# Title

# Abstract

# Keywords

# Introduction

Developmental plasticity - Incubation/nest temperature, known to change phenotypic traits in reptiles (Noble el al). Important for rising global temperatures.

Growth rate is an important life history trait, determines fitness and survival– how quickly to reach sexual maturity and how long you live for. What are the effects of incubation temperature on growth rate in trajectories? Bergmann’s Rule. Catch up growth? Do we know much?

Adaptive changes in growth can occur, but this depends on the underlying genetic variance of the phenotypic trait and how that varies with the environment/temperature. What are the theoretical expectations? How does temperature/environment change variance? Additive genetic variance?

– we don’t know much about the contributions of variance to growth rate trajectories. Is it genetic, maternal or plasticity?

Here we investigated how developmental temperatures affect contributions of variation in growth rate trajectories in an oviparous skink (*Lampropholis delicata*). Using a half-sib breeding design, we wanted to address the following questions 1) How does developmental temperature affect initial hatchling mass, growth rate, final size? 2) Does developmental temperature affect the relative contributions of variance of growth? 3) Is growth determined largely by genes, maternal effects or the environment? Do contributions change throughout the trajectory?

# Materials and Methods

## Lizard husbandry and egg collection

We established a breeding colony of adult *L. delicata* (nfemales = 100, nmales = 50) using wild individuals collected across five populations between 28 August and 8 September 2015, throughout the Sydney region. Depending on the number of natural deaths we had over the winter period, each year we further collected wild adults to supplement the breeding colony prior to the breeding season (nwild = 44).

We used a half-sib breeding design where up to three females were housed with a single male in an opaque plastic enclosure measuring 35cm 25cm 15cm (L W H). Enclosures were kept under UV lights (12L:12D) in a temperature control room set at 24ºC. Each enclosure was lined with newspaper and lizards were given access to a basking lamp, water bowl and tree bark as refuge. Lizards were fed three small crickets (*Acheta domestica*) dusted with calcium powder and multi-vitamin every two days. From October of each year, we placed an opaque plastic box (12 cm 17.5 cm 4.3 cm) containing moistened vermiculite in each enclosure for females to oviposit their eggs. From October to November, these ‘egg boxes’ were checked every day. Egg box checking increased to twice daily from December to February. We took tail tip tissue samples from adults that were from enclosures that produced eggs for DNA extraction (see below). All tissues were stored in 70% ethanol. Animal collection was approved by the New South Wales National Parks and Wildlife Service (SL101549) and procedures were approved by the Macquarie University Ethics committee (ARA 2015/015) and University of New South Wales Animal Care and Ethics committee (ACEC 15/51A).

## Incubation treatment

Eggs were collected over two years (2016 and 2017) and were typically laid between October –March. As soon as eggs were found, they were weighed using a digital scale to the nearest 0.01g (Ohaus Scout SKX123). We also measured egg length (distance between the furthest points along the longest axis of the egg) and egg width (distance between the widest points along the axis perpendicular to the longest axis of the egg) using digital callipers to the nearest 0.01mm. Following measurements, each egg was placed in a plastic cup (80ml) containing three grams of vermiculite and four grams of water. Each cup was then covered using cling wrap, secured by an elastic band. We assumed that eggs that were found together in the substrate box was from the same clutch. Each clutch was pseudo-randomly assigned to one of two incubation treatments. We used two incubators the precisely control the temperature of eggs (LabWit, ZXSD-R1090). The ‘hot’ incubation treatment was exposed to a mean temperature of 29ºC whereas the ‘cold’ incubation treatment was exposed to a mean temperature of 23ºC. Incubators fluctuated +/- 3ºC over 24 hours. These treatments represent the temperature extremes of natural nest sites of *L. delicata* (Cheetham et al., 2011). Egg cups were rotated within each incubator to case of uneven heat circulation, they were also checked daily for mould and emergant hatchlings. On average, the incubation period for the ‘hot’ treatment was 29.36 days (SD = 2.17, range = 15 - 49) days and 48.48 days (SD = 4.18, range = 25 - 56) for the ‘cold’ treatment.

## Quantifying growth rate

Newly emerged hatchlings were weighed to the nearest 0.01g and ventral photographs were taken for digital measurement (Nikon Coolpix A900). A small tail tip clipping (~2mm) was taken for genetic analyses (see below). Using ImageJ software, we measured snout-vent-length (SVL; from tip of snout to the beginning of the cloaca opening). We took monthly photographs of each hatchling for approximately six months following emergence after which we used a ruler to manually measure SVL. For the first initial nine months, hatchlings were housed individually in opaque plastic enclosures (32.3cm x 18.5cm x 6cm) lined with newspaper. Hatchlings had access to a tree bark refuge and a water bowl and were placed in temperature control room with same conditions as the adult colony. For logistical reasons, at approximately nine months, hatchlings were housed in groups of five in opaque bins with the same measurements as the adult enclosures. We pseudo-randomised individuals to each shared enclosure while maintaining similar number of individuals from each treatment. We were unable to balance sex across enclosures as hatchlings were still too young to determine their sex, however sex was later determined using SNP markers and accounted for in statistical analyses (see below). Growth measurements continued until we had approximately 16 measures per individual (mean, range.)

