

Seasonal shifts along the oviparity–viviparity continuum in a cold-climate lizard population

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Abstract

Squamate embryos require weeks of high temperature to complete development, with the result that cool climatic areas are dominated by viviparous taxa (in which gravid females can sun-bask to keep embryos warm) rather than oviparous taxa (which rely on warm soil to incubate their eggs). How, then, can some oviparous taxa reproduce successfully in cool climates – especially late in summer, when soil temperatures are falling? Near the northern limit of their distribution (in Sweden), sand lizards (*Lacerta agilis*) shift tactics seasonally, such that the eggs in late clutches complete development more quickly (when incubated at a standard temperature) than do those of early clutches. That acceleration is achieved by a reduction in egg size and by an increase in the duration of uterine retention of eggs (especially, after cool weather). Our results clarify the ability of oviparous reptiles to reproduce successfully in cool climates and suggest a novel advantage to reptilian viviparity in such conditions: by maintaining high body temperatures, viviparous females may escape the need to reduce offspring size in late-season litters.

Introduction

Most species of reptiles are oviparous (egg-laying), but viviparity (live-bearing) has evolved > 100 times in diverse lineages of squamate reptiles (Shine, 1985; Blackburn & Stewart, 2011; Stewart & Blackburn, 2014). A recent counter-hypothesis – invoking early origin of viviparity and numerous reversions to oviparity (Pyron & Burbrink, 2014) – has been strongly falsified by reanalyses (e.g. Blackburn, 2015; King & Lee, 2015; Shine, 2015). The transition between alternative modes of reproduction appears to have been driven primarily by selective forces associated with ambient thermal environments. Rates of embryogenesis in squamates are closely tied to temperature, and viviparity has evolved in regions where soil temperatures are too low for eggs to complete development prior to the onset of winter (Sergeev, 1940; Shine, 1985) or to develop successfully (i.e. low

incubation temperatures can generate offspring with suboptimal phenotypic traits: Shine *et al.*, 1997; Qualls & Andrews, 1999; Amiel & Shine, 2012; Noble *et al.*, 2017). This ‘cold-climate’ model for the adaptive significance of reptilian viviparity has extensive empirical support but several issues remain unclear. Notably, the correlation between reptilian reproductive modes and climates is strong, but not perfect (Tinkle & Gibbons, 1977; Shine & Berry, 1978). The success of viviparous taxa in warm as well as cool climates is easily explained by advantages that accrue after viviparity has evolved, rather than factors that initially drove the transition from oviparity to viviparity (e.g. Sergeev, 1940; Shine & Bull, 1979; Webb *et al.*, 2006). For example, viviparity may benefit a tropical aquatic species because reproducing females do not need to leave the water to produce their offspring, an important advantage for a seasnake, but one that provides no fitness benefit to females that exhibit intermediate stages in the progression from oviparity to viviparity (Shine & Bull, 1979).

The reverse exception to the correlation between cold climates and viviparity involves the success of a small

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number of oviparous taxa in cold climates. That situation is more puzzling, because it involves overcoming a direct abiotic constraint. How can an oviparous species reproduce in an area in which the seasonal period of suitably high soil temperatures is too short to allow an embryo in an external nest to develop to hatching? There are two evolutionary solutions to that problem. First, the oviparous taxon can evade cold soil temperatures by selecting unusually warm sites for nesting and/or by prolonging the duration of uterine retention of eggs at high maternal temperatures (and thus, reducing the subsequent incubation period post-laying; Mathies & Andrews, 1995; Shine *et al.*, 1997). Second, the oviparous taxon can evolve accelerated developmental rates (reduced incubation periods), especially at low temperatures (Shine, 1999; While *et al.*, 2015). These are complementary rather than alternative tactics, and oviparous squamates that breed in cool areas may well exhibit both of these shifts.

Although we might thus predict abbreviated incubation periods in cool-climate oviparous squamates (for theoretical models see: Shine & Bull, 1979; Roff, 1980), the proximate mechanisms generating accelerated development remain poorly understood. One intriguing hypothesis is that egg size may be under selection in such situations. Larger eggs generally require longer to develop, presumably due to the time needed to transform the extra yolk into progeny tissue (Deeming *et al.*, 2006; but see While *et al.*, 2015). In keeping with that interpretation, experimental reduction in lizard egg size reduces incubation period (Sinervo, 1990; Sinervo & Doughty, 1996). Thus, a reduction in offspring size (and hence developmental period) might enable successful reproduction even in an area where suitably warm incubation conditions are available only briefly each year (Rykena, 1987; Roitberg *et al.*, 2015).

