (13)	47.9 $\pm$ 0.6 (16)	84.4 $\pm$ 0.9 (13)	45.4 $\pm$ 0.5 (11)	81.8 $\pm$ 0.6 (11)	42.9 $\pm$ 0.4 (9)	79.4 $\pm$ 0.6 (10)	40.5 $\pm$ 0.6 (10)
	80%	88.9 $\pm$ 0.7 (15)	48.0 $\pm$ 0.3 (11)	85.1 $\pm$ 0.6 (13)	46.3 $\pm$ 0.7 (12)	83.2 $\pm$ 0.8 (9)	42.7 $\pm$ 0.3 (9)	79.3 $\pm$ 0.5 (9)	40.9 $\pm$ 0.2 (10)
Body mass₀ (g)	10%	0.54 $\pm$ 0.02 (13)	0.54 $\pm$ 0.01 (16)	0.48 $\pm$ 0.02 (13)	0.49 $\pm$ 0.01 (11)	0.49 $\pm$ 0.02 (11)	0.48 $\pm$ 0.03 (9)	0.47 $\pm$ 0.03 (10)	0.43 $\pm$ 0.03 (10)
	80%	0.57 $\pm$ 0.01 (15)	0.54 $\pm$ 0.02 (11)	0.50 $\pm$ 0.01 (13)	0.49 $\pm$ 0.02 (12)	0.51 $\pm$ 0.02 (9)	0.51 $\pm$ 0.02 (9)	0.48 $\pm$ 0.02 (9)	0.48 $\pm$ 0.02 (10)
SVL₀ (mm)	10%	29.6 $\pm$ 0.5 (13)	27.9 $\pm$ 0.3 (16)	28.7 $\pm$ 0.4 (13)	26.8 $\pm$ 0.4 (11)	28.4 $\pm$ 0.3 (11)	26.9 $\pm$ 0.5 (9)	28.5 $\pm$ 0.5 (10)	26.0 $\pm$ 0.8 (10)
	80%	30.2 $\pm$ 0.2 (15)	27.9 $\pm$ 0.3 (11)	28.9 $\pm$ 0.3 (13)	26.8 $\pm$ 0.3 (12)	29.1 $\pm$ 0.4 (9)	27.2 $\pm$ 0.5 (9)	28.7 $\pm$ 0.5 (9)	26.5 $\pm$ 0.4 (10)
Tail₀ (mm)	10%	50.5 $\pm$ 1.1 (13)	52.4 $\pm$ 1.4 (16)	49.4 $\pm$ 1.1 (13)	51.5 $\pm$ 0.6 (11)	50.2 $\pm$ 1.0 (11)	54.3 $\pm$ 1.5 (9)	46.1 $\pm$ 1.8 (10)	50.6 $\pm$ 2.2 (10)
	80%	53.2 $\pm$ 1.0 (15)	53.8 $\pm$ 1.2 (11)	49.1 $\pm$ 0.6 (13)	48.9 $\pm$ 1.5 (12)	52.2 $\pm$ 1.4 (9)	53.7 $\pm$ 1.1 (9)	48.9 $\pm$ 1.0 (9)	51.4 $\pm$ 0.9 (10)
Food intake (crickets/day)	10%	2.0 $\pm$ 0.1 (13)	2.0 $\pm$ 0.1 (16)	2.1 $\pm$ 0.1 (10)	1.7 $\pm$ 0.1 (11)	1.6 $\pm$ 0.2 (7)	2.0 $\pm$ 0.1 (9)	1.7 $\pm$ 0.1 (8)	1.6 $\pm$ 0.2 (9)
	80%	2.0 $\pm$ 0.1 (14)	2.0 $\pm$ 0.1 (11)	2.3 $\pm$ 0.1 (10)	1.8 $\pm$ 0.1 (11)	2.0 $\pm$ 0.1 (4)	2.0 $\pm$ 0.1 (8)	1.6 $\pm$ 0.1 (5)	1.7 $\pm$ 0.1 (9)
Growth rate SVL (days⁻¹ x 10³)	10%	4.5 $\pm$ 0.3 (13)	6.8 $\pm$ 0.5 (16)	4.1 $\pm$ 0.5 (10)	6.3 $\pm$ 0.4 (11)	3.2 $\pm$ 0.6 (7)	6.3 $\pm$ 0.4 (9)	2.7 $\pm$ 0.4 (8)	4.8 $\pm$ 0.7 (9)
