Estimation of sex ratio heritability in the common snapping turtle

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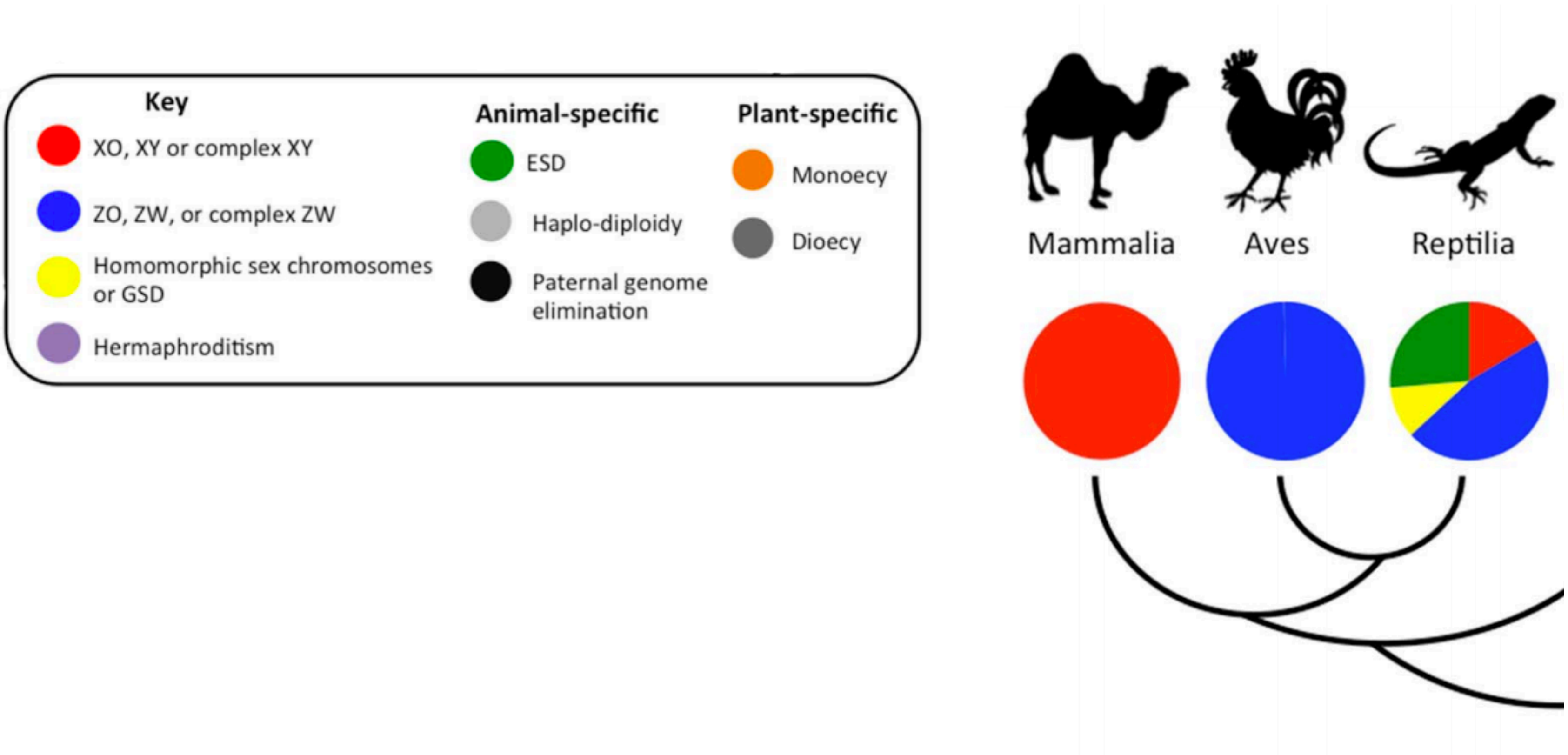
Master's Defense Seminar

November 26, 2019

Abbott Hall 101, 9 a.m.

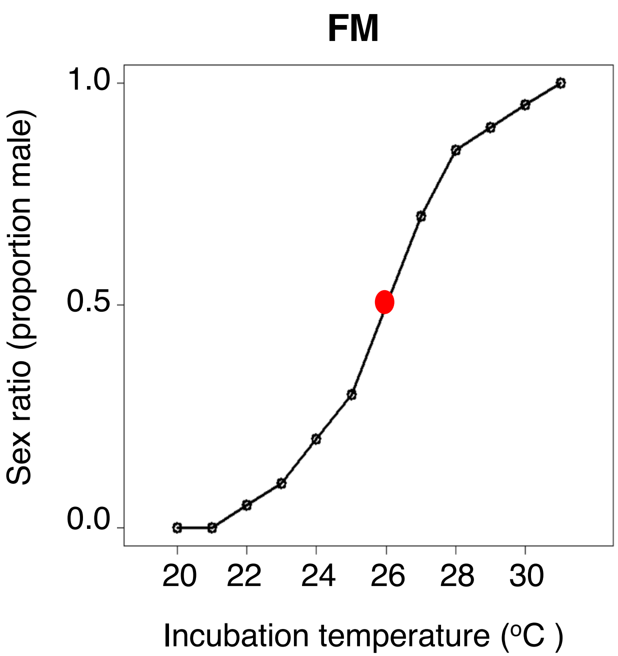
Sex determination

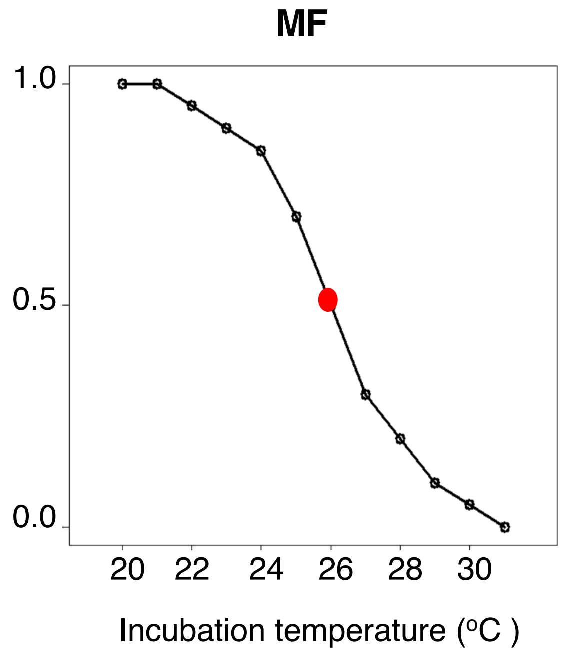
- Genotypic sex determination (GSD)
- Environmental sex determination (ESD)
  - Temperature-dependent sex determination (TSD)

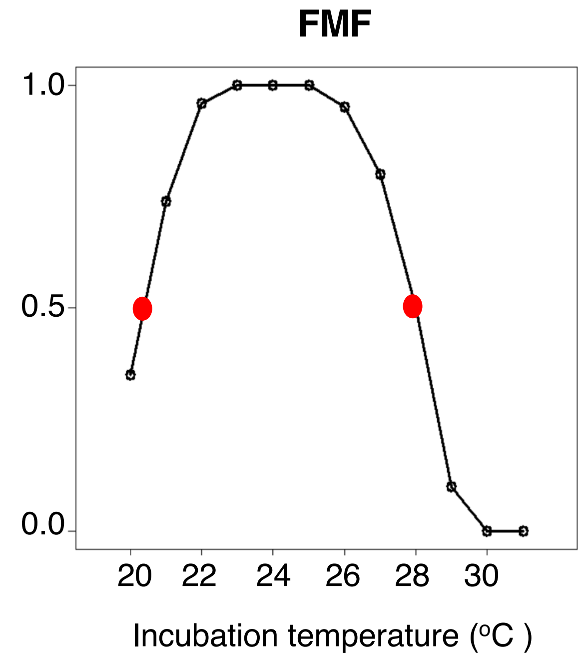


Bachtrog D., Mank J.E., Peichel C.L., Kirkpatrick M., Otto S.P. et al. (2014). Sex determination: why so many ways of doing it? *PLoS Biology* 12: e1001899.