The hypothesis that a reduction in egg size evolves as an adaptation to cool climates arose from studies on sand lizards (*Lacerta agilis*), a widespread European species that shows substantial (and correlated) geographic variation in egg size and incubation period (Rykena, 1987; Roitberg *et al.*, 2015). A trend for cool-climate lineages to have smaller eggs, that hatch sooner, has been interpreted as an adaptation to breeding in sites where soil temperatures remain high only briefly each year (Roitberg *et al.*, 2015). However, previous studies have relied upon comparisons through space, rather than through time within a season. Based on Roitberg *et al.*'s hypothesis, we predicted that sand lizards in these cool-climate extremes also should exhibit seasonal variation in egg sizes and incubation periods. A female lizard nesting early in the year can 'afford' to produce large eggs with a long incubation period, because these will have access to a relatively long window of high soil temperatures. In contrast, a female nesting later in the season would need to produce eggs that hatch quickly – perhaps by adjusting the duration

of uterine retention prior to oviposition, or by hatching in a more altricial state (i.e. by completing less development prior to hatching), or by accelerating embryogenesis at low temperatures, or alternatively or additionally, by reducing egg size in late-season clutches. Plausibly, year-to-year variation in weather conditions might intensify or reduce the selective advantages of reducing incubation periods. Our long-term field studies on a population of sand lizards (*Lacerta agilis* Linnaeus, 1758) near the northern limit of the species' distribution provide data with which to test these predictions.

Materials and methods

Study species and area

Sand lizards are medium-sized lacertids [in our study population, males average 70 mm snout-vent length (SVL), females average 78 mm, maximum 20 g] with a massive geographic range (the second largest among terrestrial reptile species) across the temperate Palearctic (Roitberg *et al.*, 2015). The species' range extends further north than that of any other oviparous lizard in Europe, and our study population at Asketunnan, on the west coast of Sweden (57°22' N, 11°59' E), is close to the northernmost edge. We have never found natural nests of this species at our study site, but thermal regimes in shaded areas of soil in such sites during the period when sand lizard eggs are incubating (May to July) remain below 15 °C in shaded areas even in mid-summer (Jungqvist *et al.*, 2014). In the Gothenburg area, soils typically experience frosts from mid-October to mid-April (<https://garden.org/apps/calendar/?q=Gothenburg,%20Sweden>). Doubtless, female lizards can find sun-exposed microhabitats that provide warmer conditions; but even there, the available period of temperatures > 25 °C must be brief. At this site, female lizards produce only a single clutch per year. Clutches produced earlier in the season contain larger offspring than do late-season clutches (Olsson & Shine, 1997a,b; Ljungström *et al.*, 2015, 2016).

Methods for data collection

Throughout the lizards' activity season, we monitored the study site on every day with sunny weather in 1987–1991 and again in 1998–2007, to locate and capture lizards. Captured animals were photographed, given a unique toe-clip, weighed (to the nearest 0.1 g) and measured (SVL to the nearest 1 mm). When females became visibly distended with eggs, we brought them into captivity and kept them in individual cages (400 × 600 × 400 mm) containing a sand substrate and a flat rock over a moist patch of soil where all females laid their eggs. Ambient temperature was maintained at 18 °C, but a 40 W spotlight at one end of

each cage enabled females to bask and thus attain body temperatures of up to 40 °C if they chose to do so. Cages were checked twice daily for newly laid eggs, which were immediately removed and incubated (with one clutch per container) in moist vermiculite (1 : 10 water to vermiculite by volume). All incubators were maintained at 25 °C, a temperature that minimizes developmental abnormalities (Zakharov, 1989). Egg mass immediately post-oviposition was recorded only in the first year of the study (1987), but hatchling traits were measured in all years. When daily checks revealed hatchling lizards, these were processed (measured, weighed, marked) in the same way as their parents and then released at the field site. Our published papers on this study provide extensive additional detail on methods (Olsson & Shine, 1997a,b; Ljungström *et al.*, 2015, 2016).