## Pedigree and genomic relatedness

Tissue samples from adults and hatchlings were used to determine parentage of offspring. DNA extraction and single nucleotide polymorphism (SNP) genotyping was performed by Diversity Arrays Technology, a commercial company which utilises a technique called DArTseq™. For more details on DNA extraction and SNP genotyping see ESM.

[For ESM: We submitted a total of 437 tissues samples, five samples experienced issues during extraction and sequencing and were therefore excluded from the final dataset (n = 432).

DNA was extracted from tissue samples using a Qiagen DNeasy Blood and Tissue Kits following the manufacturer’s instructions. Diversity Arrays Technology (DArT) combines next generation sequencing platforms and genome complexity reduction methods (Kilian et al., 2012) to select the most appropriate method for *L.delicata.*

[Insert section from DArT here on complexity reduction methods, DArT tested four methods in *Gambusia* so it’s unclear what method was used for us].

Sequences from all lanes were then processed using DArT specific pipelines. The main pipeline filters our ‘poor’ quality sequences [Details needed from DArT]. For our samples, this filtering process by DArT resulted in a total of 185,963 SNPs. ]

To create a pedigree among 132 putative parents (nfemales = 69, nmales = 63) and 266 offspring, a Hamming Distance Matrix was generated from our processed SNPs. Previous studies show that as few as 30 optimized SNPs are sufficient to differentiate among 100,000 individuals based on Hamming Distance values (Hu *et al.*, 2015) and this has method has been readily implemented in various evolutionary studies (Marsh et al., 2017; Vega‐Trejo et al., 2017) . We compared the Hamming Distance between an offspring and all putative parents from the same enclosure. The female and male with high genetic similarity (i.e. low distance) was assigned as dam and sire, respectively. In cases where offspring cannot be assigned with males in our colony, (noffspring = 110) were most likely sired by males from wild. We therefore assigned unique sire identities to these offspring (nwild father = 43). One individual was excluded from the dataset as we were unable to assign its parents. Despite our efforts in using a half sib breeding design, levels of multiple paternity were relatively low. 94% of females had offspring with a single male which means that most of the offspring are full sibs. In conjunction with relatively small clutch sizes (mean = 3.2, range = 1-9), it was not possible to create a useful pedigree for statistical analyses.

We therefore opted to use the SNP dataset to derive a genomic relatedness matrix of all the offspring instead. This required further processing of our SNPs using the R package ‘dartR’ (Gruber et al., 2018). Using the in-built functions of ‘dartR’, we filtered the loci based on various metrics in the following order: 1) read depth; call rate by loci and then by individual; reproducibility; monomorphic loci; minor allele frequencies; Hamming Distance among loci; Hardy Weinberg Equilibruim and linkage disequilibrium. For details on the filtering thresholds, see provided code. This clean-up process resulted in a dataset of 8438 loci with an average call rate of 98.5% (see ESM and provided code).

The genomic related matrix (GRM) describes the proportion of the genome that is identical by descent (VanRaden, 2008). We calculated a GRM for all hatchlings using the ‘snpready’ (Granato et al., 2018) following VanRaden, 2008:

where *Z* is the centered matrix of SNP genotypes of all individuals. This is calculated from a matrix of SNP genotypes coded as -1, 0, 1 for homozygote for the reference allele, heterozygote and homozygote for the SNP allele. *pi*is the frequency of the second locus at position locus *i.* The denominator scales the GRM matrix so that the values approximates to a relatedness matrix derived from a pedigree. The GRM was then inverted for modelling fitting (see ESM and provided code).

# Statistical Analyses

All analyses were performed using ‘R’ (Core Team, 2013). We checked the data for potential input errors using histograms, scatterplots and Cleveland plots.

We fitted quadratic Bayesian generalised mixed effects models in ‘MCMCglmm’ (Hadfield, 2010). First, to test whether developmental temperatures impacted growth trajectories of lizards, we fittedmass as the response and included an interaction between age and age^2 with incubation treatment as fixed effects. The timing of when a lizard hatches may influence its growth rate (ref). In order to account for this effect, we calculated ‘days since first emergence’, which represented the difference in days between the hatch date of the first lizard of the season and a given lizard’s hatch date. We included ‘days since first emergence’ and egg mass as covariates because the amount of resources invested in the egg by a mother can also influence offspring growth (Can cite our comparative analysis paper here and empirical papers). Mass was log-transformed. Age (days since hatch), days since first emergence and egg mass were all z-transformed. We included lizard ID and Dam ID as random intercepts and age as random quadratic slope terms. We removed non-significant interactions and refit the model with only just main effects.

Second, to test whether additive genetic variance of growth parameters change with incubation treatment, we ran separate models for each incubation treatment. We fitted a model with the same structure as one describe above, except that treatment is no longer included as a fixed effect.

For all models we used uninformative priors (see provided code). All models ran for 93,000 iterations with a burn in of 3000 and a thinning interval of 60. We checked for model convergence by inspecting trace plots and ensured samples were not strongly auto-correlated using the autocorr function from ‘coda’ (Plummer et al., 2006). We reported represent posterior means and 95% credible intervals.

# Results

# Discussion

# Conclusion

# Author contributions

# Data accessibility

Datasets and code used to generate results of this study is accessible via Open Science Framework (DOI: XXXXXXXXXXX)

# Acknowledgements