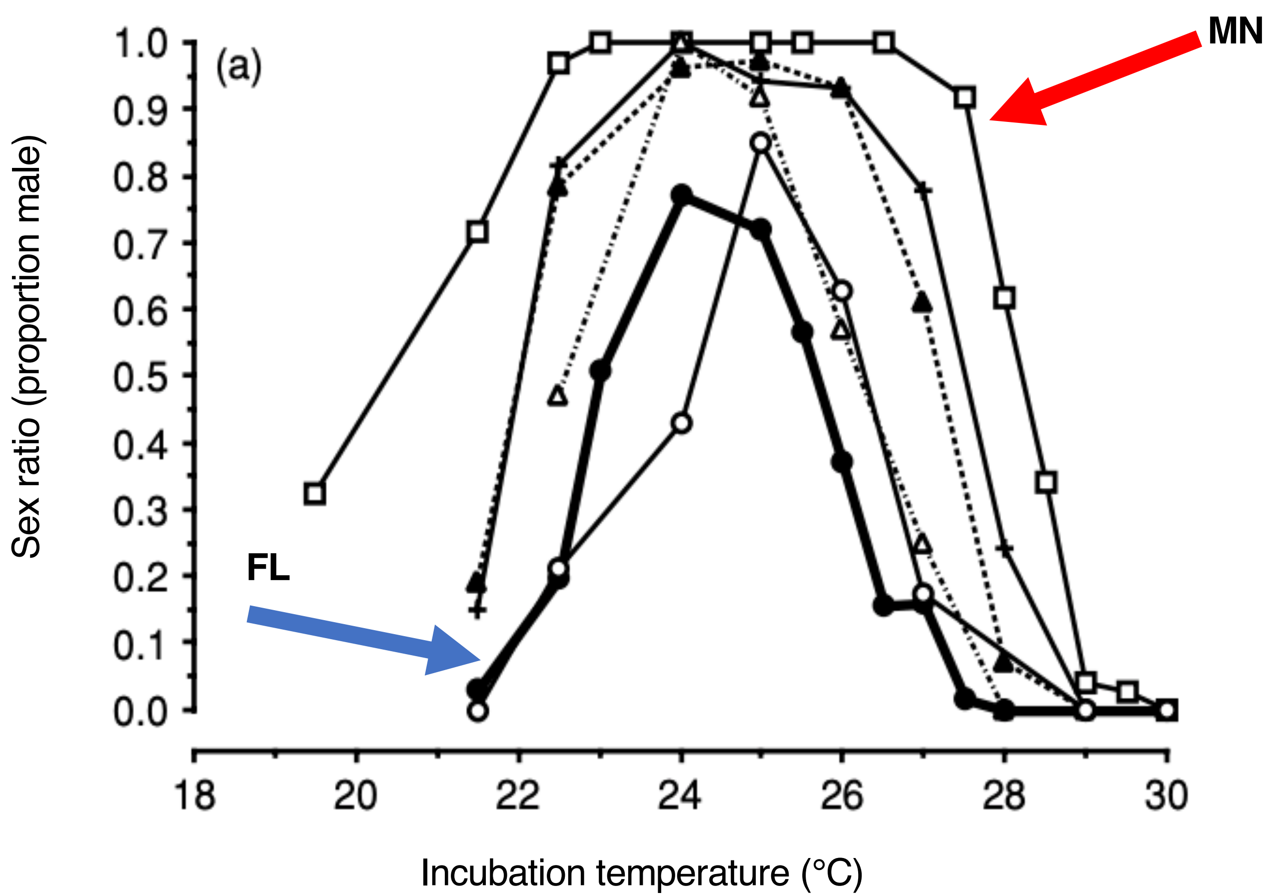
Variation in TSD among species





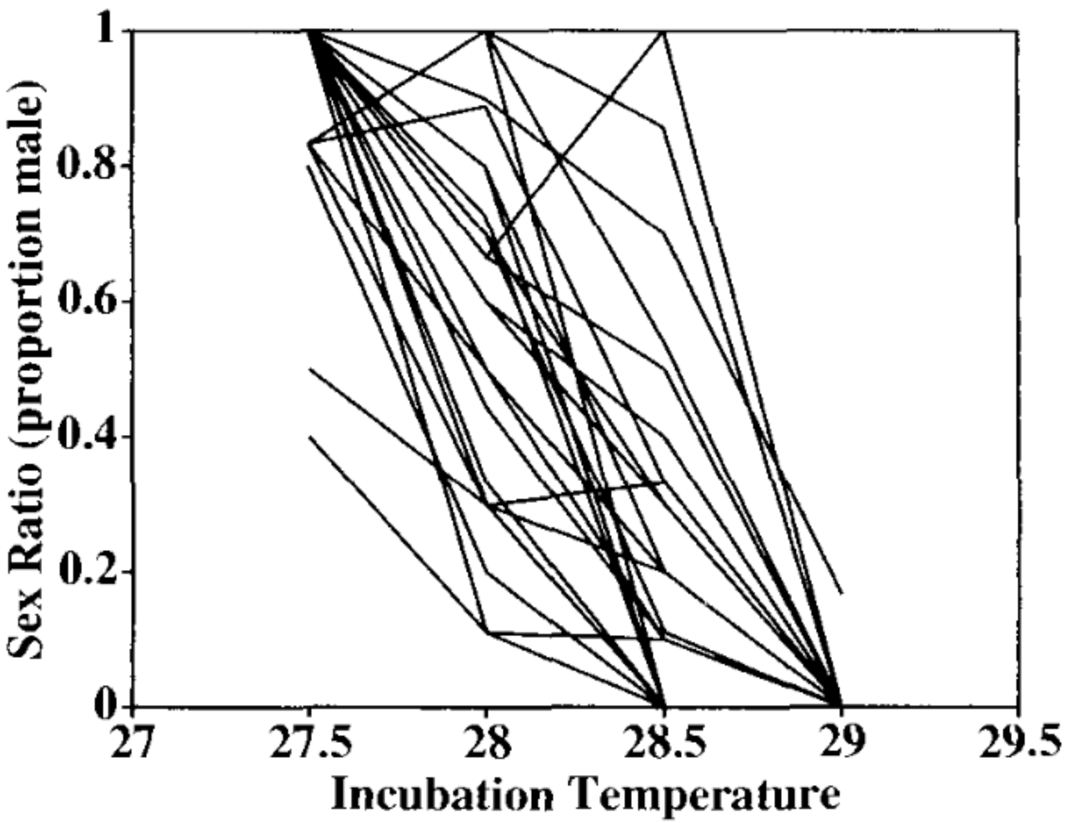


Variation in TSD among snapping turtle populations



Ewert, M.A., Lang, J.W. and Nelson, C.E. (2005). Geographic variation in the pattern of temperature-dependent sex determination in the American snapping turtle (*Chelydra serpentina*). *Journal of Zoology* 265: 81-95.

Variation in TSD within snapping turtle populations



Rhen, T. and Lang, J.W. (1998). Among-family variation for environmental sex determination in reptiles. *Evolution* 52: 1514-1520.

What causes TSD variation in the snapping turtle?

$$\underline{V_P = V_A + V_D + V_I} + \underline{V_M + V_E} + V_R$$

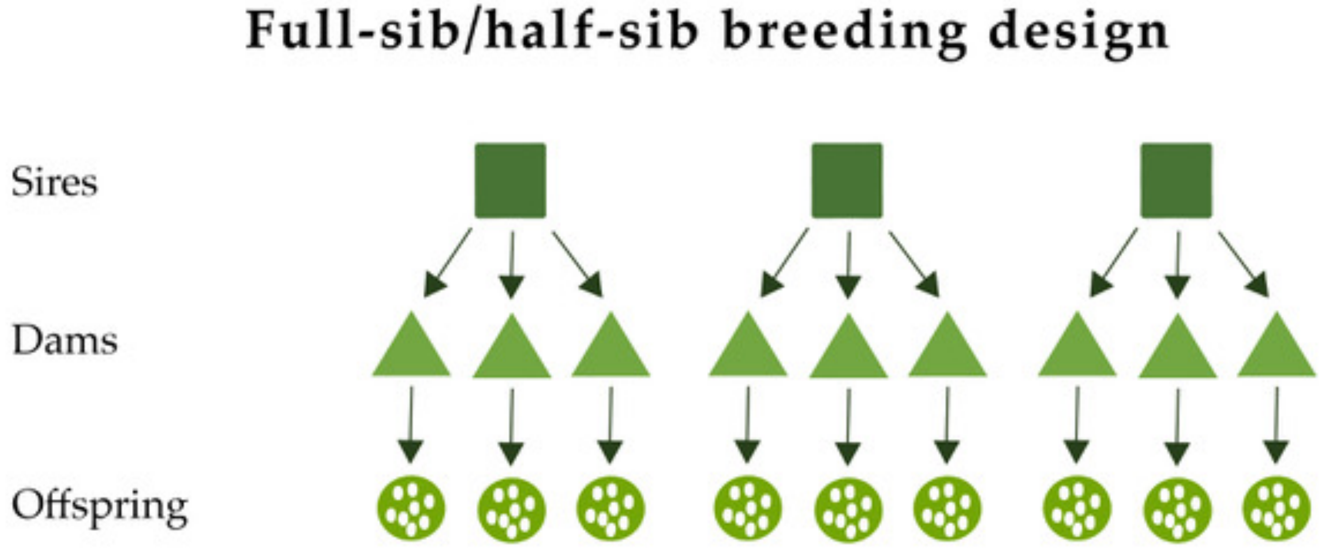
1). Genetic effects

- Embryo genotype influences temperature sensitivity
- $V_G = V_A + V_D + V_I$
  - Additive genetic effects (V_A)
    - $h^2 = V_A/V_P$