Our decision to incubate all eggs at a temperature that minimizes developmental abnormalities (25 °C) creates a mismatch between incubation conditions in the laboratory and likely incubation conditions in the field, few if any field nests would average 25 °C (see above). Importantly, however, thermal effects on total incubation periods of reptile eggs are straightforward and well understood (Andrews, 2004; Georges *et al.*, 2005; Du *et al.*, 2009). In all squamate species studied to date, including *L. agilis*, incubation periods at one temperature reliably predict incubation periods at other temperatures (e.g. Rykena, 1987). Thus, we can use incubation period at 25 °C (or any other standard temperature) to quantify the number of degree-days required to complete embryonic development, without needing to mimic field nest temperatures accurately. Given the unknown and undoubtedly variable thermal regimes inside natural nests, and the fact that thermal effects on incubation period are of much greater magnitude than the seasonal effects we have documented (e.g. Rykena, 1987), using a standard (fixed) incubation temperature offers the most powerful way to quantify intrinsic (rather than environmentally forced) among-clutch variation in the duration of incubation.

Statistical analyses

Using JMP Pro v11 (SAS Institute, Cary, NC, USA), we checked assumptions of normality and variance homogeneity prior to analysis. We ln-transformed data on survival rates (proportion of hatchlings surviving per clutch), after adding 0.5, to achieve normality. For data gathered in 1987 (when we recorded egg mass post-oviposition), we report tests based on individual eggs, nested within maternal ID #. For comparisons with weather conditions, we used annual mean values (with maternal identity and year as random effects). For all other analyses, we used each clutch as the unit of replication (i.e. mean values per trait per clutch). Our mixed models included female ID # as a random effect.

The results above are based on pairwise comparisons, and intercorrelations among variables might produce spuriously significant results (i.e. incubation period might be associated with another trait only because both are correlated with a third trait). To overcome this problem, we conducted multiple regression with incubation period as the dependent variable, and each of the factors above (lay date, hatchling SVL, duration of time in captivity prior to oviposition, mean summer temperature) as independent variables. Variance inflation factors were all < 1.02, indicating no problem with collinearity.

Results

Seasonal reproductive timing vs. subsequent survival of offspring

Although all eggs in this study were incubated at 25 °C, a higher and more stable temperature than would be available in natural nests at Asketunnan, the substantial range in dates of oviposition (overall, 29 May to 18 July; mean duration of egg-laying season per year = 26.2 days) resulted in a similarly broad range of dates of hatching (overall, 17 June to 30 August; mean interval between hatching of first and last clutches within a season = 26.2 days). Recapture data show that offspring from later-laid clutches were less likely to survive to the following year than were offspring from clutches produced earlier in the season [oviposition date vs. proportion of clutch surviving to Year X + 1: $n = 467$ clutches, $r^2 = 0.14$, $F_{1,467} = 29.61$ (estimate = -0.02 , 95% CI = -0.03 to -0.01), $P < 0.0001$; Fig. 1a].

Seasonal reproductive timing and weather vs. incubation periods of eggs

Overall incubation periods ranged from 28 to 50 days (all at 25 °C), with the range in incubation periods within any given year averaging 10.5 days. Consistent with our predictions (see Introduction), mean incubation periods per clutch were shorter for eggs laid later within a season [using Julian day to quantify oviposition date, $n = 419$ clutches, $r^2 = 0.49$, $F_{1,414.6} = 8.85$ (estimate = -0.06 , 95% CI = -0.09 to -0.02), $P < 0.001$; Fig. 1b] and were shorter for eggs laid in years with cooler summers [mean ambient temperature in May–June vs. incubation period: $n = 10$ years, $r^2 = 0.41$ (estimate = 1.37, 95% CI = 0.27 to 2.48), $P < 0.05$; Fig. 1c].

Offspring size vs. incubation periods of eggs

Longer incubation was associated with an increased size of eggs (1987 data only, $n = 150$ eggs, $r^2 = 0.16$, $P < 0.0001$) and hatchlings [$n = 417$ clutches, $r^2 = 0.02$,