	80%	4.8 $\pm$ 0.3 (14)	7.2 $\pm$ 0.4 (11)	5.3 $\pm$ 0.7 (10)	5.9 $\pm$ 0.5 (11)	3.6 $\pm$ 0.5 (4)	6.0 $\pm$ 0.4 (8)	2.9 $\pm$ 0.5 (5)	5.6 $\pm$ 0.7 (9)
Growth rate mass (days⁻¹ x 10³)	10%	14.3 $\pm$ 1.6 (13)	16.6 $\pm$ 1.4 (16)	17.1 $\pm$ 0.9 (10)	13.1 $\pm$ 1.6 (11)	2.3 $\pm$ 3.3 (7)	15.1 $\pm$ 1.6 (9)	4.5 $\pm$ 2.3 (8)	6.1 $\pm$ 2.7 (9)
	80%	14.7 $\pm$ 1.3 (14)	17.7 $\pm$ 1.6 (11)	17.5 $\pm$ 1.6 (10)	13.9 $\pm$ 1.8 (11)	9.4 $\pm$ 2.3 (4)	10.7 $\pm$ 2.2 (8)	3.1 $\pm$ 2.7 (5)	8.0 $\pm$ 2.3 (9)
Body mass₃₀ (g)	10%	0.83 $\pm$ 0.05 (13)	0.90 $\pm$ 0.04 (16)	0.77 $\pm$ 0.03 (10)	0.74 $\pm$ 0.04 (11)	0.55 $\pm$ 0.05 (7)	0.75 $\pm$ 0.05 (9)	0.56 $\pm$ 0.05 (8)	0.52 $\pm$ 0.05 (9)
	80%	0.89 $\pm$ 0.02 (14)	0.92 $\pm$ 0.03 (11)	0.84 $\pm$ 0.03 (10)	0.73 $\pm$ 0.02 (11)	0.67 $\pm$ 0.06 (4)	0.72 $\pm$ 0.05 (8)	0.56 $\pm$ 0.04 (5)	0.60 $\pm$ 0.03 (9)
SVL₃₀ (mm)	10%	33.9 $\pm$ 0.5 (13)	34.3 $\pm$ 0.7 (16)	32.1 $\pm$ 0.4 (10)	32.4 $\pm$ 0.6 (11)	31.1 $\pm$ 0.6 (7)	32.4 $\pm$ 0.6 (9)	31.1 $\pm$ 0.6 (8)	29.7 $\pm$ 0.9 (9)
	80%	34.9 $\pm$ 0.3 (14)	34.6 $\pm$ 0.3 (11)	33.7 $\pm$ 0.5 (10)	32.0 $\pm$ 0.5 (11)	32.0 $\pm$ 0.8 (4)	32.9 $\pm$ 0.4 (8)	31.8 $\pm$ 0.4 (5)	31.4 $\pm$ 0.6 (9)
Tail₃₀ (mm)	10%	63.8 $\pm$ 1.7 (13)	67.6 $\pm$ 1.8 (16)	60.4 $\pm$ 1.4 (10)	62.0 $\pm$ 1.3 (11)	59.9 $\pm$ 2.1 (7)	65.2 $\pm$ 2.9 (9)	54.6 $\pm$ 3.0 (8)	60.1 $\pm$ 2.9 (9)
	80%	65.6 $\pm$ 1.1 (14)	69.4 $\pm$ 1.6 (11)	62.9 $\pm$ 1.4 (10)	60.2 $\pm$ 2.5 (11)	61.8 $\pm$ 1.8 (4)	66.4 $\pm$ 1.8 (8)	58.8 $\pm$ 1.8 (5)	63.2 $\pm$ 2.0 (9)

Table 3. Results of mixed-model ANOVAS for the effects of female (nested within population and lineage), population (nested within lineage), lineage, and incubation treatments (temperature and moisture, see text for details) on the phenotypic traits of hatchlings and juveniles shown in Table 2. The effects of double interactions between fixed factors (lineage, temperature, and moisture) are also shown.