2). Non-genetic maternal effects

- Maternally-derived yolk steroids influence temperature sensitivity

Breeding design



Quantitative genetic analysis

- Additive genetic effects v.s. non-genetic maternal effects

- Egg mass
- Egg diameter
- Hatchling mass
- Hatchling carapace length
- Hatchling plastron length
- **Hatchling sex**

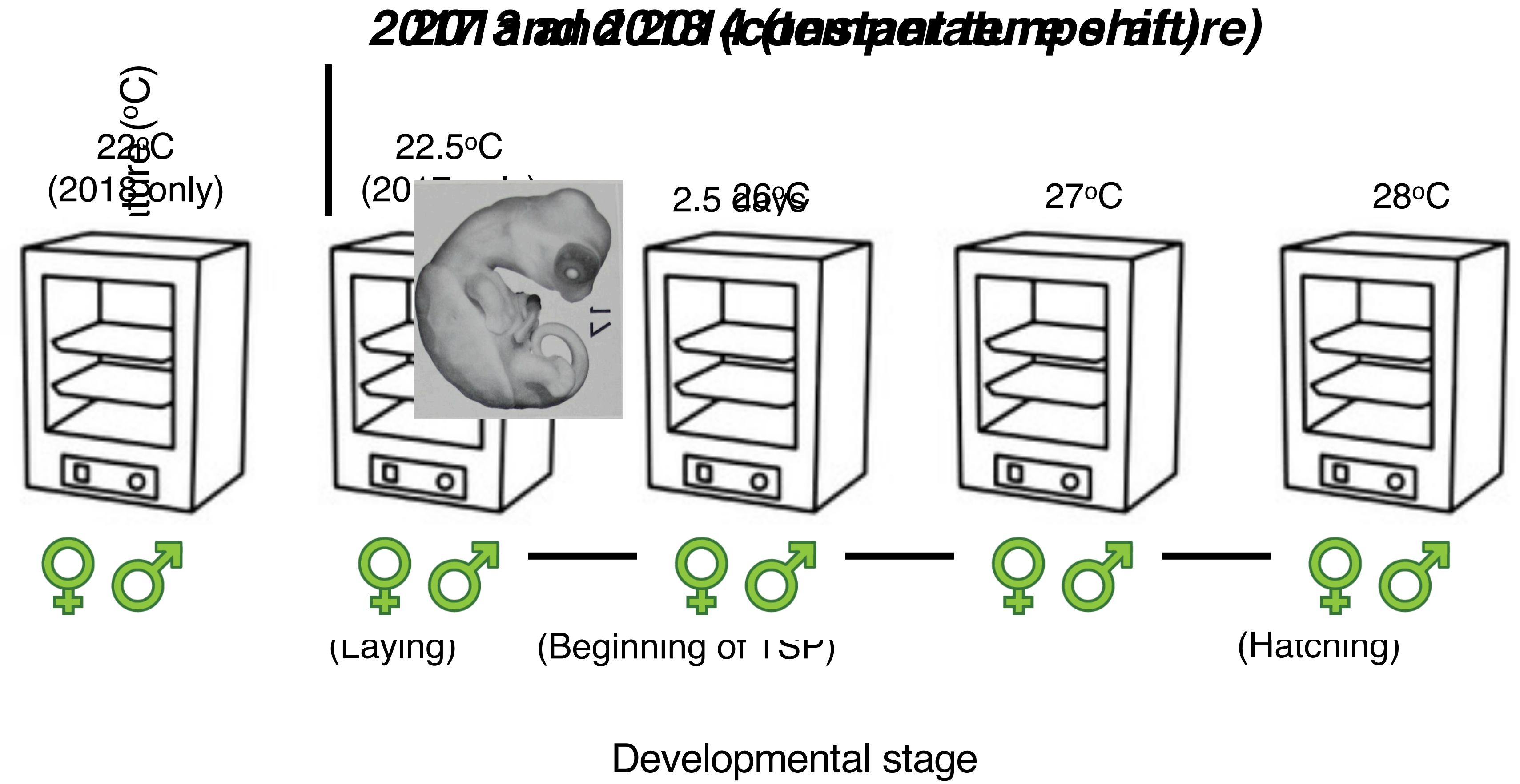
TABLE 10.4

Interpretation of the observational components of variance in a sib analysis

Observational component		*Covariance and causal components estimated*	
Sires:	$\sigma_S^2 =$	$cov_{(HS)}$	$\boxed{= \frac{1}{4} V_A}$
Dams:	$\sigma_D^2 =$	$cov_{(FS)} - cov_{(HS)}$	$\boxed{= \frac{1}{4} V_A + \frac{1}{4} V_D + V_{Ec}}$
Progenies:	$\sigma_W^2 =$	$V_P - cov_{(FS)}$	$= \frac{1}{2} V_A + \frac{3}{4} V_D + V_{Ew}$
Total:	$\sigma_T^2 = \sigma_S^2 + \sigma_D^2 + \sigma_W^2 =$	V_P	$= V_A + V_D + V_{Ec} + V_{Ew}$
Sires + Dams:	$\sigma_S^2 + \sigma_D^2 =$	$cov_{(FS)}$	$= \frac{1}{2} V_A + \frac{1}{4} V_D + V_{Ec}$

Falconer, D.S. and Mackay, T.F.C. (1996). *Introduction to Quantitative Genetics.* 4th edition Longman House: Essex, England.

Egg collection and incubation



Measurement of phenotypic traits and tissue collection

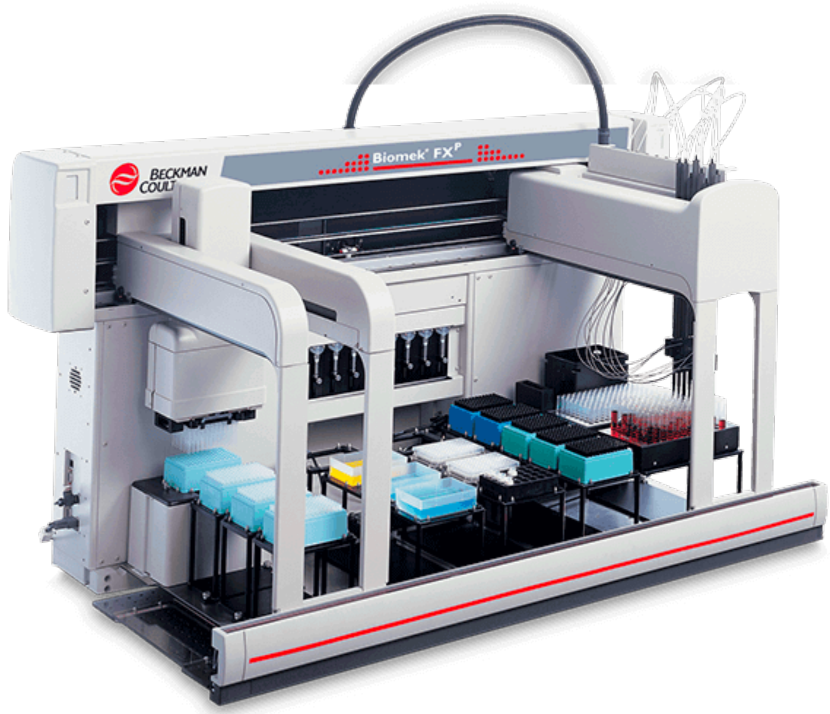
- In 2013, 2014, 2017 and 2018 we sexed hatchling turtles and collected tissue for genotyping
- In 2017 and 2018, we also collected egg and hatchling size data

Sample sizes

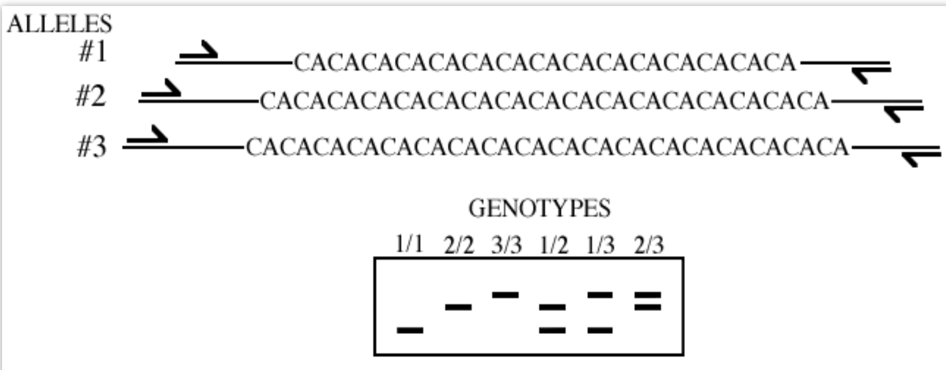
2013: 1062
2014: 1035
2017: 1358
2018: 1422