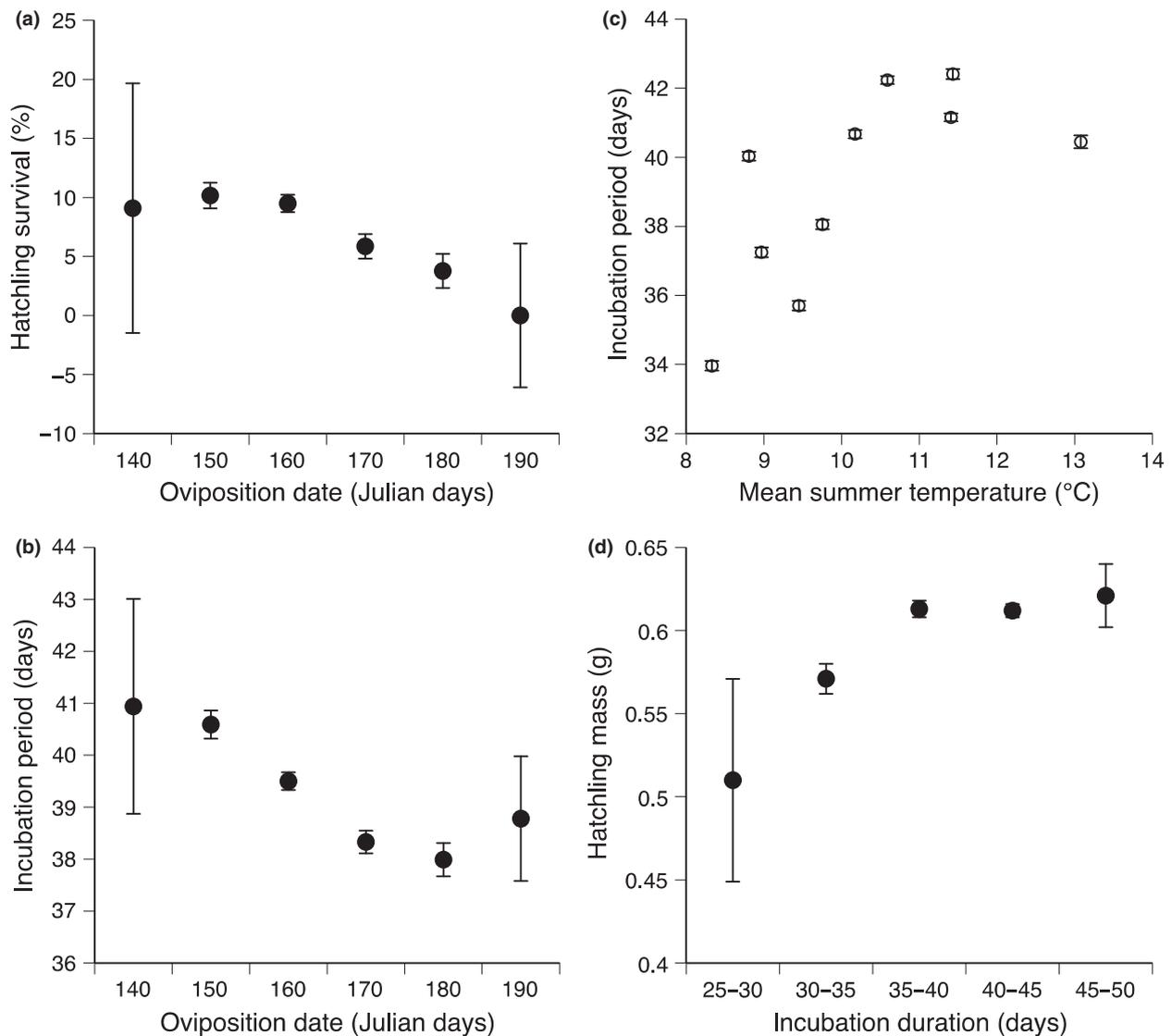


Fig. 1 Seasonal shifts in offspring viability, incubation period and ambient temperatures in sand lizards *Lacerta agilis* from a cool-climate population near the northern limit of the species' range. The top panel (a) shows that offspring produced relatively early in the season are more likely to survive into the second year of life (based on recapture data). The second panel (b) shows that incubation periods (measured in the laboratory at a standard 25 °C) decline for clutches laid later in the season. The third panel (c) shows that incubation periods tend to be longer in warmer summers. The fourth panel (d) shows that larger offspring hatch after longer incubation periods. For simplicity, panel (d) shows 5-day categories of the independent variable. Statistical tests in the text use raw data (as continuous variables rather than categories), but analyses based on these grouped categories are as follows: (a) ANOVA $F_{5,466} = 4.53$, $P < 0.0005$; (b) $F_{5,418} = 176.69$, $P < 0.0001$; (d) $F_{5,418} = 4.58$, $P < 0.0004$. Panels (a), (b) and (d) show mean values and associated standard errors. Sample sizes (left to right): (a) and (b) = 27, 200, 179, 108, 25, 9; (c) = 21, 16, 74, 241, 37, 138, 21; (d) = 2, 43, 136, 211, 27.

$F_{1,414.3} = 11.19$ (estimate = 9.30, 95% CI = 3.83 to 14.76), $P < 0.005$; Fig. 1d].

Season vs. sizes of eggs and offspring

Clutches laid later in the season consisted of smaller eggs (1987 data only, $n = 150$ eggs, $r^2 = 0.89$, $P < 0.0001$; Fig. 2a) and produced smaller hatchlings

[lay date vs. offspring SVL, $n = 546$ clutches, $r^2 = 0.67$, $F_{1,516} = 29.27$ (estimate = -0.02 , 95% CI = -0.03 to -0.01), $P < 0.0001$; Fig. 2b].