	Incubation time	Body mass ₀	SVL ₀	Tail ₀	Food intake	Growth rate SVL	Growth rate mass	Body mass ₃₀	SVL ₃₀	Tail ₃₀
Dam (population x lineage)	$F_{29, 143} = 3.91$ $P < 0.001$	$F_{29, 143} = 14.75$ $P < 0.001$	$F_{29, 143} = 6.31$ $P < 0.001$	$F_{29, 143} = 3.93$ $P < 0.001$	$F_{29, 117} = 1.87$ $P = 0.011$	$F_{29, 117} = 0.97$ $P = 0.520$	$F_{29, 117} = 1.83$ $P = 0.013$	$F_{29, 117} = 2.24$ $P = 0.001$	$F_{29, 117} = 2.33$ $P = 0.001$	$F_{29, 117} = 2.63$ $P < 0.001$
Population (lineage)	$F_{2, 29.16} = 15.95$ $P < 0.001$	$F_{2, 29.04} = 3.32$ $P = 0.050$	$F_{2, 29.1} = 3.23$ $P = 0.054$	$F_{2, 29.16} = 5.07$ $P = 0.013$	$F_{2, 33.68} = 0.49$ $P = 0.618$	$F_{2, 40.23} = 2.95$ $P = 0.064$	$F_{2, 34.82} = 1.17$ $P = 0.322$	$F_{2, 33.72} = 7.4$ $P = 0.002$	$F_{2, 33.53} = 7.92$ $P = 0.002$	$F_{2, 33} = 7.36$ $P = 0.002$
Lineage	$F_{1, 2} = 6.63$ $P = 0.124$	$F_{1, 1.99} = 1.03$ $P = 0.417$	$F_{1, 2} = 0.86$ $P = 0.451$	$F_{1, 2} = 0$ $P = 0.982$	$F_{1, 2.64} = 9.89$ $P = 0.061$	$F_{1, 2.19} = 4.51$ $P = 0.156$	$F_{1, 2.26} = 25.77$ $P = 0.028$	$F_{1, 2.03} = 6.56$ $P = 0.123$	$F_{1, 2.03} = 2.63$ $P = 0.244$	$F_{1, 2.03} = 0.21$ $P = 0.689$
Temperature	$F_{1, 143} = 20,437.89$ $P < 0.001$	$F_{1, 143} = 5.93$ $P = 0.016$	$F_{1, 143} = 168.78$ $P < 0.001$	$F_{1, 143} = 15.53$ $P < 0.001$	$F_{1, 117} = 0.2$ $P = 0.656$	$F_{1, 117} = 54.17$ $P < 0.001$	$F_{1, 117} = 3.77$ $P = 0.055$	$F_{1, 117} = 1.1$ $P = 0.296$	$F_{1, 117} = 1.05$ $P = 0.307$	$F_{1, 117} = 13.8$ $P < 0.001$
Moisture	$F_{1, 143} = 5.92$ $P = 0.016$	$F_{1, 143} = 12.31$ $P < 0.001$	$F_{1, 143} = 3.11$ $P = 0.080$	$F_{1, 143} = 1.48$ $P = 0.226$	$F_{1, 117} = 1.45$ $P = 0.23$	$F_{1, 117} = 2.05$ $P = 0.155$	$F_{1, 117} = 0.07$ $P = 0.788$	$F_{1, 117} = 2.43$ $P = 0.121$	$F_{1, 117} = 7.59$ $P = 0.007$	$F_{1, 117} = 2.88$ $P = 0.092$
Lineage x temperature	$F_{1, 143} = 0.08$ $P = 0.784$	$F_{1, 143} = 1.09$ $P = 0.299$	$F_{1, 143} = 0.07$ $P = 0.785$	$F_{1, 143} = 5$ $P = 0.027$	$F_{1, 117} = 4.42$ $P = 0.038$	$F_{1, 117} = 0.17$ $P = 0.685$	$F_{1, 117} = 5.46$ $P = 0.021$	$F_{1, 117} = 2.9$ $P = 0.091$	$F_{1, 117} = 0.08$ $P = 0.78$	$F_{1, 117} = 3.1$ $P = 0.081$
Lineage x moisture	$F_{1, 143} = 0.21$ $P = 0.650$	$F_{1, 143} = 0.02$ $P = 0.891$	$F_{1, 143} = 0.03$ $P = 0.872$	$F_{1, 143} = 0.02$ $P = 0.895$	$F_{1, 117} = 0.25$ $P = 0.617$	$F_{1, 117} = 0.12$ $P = 0.725$	$F_{1, 117} = 0$ $P = 0.987$	$F_{1, 117} = 0.04$ $P = 0.847$	$F_{1, 117} = 0.05$ $P = 0.821$	$F_{1, 117} = 0.17$ $P = 0.679$
Temperature x moisture	$F_{1, 143} = 0.01$ $P = 0.928$	$F_{1, 143} = 0.33$ $P = 0.567$	$F_{1, 143} = 0.01$ $P = 0.938$	$F_{1, 143} = 1.16$ $P = 0.283$	$F_{1, 117} = 0.45$ $P = 0.506$	$F_{1, 117} = 1.46$ $P = 0.229$	$F_{1, 117} = 0.51$ $P = 0.476$	$F_{1, 117} = 0.85$ $P = 0.358$	$F_{1, 117} = 0.77$ $P = 0.381$	$F_{1, 117} = 0.25$ $P = 0.615$

Figure 1.