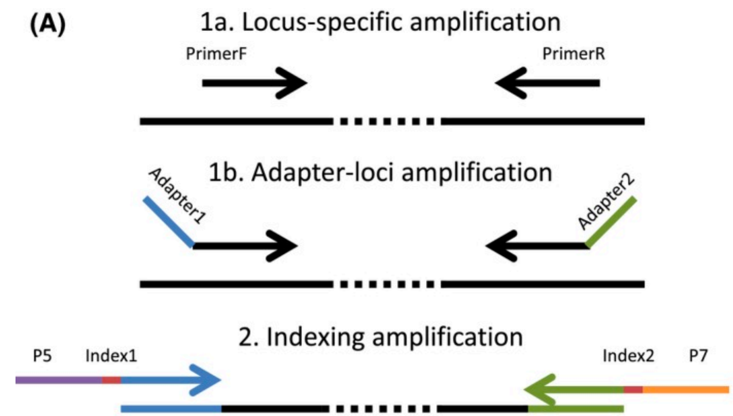
Total: 4877

DNA isolation, quantification, normalization



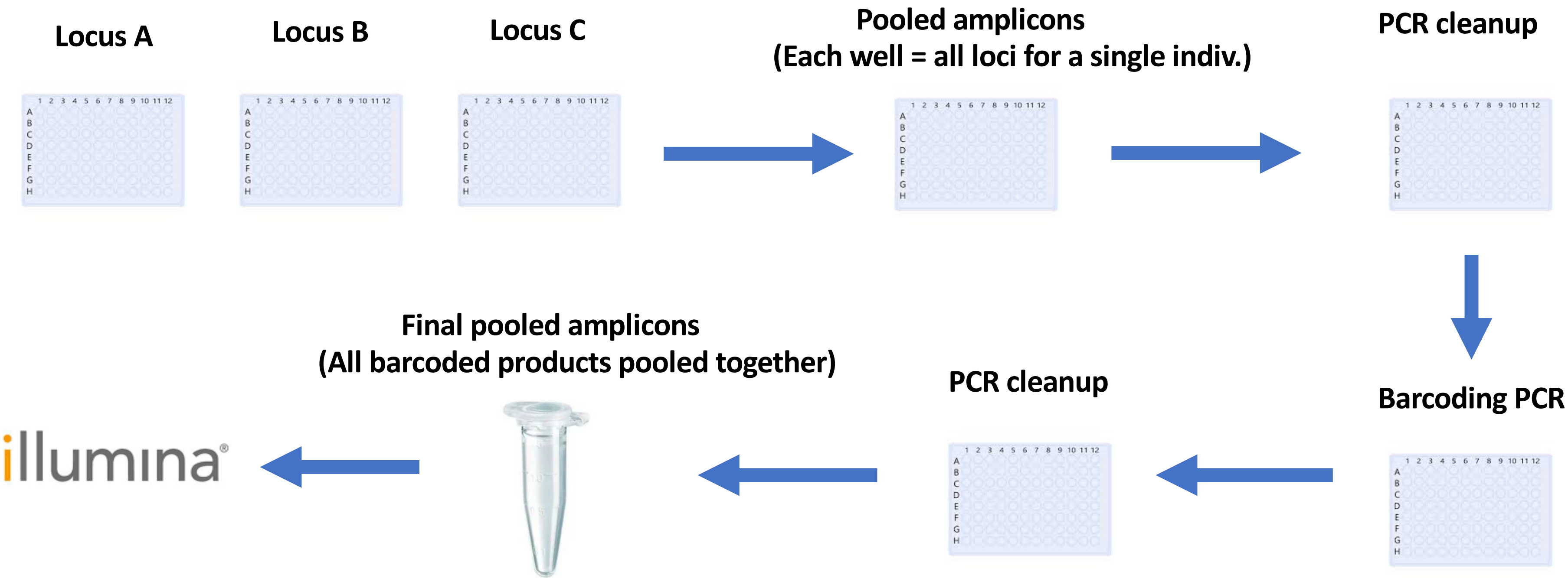
Amplicon sequencing





Darby B.J., Erickson S.F., Hervey S.D., Ellis-Felege S.N. (2016). Digital fragment analysis of short tandem repeats by high-throughput amplicon sequencing. *Ecology and Evolution* 6: 4502–4512.

Amplicon sequencing



Bioinformatics and data processing

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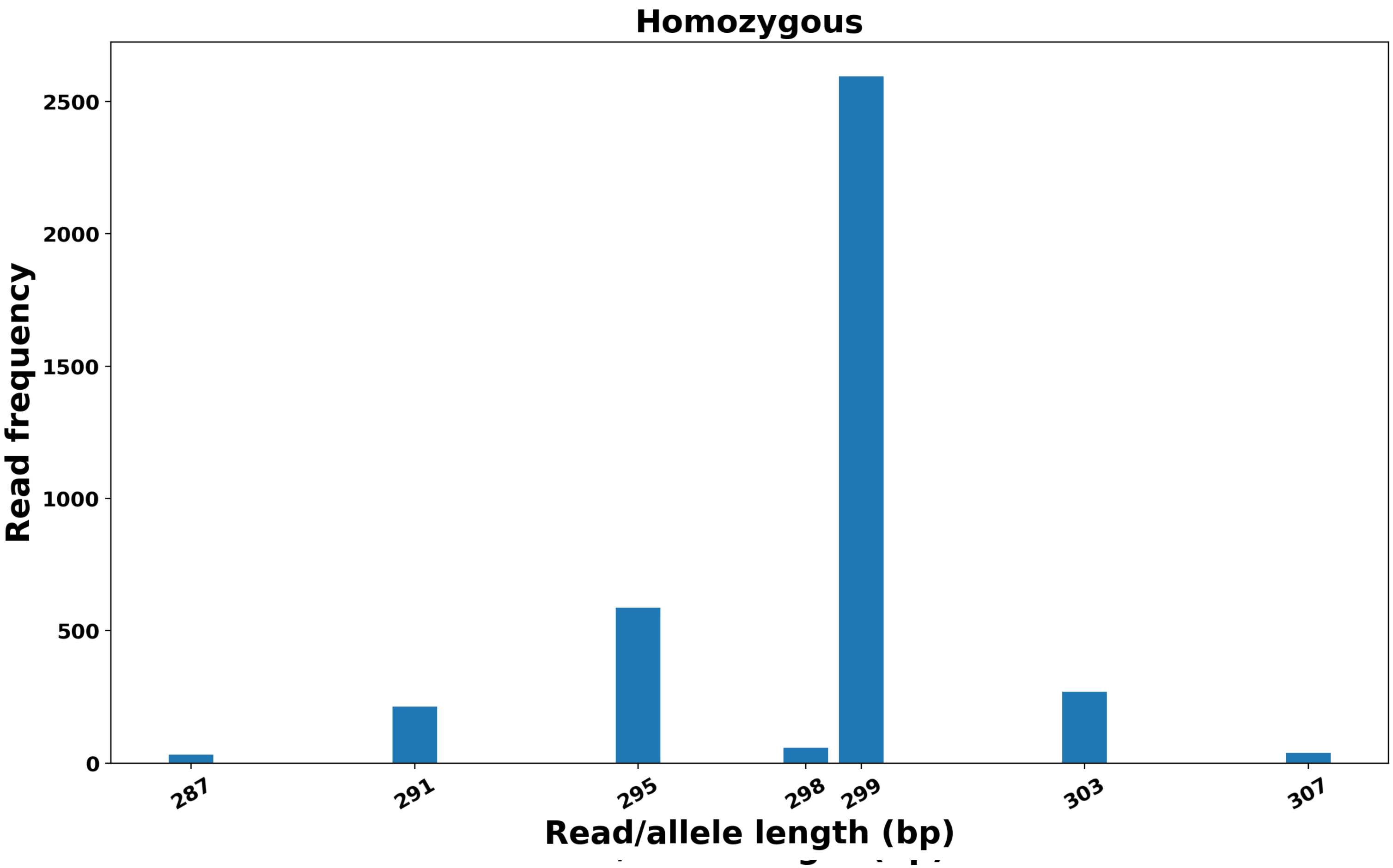
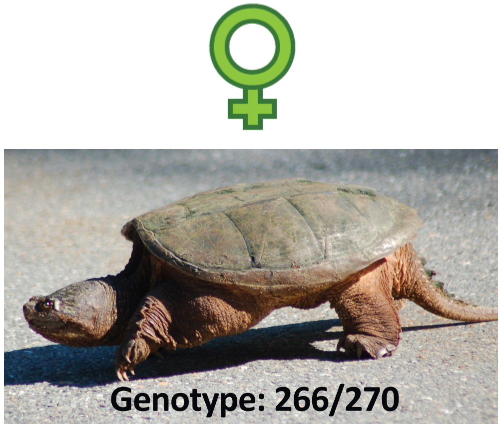
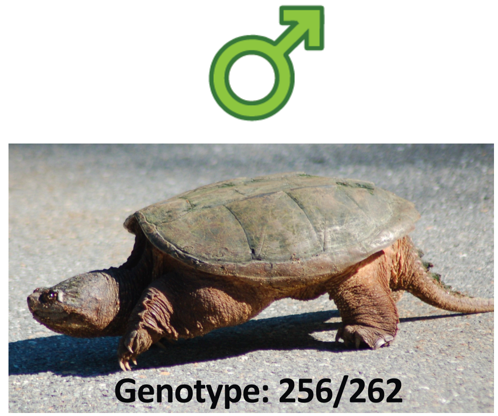
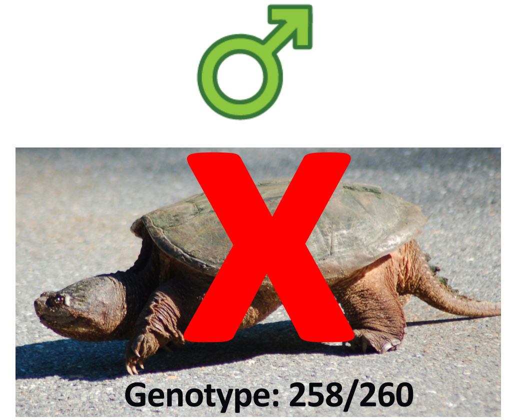
Microsatellite genotyping