Season and weather vs. uterine retention of eggs

In all years of the study, female lizards were brought into the laboratory as soon as we found them visibly

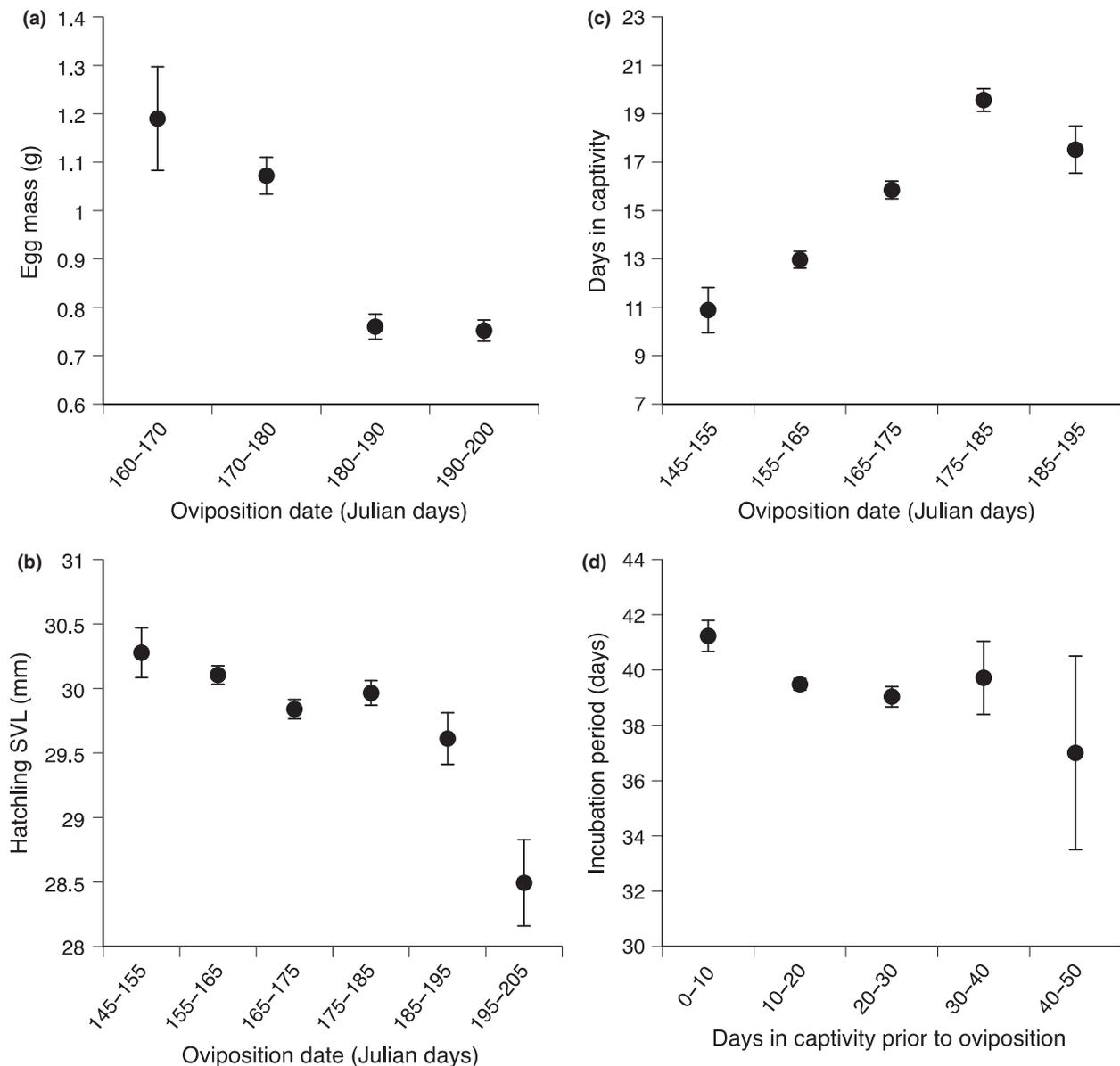


Fig. 2 In a Swedish population of sand lizards *Lacerta agilis*, eggs laid later in the season are smaller (a) and produce smaller hatchlings (b). Females brought into the laboratory later in the season tend to delay laying their eggs (c), and longer delays result in eggs with briefer incubation periods (d). For simplicity, the graphs show 10-day categories of the independent variable. Statistical tests in the text use raw data (as continuous variables rather than categories), but analyses based on these grouped categories are as follows: (a) ANOVA $F_{4,418} = 17.37$, $P < 0.0001$; (b) $F_{5,545} = 6.33$, $P < 0.0001$; (c) $F_{5,546} = 31.82$, $P < 0.0001$; (d) $F_{4,418} = 2.91$, $P < 0.03$. Graphs show mean values and associated standard errors. Sample sizes (left to right): (a), (b) and (c) = 27, 200, 179, 108, 25, 9; (d) = 67, 372, 98, 8, 2.

