

# CLIMATE CHANGE, MULTIPLE PATERNITY AND OFFSPRING SURVIVAL IN LIZARDS

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Recent work suggests that rising spring temperatures over recent decades have eliminated many lizard populations, and threaten many more worldwide. However, because ambient temperatures constrain activity times in ectotherms, warming conditions (as expected under global climate change scenarios) can increase the duration of seasonal opportunities for courtship and mating. Thus, in species where polyandry results in enhanced offspring viability, a warming climate may not necessarily impair long-term survival. Our nine-year study of a sand lizard (*Lacerta agilis*) population near the northern range limit in Sweden revealed consistently higher incidence of multiple paternity of clutches in warmer years, and higher viability of offspring from multiply-sired clutches (presumably reflecting the advantages of more intense sperm competition). Any trend to warmer spring temperatures likely will benefit offspring viability in this system, by increasing a female's opportunities to mate with additional males.

Many critical ecological traits are highly sensitive to ambient thermal conditions, and hence potentially will be modified by cli-

mate change. A growing literature documents such effects on traits such as seasonal phenology, growth rates, and sex-determining systems (Kearney, Porter and Shine 2009; Telemeco, Elphick and Shine 2010). Ectotherms (cold-blooded animals) may be especially vulnerable in such respect, because they are critically dependent upon ambient thermal heterogeneity for behavioral regulation of body temperatures and thus, local climatic conditions constrain the timing and intensity of fitness-relevant activities (Kearney, Porter and Shine 2009). Sinervo et al. (2010) suggest that rising spring temperatures over recent decades have eliminated many lizard populations, and threaten many more worldwide. However, the impact of this thermal shift may be very different in a cold-climate area (such as Sweden) than in a hotter area (such as Mexico, the basis for most of their analyses).

In sites where operative temperatures are already close to critical thermal maxima for local reptiles, higher temperatures could be devastating. For example, animals in such an area may have little time per day when conditions are cool enough to allow activities such as mate-searching and foraging (Kearney, Porter

and Shine 2009; Sinervo et al. 2010). In contrast, reptiles in cooler areas may benefit from higher temperatures, because the consequent ability to maintain relatively high body temperatures over longer periods may enhance organismal fitness via extended activity times and enhanced performance (Kearney, Porter and Shine 2009). Here we test the hypothesis that warmer weather increases offspring survival via elevated sperm competition (and not just as an effect of higher temperature per se).

## Methods

We studied sand lizards (*L. agilis*) at a coastal site in western Sweden (Asketunnan, ~57°22'N, 11°58'E), close to the northern (cold-climate) edge of this widespread species' geographic range (Olsson and Madsen 2001a; Olsson et al. 2010). At this site, the lizards attain sexual maturity at about three years of age, and females produce a single annual clutch of 4 to 15 eggs thereafter (Olsson and Madsen 2001a; Olsson et al. 2010). Lizards emerge from their overwintering sites in April–May, mate in May–early June, and oviposit in May–July (depending on weather conditions), and then reenter their winter retreats in September (Olsson and Madsen 2001a; Olsson et al. 2010). Climatic data were purchased from the Swedish Bureau of Meteorology and Hydrology (SMHI). We used data from the Varberg climate data logger (closest to our field site and at the same altitude [sea level], ca 50 km along the coast S of Asketunnan). Although the data were not collected on the immediate field site, any variation among years should be reflected in corresponding differences at the Asketunnan site. We then calculated the mean temperature (in the major part of the mating season, the month of May, during which approximately 90% of copulations take place; Olsson et al. 1996a), and used this estimate in our analysis of climatic effects on mating system variation.

Each year from 1998 to 2006, we spent an average of seven weeks at the study site to monitor lizard reproductive biology. We captured females as soon as bodily distension indicated that they were close to oviposition. The eggs were incubated at 25°C (a temperature that minimizes the rate of hatchling deformities; Zakharov 1989). Hatchlings and potential parents were blood-sampled (hatchlings ca 10 µl, adults ca 50 µl) from the *vena angularis* (corner of the mouth) and paternity-assigned using up to 21 microsatellite loci (Schwartz and Olsson 2008; Olsson et al. 2010). Hatchlings were individually marked for identification by toe-clipping, then released; recaptures in subsequent years provided data on rates of survival over the (crucial) first year of life (Olsson and Madsen 2001b). A 0.6 km corridor surrounding the study site, corresponding to the combined width of more than 60 average female home ranges, was routinely monitored for migration, removing any bias of mortality estimates due to undetected migration (Olsson et al. 1996b).