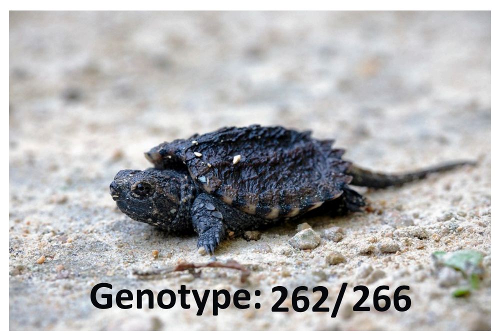


Parentage analysis









Quantitative genetic analysis

Traits

- Egg mass
- Egg diameter
- Hatchling mass
- Hatchling carapace length
- Hatchling plastron length
- Hatchling sex

Model parameters

- **Incubation temperature** → factor, fixed effect
- **Year** → factor, random effect
- **Sire** → factor, random effect
- **Dam** → (nested within sire) → factor, random effect
- **Sire x temperature** → random effect
- **Dam x temperature** (nested within sire) → random effect

ASReml® R

jmp® Statistical Discovery.™ From SAS.

Fixed effects tests = Type III ANOVA, Tukey HSD

Random effects tests = Likelihood ratio test

Analysis of egg mass

Table 3. Likelihood ratio tests of random effects on egg mass in snapping turtles.

Effect	LR_{X^2}	*Df*	*p*
Sire	0	1	1.0
Dam (nested within sire)	1719.06	1	<0.0001
Year	91.31	1	<0.0001

Table 5. Estimates of narrow-sense heritability (h^2) and maximum maternal effects (m^2_{Max}) of egg mass ± standard error.

Parameter	*Estimate*
h^2	0.00 ± 0.48
m^2_{Max}	0.59 ± 0.24

Analysis of egg diameter

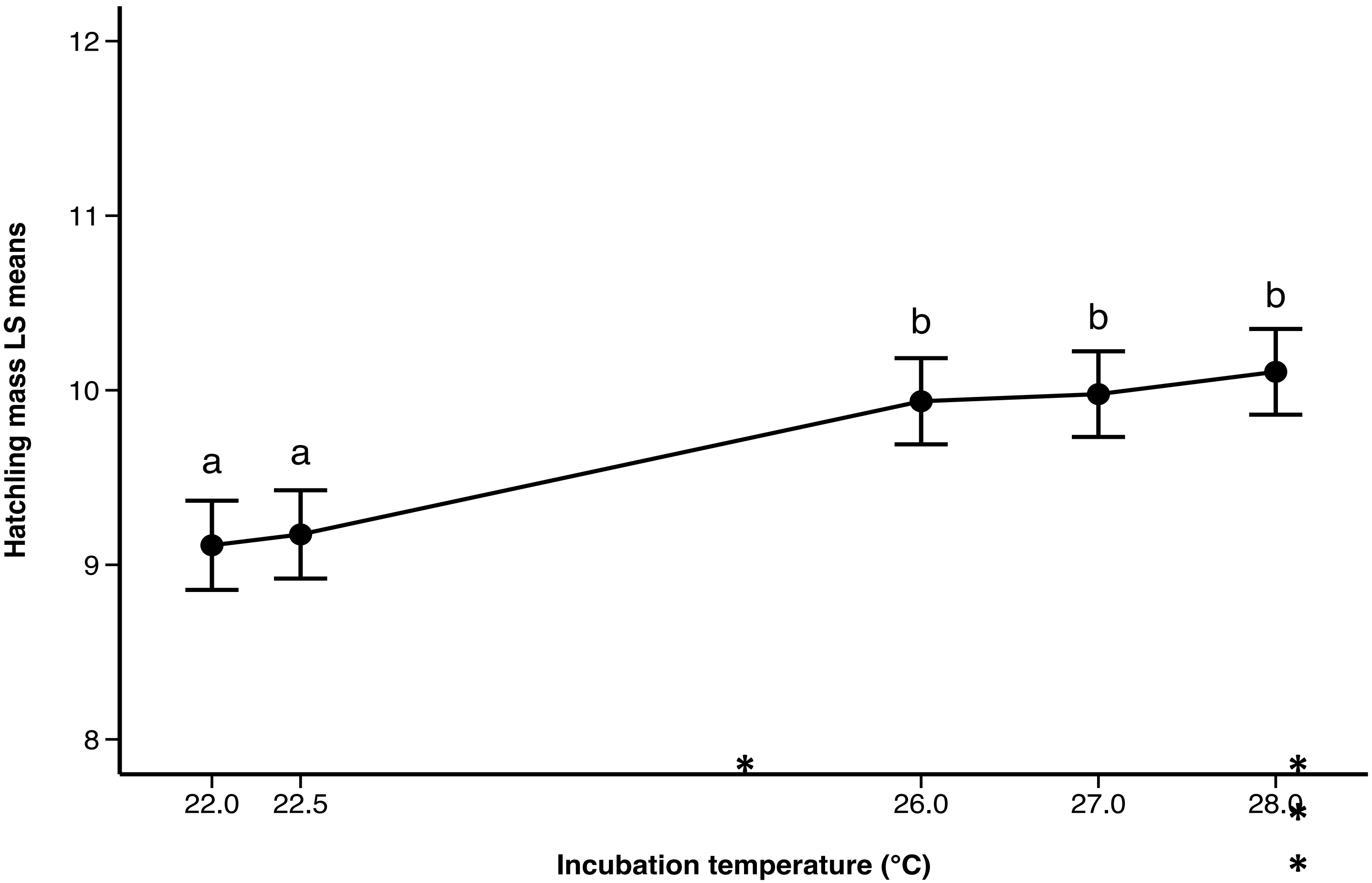
Table 4. Likelihood ratio tests of random effects on egg diameter in snapping turtles.

Effect	LR_{X^2}	*Df*	*p*
Sire	0	1	1.0
Dam (nested within sire)	1211.20	1	<0.0001
Year	202.00	1	<0.0001

Table 6. Estimates of narrow-sense heritability (h^2) and maximum maternal effects (m^2_{Max}) of egg diameter ± standard error.