distended, and could palpate the developing eggs. However, the duration of time they were held in captivity before laying those eggs increased over the course of the season [$n = 546$ clutches, $r^2 = 0.32$, $F_{1,542.1} = 109.70$ (estimate = 0.23, 95% CI = 0.19 to 0.27), $P < 0.0001$; Fig. 2c], consistent with more prolonged uterine retention of late-season clutches. An increased duration of time in captivity prior to

oviposition was associated with a reduction in incubation period [$n = 418$ clutches, $r^2 = 0.04$, $F_{1,405.5} = 21.61$ (estimate = -0.14 , 95% CI = -0.20 to -0.08), $P < 0.0001$; Fig. 2d]. Broadly, an increase of 5 days in uterine retention was associated with a decrease of about 1 day in subsequent incubation period (Fig. 2d). We found no significant relationship between annual weather variation and either hatchling mass

[$n = 15$ years, $r^2 = 0.02$, $F_{1,12.9} = 0.68$ (estimate = 0.003, 95% CI = -0.005 to -0.02), $P = 0.012$] or the duration of captivity prior to oviposition [$n = 15$ years, $r^2 = 0.16$, $F_{1,14.8} = 1.68$ (estimate = 0.42, 95% CI = -0.27 to 1.12), $P = 0.14$].

Multivariate analysis

This procedure showed independent effects of all of the variables considered, even after the effects of the other variables had been factored out of the analysis [$n = 417$ clutches, overall $r^2 = 0.60$; effect of lay date $F_{1,413} = 4.98$, $P < 0.03$ (estimate = -0.04 , 95% CI = -0.08 to -0.005); effect of summer ambient temperature $F_{1,413} = 282.92$, $P < 0.0001$ (estimate = 1.70, 1.50 to 1.90); effect of days in captivity prior to oviposition $F_{1,413} = 15.66$, $P < 0.0001$ (estimate = -0.11 , -0.17 to -0.06); effect of hatchling mass $F_{1,413} = 6.99$, $P < 0.009$ (estimate = 5.52, 1.42 to 9.62)].

Discussion

In a population close to the northern (cold-climate) range limit of the species, oviparous sand lizards modify the incubation periods of their eggs in ways that enable their eggs to hatch before the onset of winter. Eggs laid late in the season, or during unusually cool summers, hatch after a shorter period at 25 °C than do eggs laid earlier in the season, or during warmer summers. By incubating eggs in the laboratory at a standard temperature, we eliminated a major source of variation in incubation periods in natural nests. In the field, the eggs laid late in the season and in cool summers may incubate for longer, not shorter, periods than do those laid in warmer conditions, because low nest temperatures slow embryogenesis (Rykena, 1987). In short, our data strongly support the hypothesis (Rykena, 1987; Roitberg *et al.*, 2015) that the northern range limits for *Lacerta agilis* in Europe are determined by the need for a prolonged period of warm soil temperatures for egg incubation, and hence, that populations close to that northern limit are under strong selection to reduce the length of time between oviposition and hatching. Studies on populations of wall lizards (*Podarcus muralis*) introduced to the UK from warmer European sites also strongly support this model: lizards from the non-native populations produce smaller eggs, with more advanced embryos at laying, and that develop faster at low temperatures (While *et al.*, 2015).

The earlier analyses on *Lacerta agilis* (Rykena, 1987; Roitberg *et al.*, 2015) were based on correlated geographic variation in climate, mean incubation periods and hatchling sizes. Inevitably, such comparisons are confounded by many other factors, such as differing body sizes and phylogenetic histories of the populations involved (Roitberg *et al.*, 2015). By examining patterns through time (within seasons, and among years) within

a single population, we eliminated many potentially confounding factors – but still saw the same correlation between availability of incubation conditions and reproductive traits of our lizards. Small hatchling size and rapid incubation thus seem likely to be adaptive responses to the thermal challenges that oviparous reptiles encounter in cold climates.