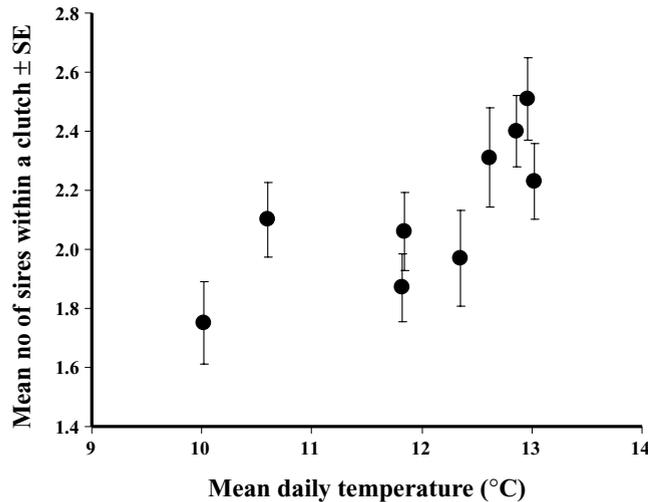
## MICROSATELLITE PATERNITY ASSIGNMENT

### Genotyping

DNA was isolated from 4543 adult and offspring samples (blood and tissue) collected over a nine-year period (1998–2006), representing 3938 individuals. Of these, we entered individuals into our analyses for which we had data on relevant parental traits. Samples were genotyped using 21 microsatellite loci: LA01, LA02, LA03E, LA04, LA10, LA27, LA37, LA40, LA45, LA47, LA50, LA55, LA58 (Schwartz and Olsson 2008); LA1-AG, LA2-AG, LA3-AG (Gullberg et al. 1997); Lvir7, Lvir17 (Bohme et al. 2005); and two loci (LV4-72 and LV4-X) (Boudjemadi et al. 1999) for which primers were redesigned to optimize multiplexing. The 21 microsatellite loci were amplified in multiplexed PCR reactions each containing three to six loci, using fluorescently labeled primers. Multiplexed PCR reactions were run in a 7-µl volume and contained 250–850 pmol of each primer, 0.22 mM dNTPs, 1.1X Qiagen PCR buffer with 1.5mM MgCl<sub>2</sub>, additional MgCl<sub>2</sub> when necessary, 0.03 U of hot start *Taq* (Qiagen), and 10–50 ng DNA. Thermal cycling conditions had a 15-min hot start at 95°C, 30 cycles of 95°C for 20 sec, 52–56°C annealing for 30 sec, 72°C for 1.15 min, and a final extension at 72°C for 30 min to 3 h. Sample electrophoresis was run on an ABI 3130xl genetic analyzer and analyzed in GeneMapper (Applied Biosystems, Carlsbad, CA) and corrected by eye.

### PATERNITY ANALYSIS

Because of the low level of genetic variability in this population (Gullberg et al. 1997), and the overlap of generations, it was necessary to use 17–21 microsatellite loci to assign paternity with high confidence. All of the adults and the offspring from years 2001–2006 were genotyped at 21 loci. The offspring from years 1998–2000 were genotyped with 17 loci except for offspring for which paternity between the two top candidate males could not be determined with 95% confidence; these offspring were genotyped at all 21 loci. Paternity analyses were conducted in Cervus 3.0 (Kalinowski et al. 2007) for the nine years of data 1998–2006. A four-year sliding window of adult genotypes was used to calculate the population allele frequencies for each year of offspring analyzed. Genotyping error rate in the final dataset is estimated to be less than 1% based on mother–offspring comparisons, repeated genotyping of the same individuals collected in multiple years, and from 30% of the data being independently scored by a second researcher. Paternity was assigned based on two simulation analyses, complete exclusion, and 1% error rate. Candidate sires included all adult males sampled in previous years, the year the offspring were born, and the subsequent year. Confidence levels of Delta (the difference between the LOD scores (the natural log of the overall likelihood ratio) of the first and second most likely candidates) were set at 95% and 80%. The paternity assignment was classified using three “confidence codes” based on the type