Parameter	*Estimate*
h^2	0.00 ± 0.38
m^2_{Max}	0.46 ± 0.20

Analysis of hatchling mass



27°C	0.25 ± 0.46	0.49 ± 0.13	*
28°C	0.00 ± 0.38	0.51 ± 0.12	*

The effect of incubation temperature on hatchling mass

Analysis of hatchling carapace length

Table 18.
using the

Effect	*p*
Incubatio	001
Sire	0
Sire x ter	828
Dam (ne	001
Dam x te	001
Year	001

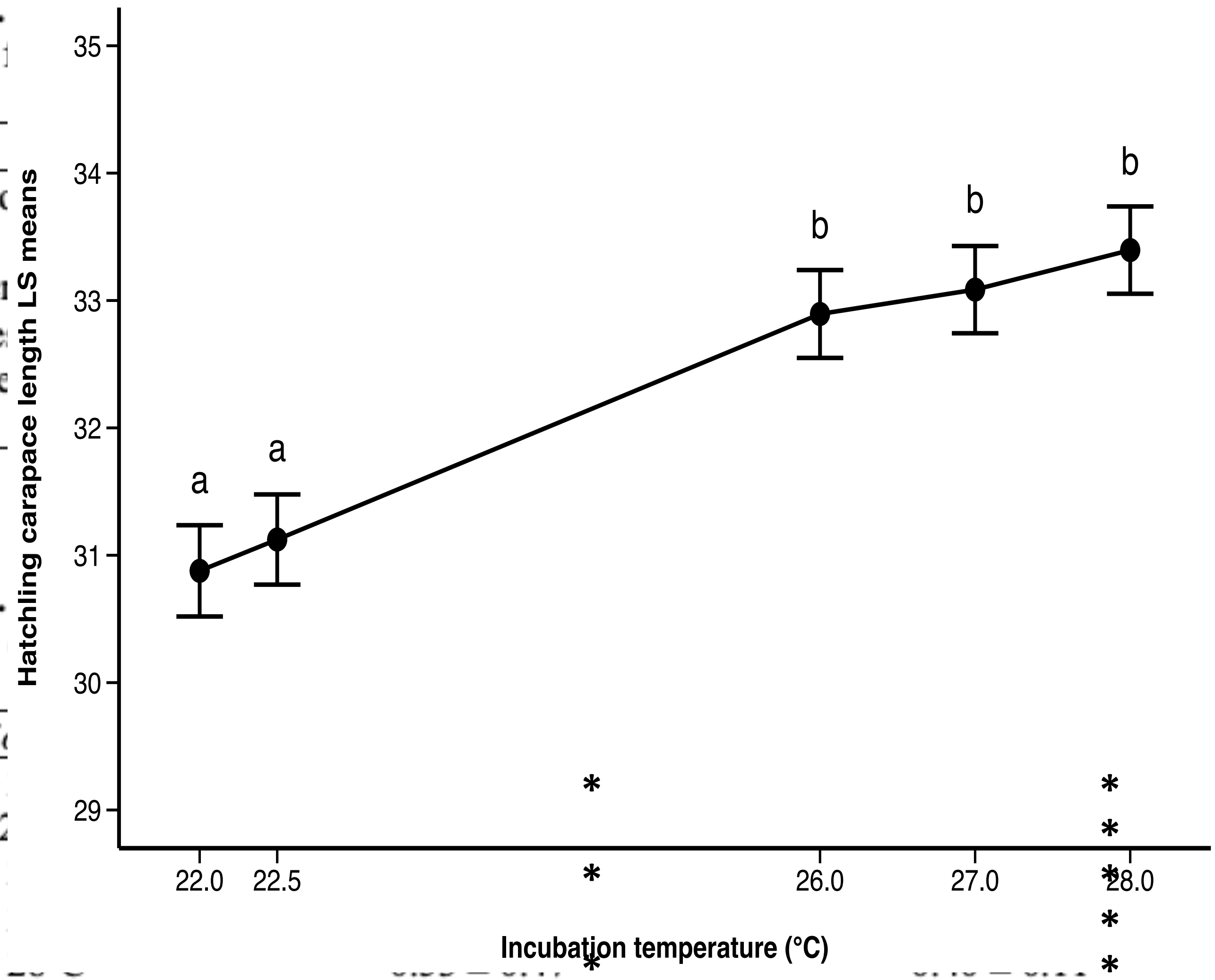


Table 24. t^2_{Max}) of
hatchling

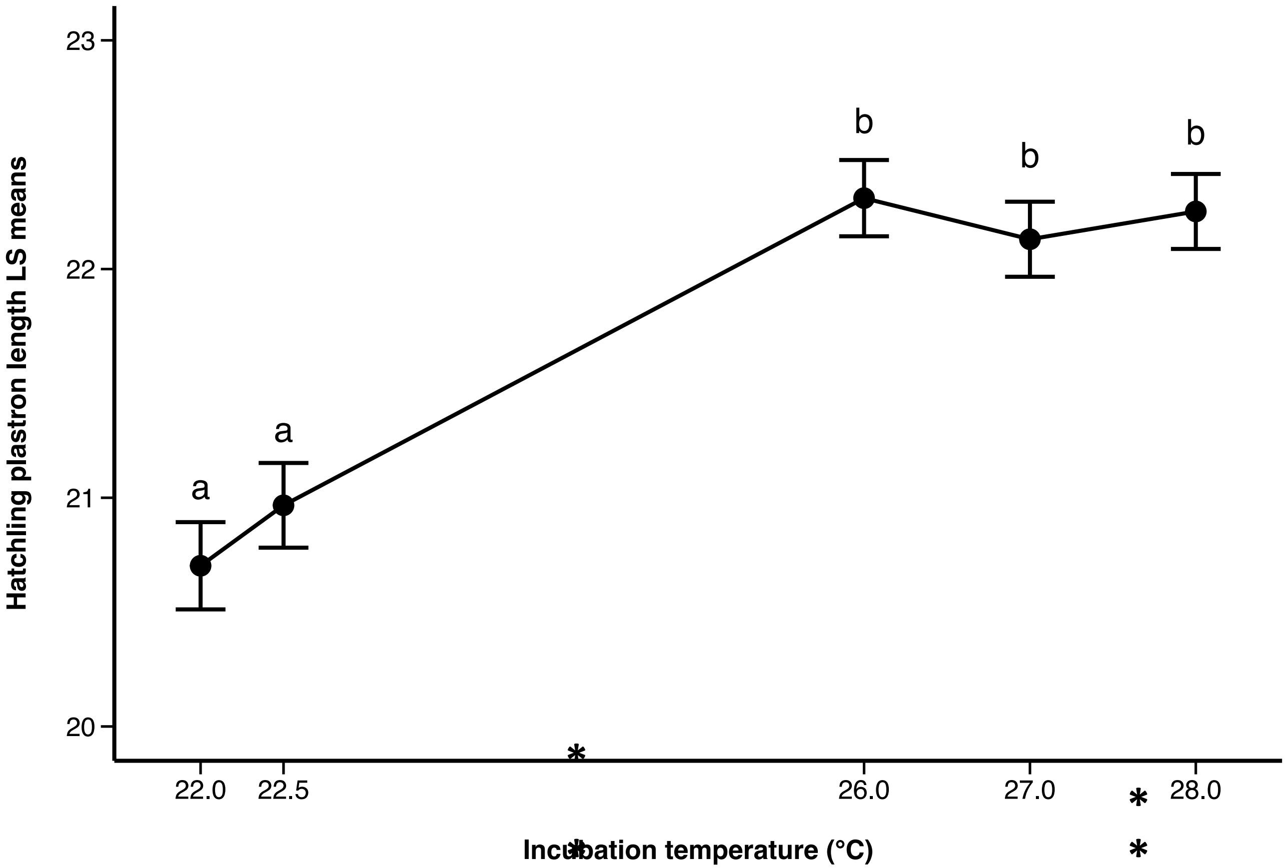
Incubati

The effect of incubation temperature on hatchling carapace length

Analysis of hatchling plastron length

Table 26. Analysis of fixed and random effects on hatchling plastron length using the full model

Effect	*p*
Incubation	.0001
Sire	1.0
Sire x tem	.0001
Dam (nest	.0001
Dam x ten	.2401
Year	.3681



The effect of incubation temperature on hatchling plastron length

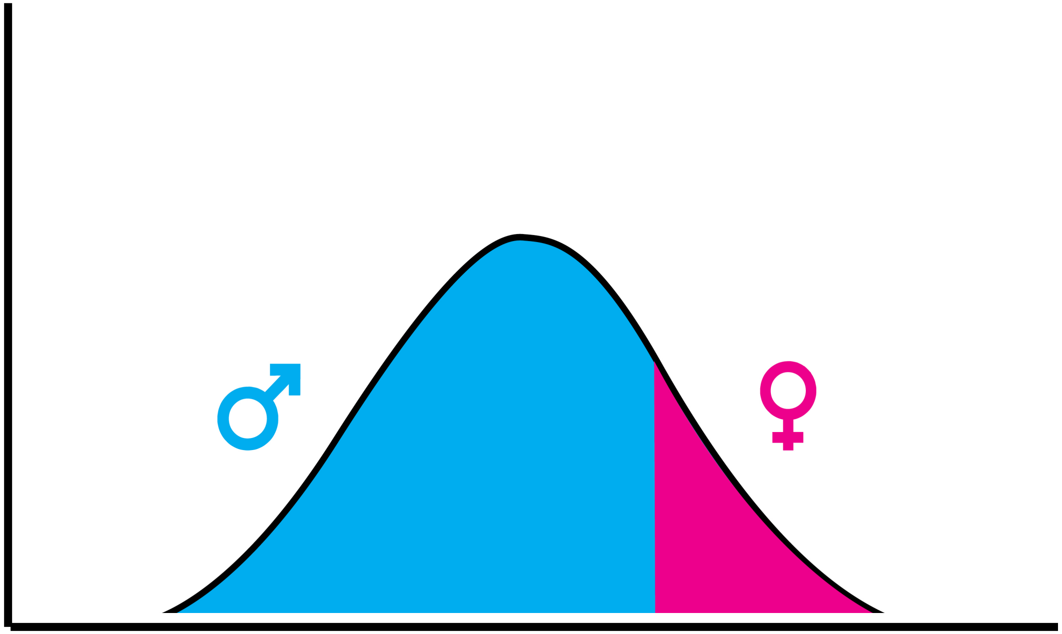
Table 32. E... (h^2_{Max}) of hatchling pl...