Our data also clarify the nature of selective pressures that have generated this seasonal shift. In keeping with the hypothesis of Roitberg *et al.* (2015), late-season hatching reduced the probability of offspring survival. The same constraint may apply broadly to oviparous reptiles in cool climates (Sergeev, 1940; Shine, 1985). Indeed, the seasonal timing of hatching may influence progeny fitness even in the absence of thermal constraints (e.g. Brown & Shine, 2006; Warner & Shine, 2007). In other species or populations, the seasonally variable selective force on offspring size may involve biotic factors such as food availability or predation risk, potentially creating complex patterns in optimal offspring sizes across the breeding season (Landa, 1992). In some warm-climate lizards, for example, offspring from late-season clutches are larger not smaller (the reverse of the pattern seen in *L. agilis*), putatively because those later hatchlings must overcome the challenges of lower food supply and higher predation risk (Nussbaum, 1981; DeMarco, 1989; Sinervo & Doughty, 1996). More generally, the seasonal timing of oviposition (and thus hatching) may be a critical dimension of life-history variation within squamate reptiles (Shine, 2003; Warner & Shine, 2007; Wapstra *et al.*, 2010; Le Henanff *et al.*, 2013). Because seasonal timing of oviposition may correlate with other important factors (e.g. maternal body size or condition: Ljungström *et al.*, 2015), associations between offspring fitness and seasonal timing of hatching also may reflect additional causal influences.

The reduction in incubation periods of eggs from late-season clutches (compared to those from early-season clutches) of our sand lizards appears to be achieved by multiple proximate mechanisms. As well as reducing egg sizes, females retained eggs *in utero* for longer before laying, as evidenced by the duration of time in captivity between capture and oviposition. This pattern fits well with reports of prolonged uterine retention of eggs in other species of oviparous squamates in cool climates (e.g. Neill, 1964; Huey, 1977; Mathies & Andrews, 1995; Smith & Shine, 1997; Telemeco *et al.*, 2010). That effect may be due at least partly to phenotypic plasticity in response to low ambient temperatures, as it is in the skink *Acritoscincus [Bassiana] duperreyi* (Telemeco *et al.*, 2010). A role for plasticity is suggested by the observation that incubation periods were reduced following cooler weather in summer. However, adaptation may also fashion canalized responses; for example, eggs of cool-climate populations of *Lacerta agilis* hatch after shorter incubation periods

than do those of warm-climate conspecifics, even at the same egg size (Rykena, 1987). The mechanism remains unknown, but recent experimental studies on turtles show that maternal allocation of thyroid hormones in egg yolk can accelerate development (and thus, hasten hatching) without any major impact on hatchling phenotypes (McGlashan *et al.*, 2017).

Intriguingly, the relationship between duration of time in captivity and incubation period was far from one-to-one; an increase of 5 days in uterine retention was associated with a decrease of only about 1 day in subsequent incubation period (Fig. 2d). At first sight, this result suggests that the date of hatching would be delayed rather than brought forward by prolonged uterine retention of eggs. However, eggs in natural nests would develop at lower temperatures than our standard laboratory conditions (25 °C), extending the duration of extra development post-oviposition. Additionally, retention of eggs at higher maternal temperatures prior to oviposition may enhance the viability of the offspring that eventually hatch from those eggs (Shine, 2006).

Our multivariate analysis suggested that an additional factor may also be involved. The duration of incubation was significantly affected by oviposition date even after the effects of hatchling size, uterine retention and summer weather had been removed statistically. What additional factor might reduce incubation period for late-season eggs? In previous laboratory-based work, we showed that incubation periods are affected by paternal genotype within *L. agilis*; even within a single clutch, eggs fathered by a male from a warmer-climate population exhibited more prolonged incubation, and completed more embryonic development prior to hatching, than did eggs fathered by males from the local (Asketunnan) population (Olsson *et al.*, 1996; Shine & Olsson, 2003). The longer incubation period of early-season eggs might be achieved by a similar mechanism (a shift towards relatively more precocial offspring), independent of paternity, although we have no way to test this hypothesis. In summary, then, the incubation periods of *L. agilis* eggs at the coldest edge of their geographic distribution may be reduced by several processes: a reduction in egg size, an acceleration of embryonic developmental rates, a prolongation of uterine retention at high maternal body temperatures and a shift towards hatching at an earlier stage of development. Data on the degree of embryogenesis at oviposition, and of offspring performance at hatching, might clarify the nature of those changes.

