# 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 affects growth trajectories and the additive genetic variation of growth rate parameters using an oviparous skink (*Lampropholis delicata*). Using 8,433 SNP markers to derive a genomic relatedness matrix, 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, breeding design and egg collection

From 2015 – 2017, we established a breeding colony of adult *L. delicata* (nfemales = 144, nmales = 50) using wild individuals collected across five sites throughout the Sydney region between 28 August and 8 September 2015. 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 and they were given access to a heat lamp that elevated temperatures to between 28-32 ºC. Each enclosure was lined with newspaper and lizards had constant access to water and tree bark as refuge. Adult lizards were fed medium sized crickets *ad libitum* (*Acheta domestica*) dusted with calcium powder and multi-vitamin every two days. From the beginning of egg laying seasons (October of each year), we replaced newpaper lining with garden potting mix and 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. During this time, enclosures were sprayed gently with water every second day to maintain a relatively humid environment. From October to November, egg boxes were checked every day. 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 all 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 from 2016 -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 and secured by an elastic band. We assumed that eggs that were found together in the substrate box were from the same clutch as eggs from a single female are often stuck together. Each clutch was pseudo-randomly assigned to one of two incubation treatments. We used two incubators to 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. However, both 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 weekly to avoid uneven heat circulation within incubators. Incubators were also checked daily for dead eggs and emergent 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 a small tail tip clipping (~2mm) was taken for genetic analyses (see below). Ventral photographs were taken for digital measurement (Nikon Coolpix A900). For the first two months, photographs of hatchlings were taken approximately every 14 days. After which, hatchlings were photographed at approximately a 30-day interval. From approximately six months onwards, we manually measured hatchling SVL using a clear ruler. Growth measurements continued until we had approximately 16 measures per individual (mean = , range =). From the photographs, we extracted snout-vent-length (SVL; from tip of snout to the beginning of the cloaca opening) using ImageJ software (Rueden et al., 2017). 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 were fed the same number of crickets every second day and had constant access to a tree bark refuge and water. Hatchling enclosures were placed in a temperature control room under the same conditions as described above for 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 a 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).

## Pedigree and genomic relatedness

Tail 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 problems 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. ]

In order to estimate quantitative genetic parameters, we derived a genomic relatedness matrix (GRM) using our SNP dataset for 261 offspring growth data (132 putative parents; nfemales = 69, nmales = 63). While our half-sib breeding design allowed us to assign parentage to derive a pedigree, high levels of sperm storage and low levels of multiple paternity (94% of females had been sired by a single male) meant our pedigree had low resolution to effectively estimate additive genetic variation. Recent studies have shown that GRM derived from SNPs have low error rates (<0.3%) and are able to reconstruct pedigree relationships when at least 200 SNP loci are used (Bérénos et al., 2014; Huisman, 2017). Moreover, both relatedness and heritability values estimated from a GRM are strongly correlated to those inferred using a pedigree (Bérénos et al., 2014; Huisman, 2017).

Prior to deriving our GRM, we filtered our SNPs using the R package *dartR* (Gruber et al., 2018). Using the in-built functions of *dartR*, we filtered loci based on various metrics in the following order: 1) read depth (8 – 40); reproducibility (> 0.996); call rate by loci (> 0.97) and then by individual (> 0.80); monomorphic loci; minor allele frequencies (> 0.02); Hamming Distance among loci (> 0.25) and Hardy Weinberg Equilibrium. This clean-up process resulted in a dataset of 8,438 loci with an average call rate of 98.5% (see ESM and provided code).

Using these 8,438 loci we derived a GRM, which describes the proportion of the genome that is identical by descent (VanRaden, 2008). We calculated a GRM for all hatchlings using the *snpReady* R package (Granato et al., 2018) following methods described by 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