Incubation		
22	*	*
22.5		*
26	*	*
27°C	0.05 ± 0.23	0.24 ± 0.08 *
28°C	0.13 ± 0.22	0.19 ± 0.07 *

Analysis of sex ratio

Approaches to analyze sex:

- Continuous model
- Logistic model
- Liability threshold model

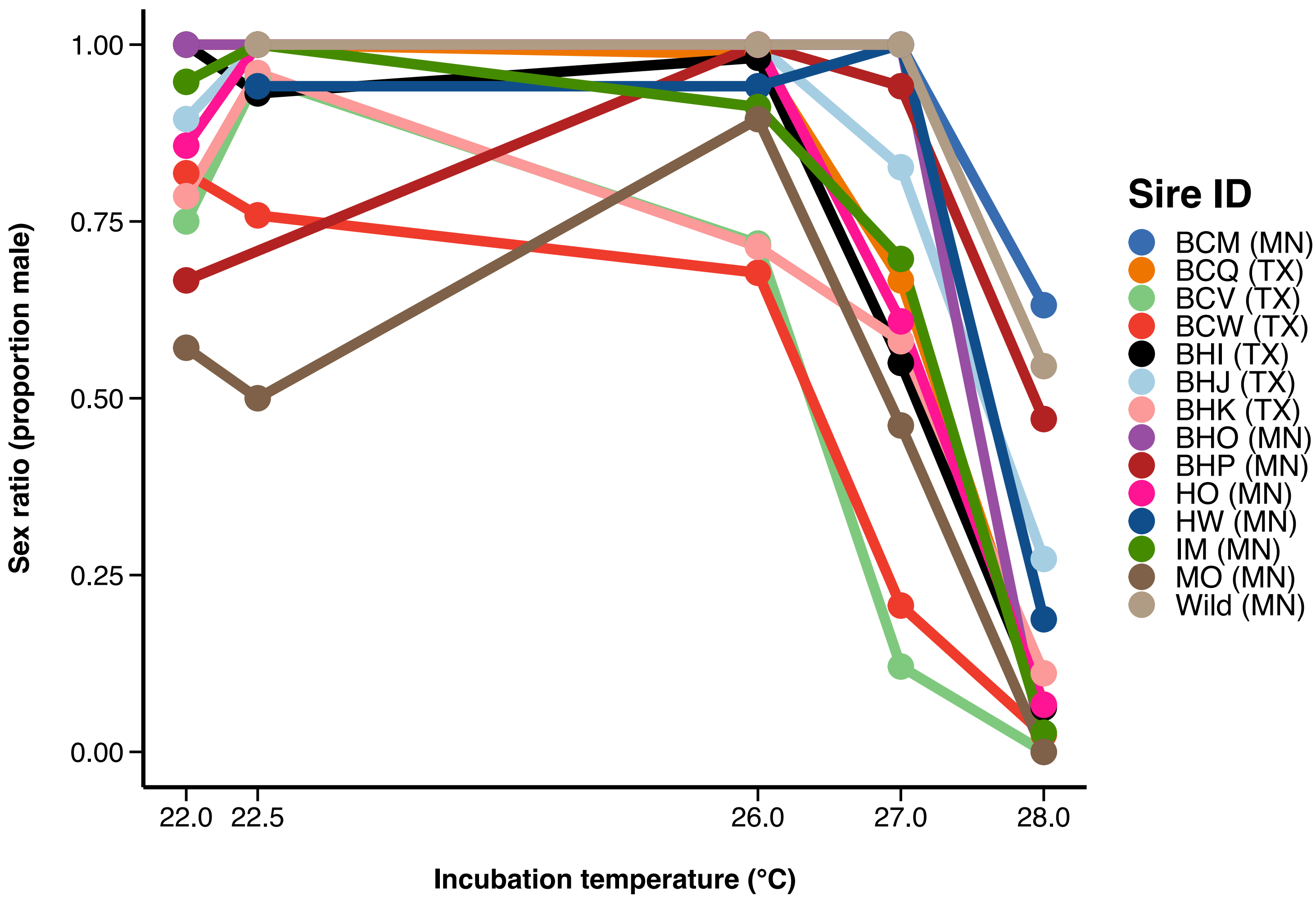


Analysis of sex ratio

Table 41. Estimates of narrow-sense heritability (h^2) of hatchling sex at individual incubation temperatures with their 95% confidence intervals. Estimates are shown using the observed scale, and two liability methods as described in the text.

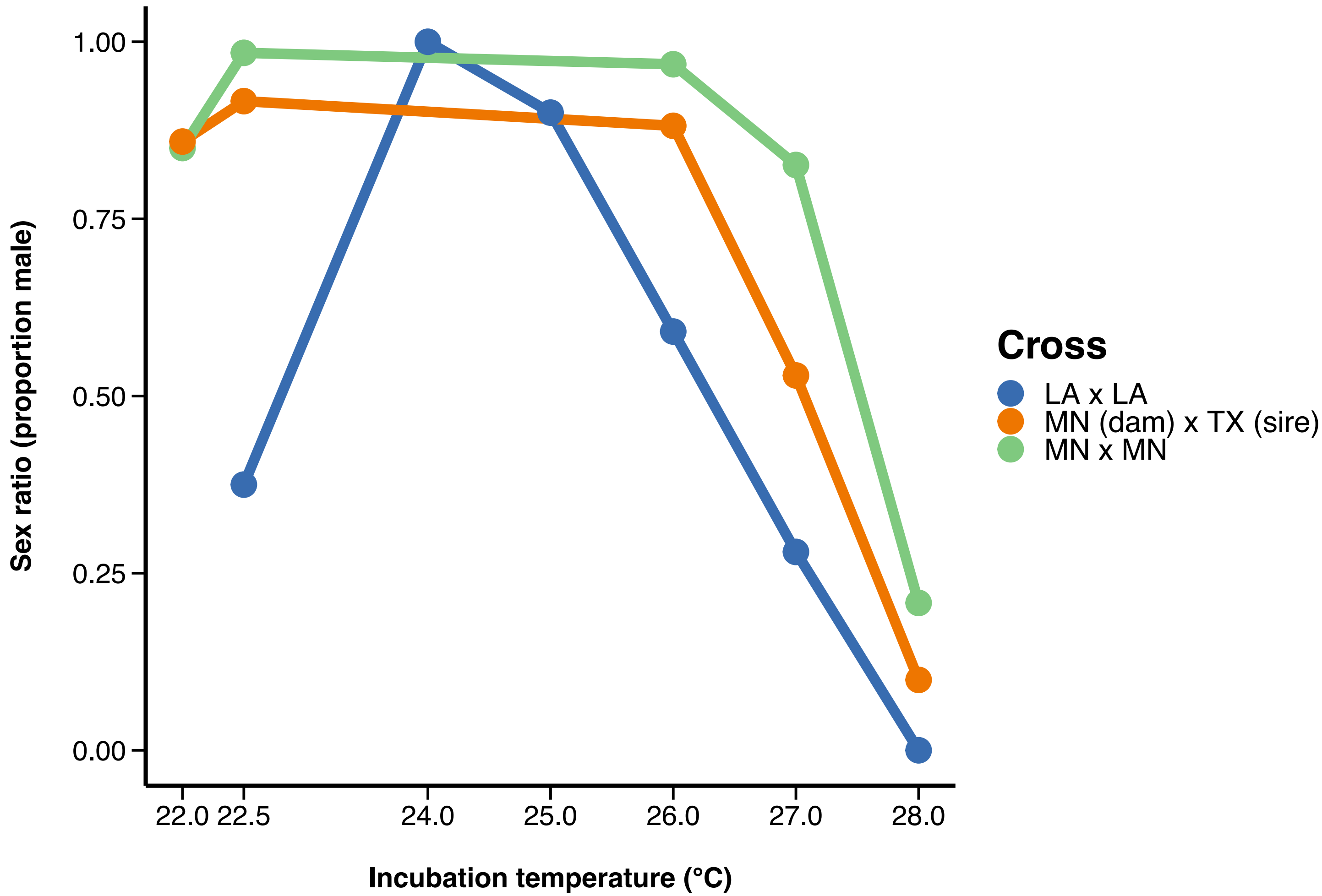
Incubation temperature	*Continuous*	*Liability (Bull)*	*Liability (F & M)*
22°C	0.34, CI = [0.11, 1.00]	0.78, CI = [0.29, 1.00]	0.80, CI = [0.27, 1.00]
22.5°C	0.61, CI = [0.20, 1.00]	1.00, CI = [0.71, 1.00]	1.00, CI = [0.76, 1.00]
26°C	0.29, CI = [0.08, 1.00]	0.80, CI = [0.34, 1.00]	0.86, CI = [0.22, 1.00]
26-31-26°C	0.50, CI = [0.21, 1.00]	1.00, CI = [0.50, 1.00]	1.00, CI = [0.50, 1.00]
27°C	1.00, CI = [0.52, 1.00]	1.00, CI = [0.80, 1.00]	1.00, CI = [0.83, 1.00]
28°C	0.90, CI = [0.36, 1.00]	1.00, CI = [0.86, 1.00]	1.00, CI = [0.92, 1.00]