Speculations on the evolution of squamate viviparity in cool climates have focused on the role of uterine retention of eggs in shortening incubation periods and hence, enabling embryonic development to occur within the brief seasonal window of sufficiently warm incubation temperatures in the soil (e.g. Sergeev, 1940; Shine, 1985). The data from sand lizards support and extend that model, by showing that egg layers in cool

climates can shorten the period of incubation required by reducing egg size, accelerating developmental rate and (potentially) by hatching at an earlier stage of development. That conclusion suggests three new perspectives on the evolution of reptilian viviparity:

- 1 First, those same shifts in egg size (and perhaps incubation period relative to egg mass, and stage of development at hatching) likely occurred in numerous lineages of oviparous squamates as they extended into cooler regions, or as climates cooled in their existing geographic range
- 2 Second, prolonged uterine retention of eggs (the intermediate step towards viviparity) may have conferred a selective advantage seasonally, by extending the range of ovulation dates that could result in viable offspring. Other phenotypic traits are correlated with earlier vs. later-season reproduction in squamates; notably, it is often the largest (oldest) females that are first to breed (Olsson & Shine, 1997a,b). Hence, selection for prolongation of uterine retention of eggs may have been more intense on some age and/or size classes within the population, with a transition towards viviparity favoured in some but not all females.
- 3 Third, the transition towards viviparity (increasing proportion of embryogenesis completed prior to oviposition) may have conferred an additional advantage: it allowed reproducing females to escape the need to reduce offspring size in late-season clutches. Under this interpretation, viviparity is advantageous because it allows a female to produce a larger offspring than would be possible if incubation occurred outside the maternal body, at lower temperatures.

Adjustment of incubation durations in response to local conditions is likely to be widespread, because fundamental aspects of our sand lizard system are seen among other types of organisms. For example, seasonal variation in offspring sizes has been reported in diverse groups (flowering plants, isopods, cladocerans, insects, fish, amphibians, reptiles, birds: see Landa, 1992; Härkönen *et al.*, 2013). Similarly, incubation periods often vary both within and among species (e.g. Ricklefs & Smeraski, 1983; Martin *et al.*, 2007), although patterns are complex and much of the variation remains unexplained (Tieleman *et al.*, 2004). A trend for larger eggs to take longer to incubate (as seen in our sand lizards) is common (e.g. for birds: Henderson, 1950; for lizards: Sinervo, 1990; but see Runde & Barrett, 1981), although the exact nature of the relationship between egg mass and incubation period varies among phylogenetic lineages of birds and reptiles (Deeming *et al.*, 2006). Relationships among incubation temperature, incubation period and offspring phenotype are widespread but again, differ phylogenetically (e.g. Qualls & Andrews, 1999; Hepp *et al.*, 2006). Seasonal shifts in

incubation duration have been reported across a broad range of taxa (e.g. seabirds: MacRoberts & MacRoberts, 1972; sea turtles: Matsuzawa *et al.*, 2002). We might thus expect to see other lineages in which cool-climate populations have evolved shorter incubation periods. In keeping with that prediction, the Japanese cricket (*Teleogryllus* sp.) exhibits briefer incubation at more northern latitudes (Masaki, 1965), as do many birds (Robinson *et al.*, 2008; but see Geffen & Yom-Tov, 2000). A seasonal reduction in egg size may contribute to that seasonal decline in incubation period in sea birds, as in our lizards (Parsons, 1972; Massaro *et al.*, 2004). Adaptive shifts in egg size might occur rapidly, as has occurred in non-native populations of the European wall lizard translocated to cooler regions within the last century (While *et al.*, 2015). Nonetheless, ambient nest conditions are not the only influence on incubation periods; for example, variation in incubation periods also may function to increase synchrony of hatching within a clutch, with no consistent seasonal decrease in egg size (MacCluskie *et al.*, 1997).

Our data suggest that as an oviparous squamate species extends its range into cooler climates, it experiences a suite of selective forces. Maternal fitness can be enhanced not only by judicious selection of warm nest-sites, but also by prolonged uterine retention of developing eggs, by bringing forward the seasonal timing of ovulation, by reducing egg size, by accelerating developmental rate for any given size of egg and by reducing the stage of development at hatching. Shifts in all of these traits can enable hatching to occur sooner after oviposition than would otherwise be possible. Hence, selection on that duration likely favoured concurrent shifts in all of those traits. From this perspective, the oviparity–viviparity continuum in reproductive modes (Shine, 1983) may be multidimensional and season-specific, rather than a simple linear transition based on incremental increases in the duration of uterine retention of eggs.

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