**Figure 1.** The influence of ambient temperatures on the incidence of multiple paternity within clutches of Swedish sand lizards. Warmer years prolonged the mating period, and thus increased the average number of males contributing to each clutch of eggs.

of data that supported the assignment. (1) 95% confidence level of the mother–offspring–father Delta based on either the zero error rate or the 1% error rate simulation. (2) 80% confidence level of the mother–offspring–father Delta, and additional evidence of partnership from field observations (witnessing copulations or mate guarding between the assigned sire and the mother). (3) 80% confidence level of the mother–offspring–father Delta and evidence of the male contributing to the clutch due to siblings being independently assigned the same father with at least 80% confidence. If paternity could not be assigned based on these confidence codes, the individual was eliminated from subsequent analyses. For a more detailed description of paternity assignment see Olsson et al. (2010).

For statistics we used Proc Mixed in SAS 9.2 to model first year offspring survivorship (mean survival of offspring per clutch). Mean daily temperature and its interaction with number of fathers were used as predictors in the original model but were backwards eliminated ( $P > 0.45$  for both). This study meets the terms of the ethics committee at the institution where the experiment was carried out under permit numbers AE03/03, AE03/04 and AE03/05.

## Results

The incidence of multiple paternity (mean number of fathers per clutch) was higher in warmer years (Spearman's rank-order correlation,  $r_s = 0.78$ ,  $P = 0.012$ ,  $n = 9$ ; Fig. 1). Offspring from multiply-sired clutches had higher survival during the first year of life (Mixed model analysis with mean survival of offspring per clutch as response variable [survived = 1, died = 0], log likelihood ratio tests for random effects: year  $\chi^2 = 9.0$ ,  $P < 0.01$ ,  $df =$

1, females ( $n = 226$ )  $\chi^2 = 0.1$ ,  $P > 0.05$ ,  $df = 1$ ; fixed effects: number of sires per clutch,  $F = 5.4$ ,  $P = 0.02$ , slope coefficient  $\beta = 0.016 \pm 0.006$ ; laying date,  $F = 9.1$ ,  $P = 0.003$ , slope coefficient  $\beta = -0.004 \pm 0.00001$ ; offspring mean mass,  $F = 0.8$ ,  $P = 0.36$ ;  $df = 1, 111$ ).

## Discussion

The current results build on our previous studies on this population, which demonstrated that offspring from multiply sired clutches suffered less from malformations (Olsson et al. 2010). Here we show that indeed offspring not only survive early embryonic development better when sired during increased sperm competition (as shown in Olsson et al. 2010), but also that their first-year survival in the subsequent year is significantly enhanced. In earlier fieldwork, we have shown that warmer weather during spring (April–May) enhances the lizards' opportunities for mate-searching and reproductive interactions prior to egg-laying (Olsson and Madsen 2001a). In experimental work with captive lizards, we have shown that mating with additional males can enhance the viability of a female's offspring, via sperm competition (Olsson et al. 1994). In combination, these results accord well with our direct field-based demonstrations of positive links between spring temperatures and multiple paternity (Fig. 1), and between multiple paternity and offspring viability.

Changing climates will affect lizards in many ways by altering patterns of selection. Some populations may be driven to extinction as temperatures become too warm for activity (Sinervo et al. 2010), whereas others may benefit from increased basking opportunities. Even within a single evolutionary lineage (such as lizards), climate change may disadvantage some species and advantage others. Our data on sand lizards show that a fundamental aspect of the mating system, closely linked to offspring fitness, is highly sensitive to ambient thermal conditions. Such sensitivity reinforces the fact that any change to climatic conditions will affect wildlife through complex pathways, and that we will need to understand the nature of such links to predict the evolutionary impacts of climate change.

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