Significant sire x temperature interaction



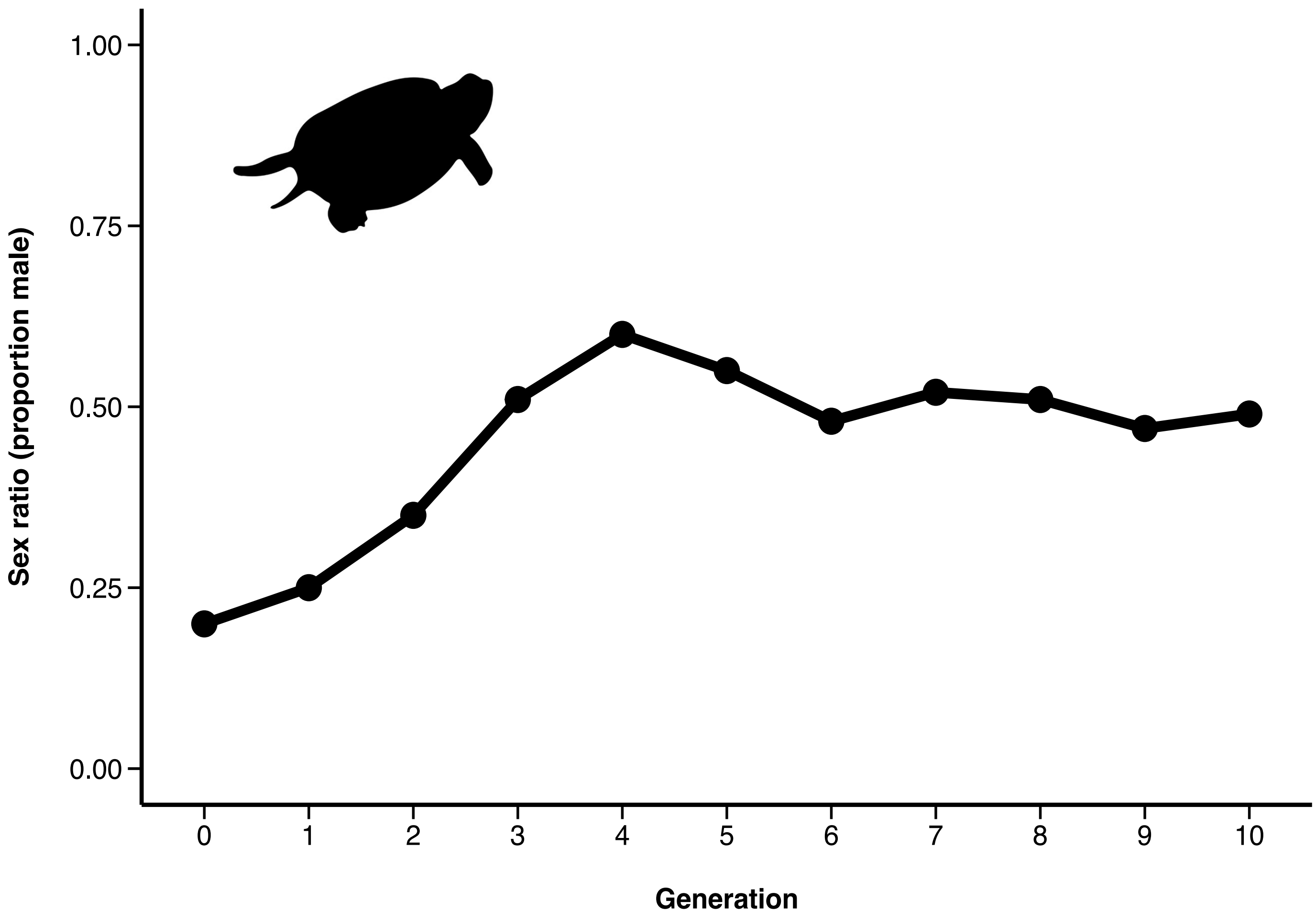
Sex ratio reaction norms as a function of incubation temperature

Hybrid status effect



Sex ratio reaction norms of three snapping turtle population crosses

Sex ratio evolution through frequency-dependent selection



Conclusion

1. Variation in egg size and hatchling size is caused by non-genetic maternal effects.

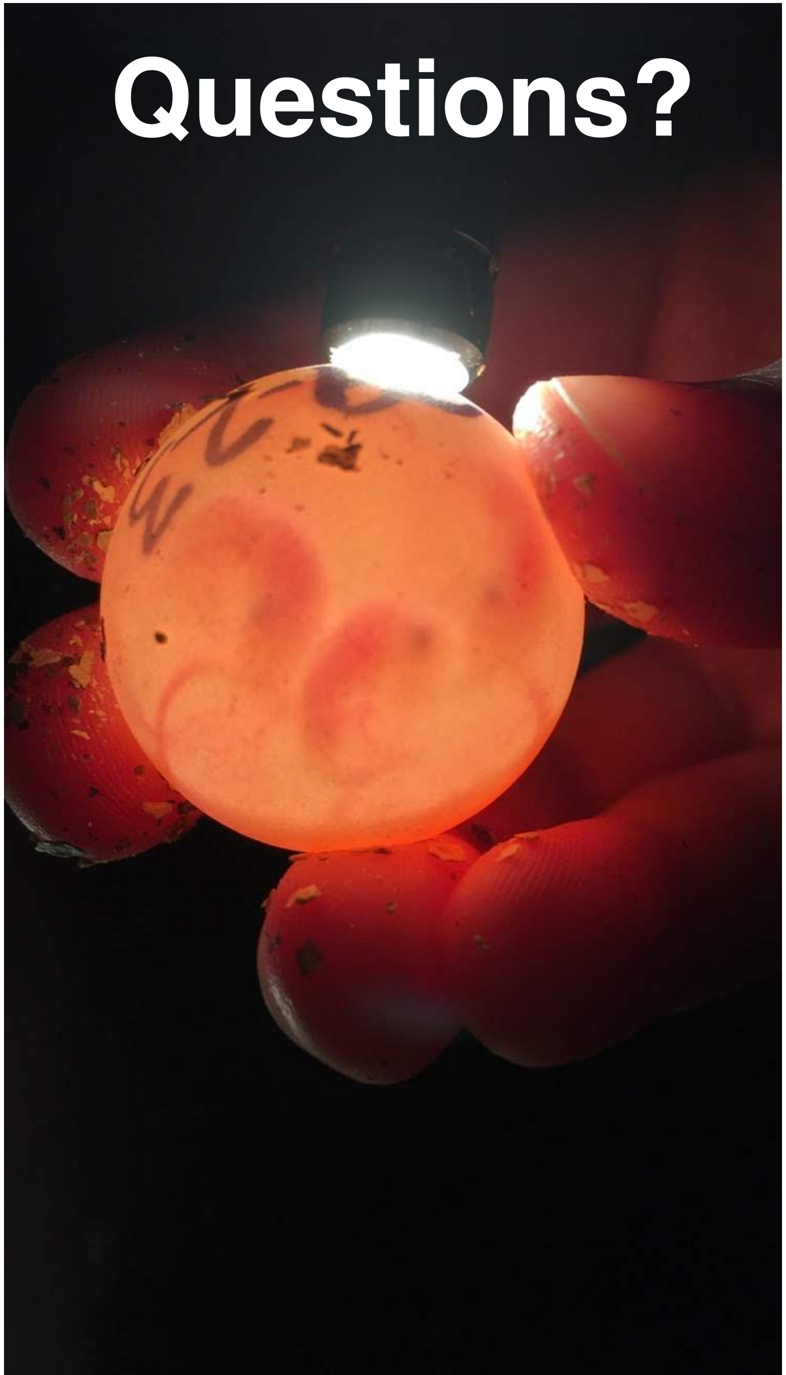
2. In the same offspring, sexual phenotype is highly heritable. We also detected a significant sire-by-temperature interaction.

3. Genetic variation in sex determination would allow the evolution of TSD pattern in response to climate change.

Acknowledgments

- Committee
    - Turk Rhen
    - Brian Darby
    - Robert Newman
- Rhen lab
- Sam Hervey
- Matt Flom
- UND Biology Department
- NSF Grants IBN IOS 0923300 and IBN IOS-1558034
- IACUC Protocol 1509-6C

Questions?



Sex ratio reaction norms: Texas v.s. Minnesota