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

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

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Abstract
Because squamate embryos require weeks of high temperature to complete development, 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 penetrate into 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 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.

Key words. – life-history evolution, reproductive mode, seasonality, thermal biology

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 and Stewart 2011; Stewart and Blackburn 2014). 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 and Andrews 1999; Amiel and Shine 2012). 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 and Gibbons 1977; Shine and 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 and Bull 1979). For example, viviparity may benefit an 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 and Bull 1979).

The reverse exception to the correlation between cold climates and viviparity involves the success of a small 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? Studies have identified 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 (Mathies and Andrews 1995; Shine et al. 1997). Second, the oviparous taxon can evolve accelerated developmental rates (reduced incubation periods), especially at low temperatures (Shine 1999). 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 and Bull 1979 and 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). In keeping with that interpretation, experimental reduction in lizard egg size reduces incubation period (Sinervo 1990; Sinervo and 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 is based on studies of 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). Based on that 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, the same 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, 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 longterm field studies on a population of *Lacerta agilis* near the northern limit of the species’ distribution provide the data with which to test these predictions.

**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 (N57°22, E11°59’), is close to the northernmost edge. We have never found natural nests of this species at our study site, but during the period when sand lizard eggs are incubating (May to **), mean soil temperatures average around **°C (**SHOULD BE ABLE TO FIND SOME WEB SITE WITH THESE DATA? – PROBABLY SIMILAR TO OCEAN TEMPERATURES**). At this site females produce only a single clutch per year. Clutches produced earlier in the season contain larger offspring than do late-season clutches (Olsson and Shine 1997a,b; Ljungstrom 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 X 600 X 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 (AS CLUTCHES??) in moist vermiculite (one volume water in 10 volumes wevrmiculite) incubators maintained at 25°C. Zakharov, V.M. (1989). *Future prospects for population phenogenetics. Sov. Sci. Rev. F. Physiol. Gen. Biol.*, 4, 1-79. Egg mass immediately post-oviposition was recorded in only the first year of the study (1987), but hatchling traits were measured in all years. When daily checks revealed hatching lizards, these were processed (measured, weighed, marked) in the same way as their parents, and then released at the field site. We obtained data on ambient temperatures from **. Our earlier papers on this study provide extensive additional detail on methods (Olsson and Shine 1997a,b; Ljungstrom et al. 2015, 2016).

**Statistical analyses.** – Using JMP Pro 11 (SAS **), we checked assumptions of normality and variance homogeneity prior to analysis. For data gathered in 1987 (when we recorded egg mass post-oviposition), we report tests based on individual eggs. For comparisons with weather conditions, we used annual mean
values. For all other analyses, we used each clutch as the unit of replication (i.e., mean values per trait per clutch).

**Results**

**Effect of seasonal reproductive timing on 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 = 23.9 days, range in annual means 13 to 36 days) resulted in a similarly broad range of dates of hatching (overall, **June to ** August; mean spread per year = ** days, range in annual means ** to ** 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, \( r^2 = 0.14 \), \( P < 0.0001 \); FIG 1a).

**Effect of seasonal reproductive timing and weather on 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 (range from of 6 to 22 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, \( r^2 = 0.69 \), \( P < 0.0001 \); Fig. 1b) and were shorter for eggs laid in years with cooler summers (year effect \( F_{9,418} = 3435.08 \), \( P < 0.0001 \); mean ambient temperature in May-June vs incubation period: n = 10 years, \( r^2 = 0.41 \), \( P < 0.05 \); Fig. 1c).

**Effect of season and offspring size on 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, \( r^2 = 0.02 \), \( P < 0.005 \); Fig 1d).

**Effect of season on sizes of eggs and offspring.** Clutches laid later in the season consisted of smaller eggs (1987 data only, n = 150 eggs, \( r^2 = 0.20 \), \( P < 0.0001 \); Fig. 2a) and produced smaller hatchlings (lay date vs offspring SVL, n = 546 clutches, \( r^2 = 0.03 \), \( P < 0.0001 \); Fig. 2b).

**Effect of season and weather on uterine retention of eggs.** Female lizards were brought into the laboratory as soon as we found them visibly 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 = 547 clutches, \( r^2 = 0.18 \), \( P < 0.0001 \); 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, \( r^2 = 0.04 \), \( P < 0.0001 \); Fig. 2d). Broadly, an increase of five days in uterine retention was associated with a decrease of about one day in subsequent incubation period (Fig. 2d). We saw no significant relationship between annual weather variation and either hatchling mass (n = 15 years, \( r^2 = \)
0.02, \( P = 0.64 \) or the duration of captivity prior to oviposition \( (n = 15 \text{ years}, r^2 = 0.16, P = 0.14) \).

**Multivariate analysis.** – The results above are based on pairwise comparisons, and intercorrelations among variables might produce spuriously significant results (i.e., trait A might be associated with trait B only because both are correlated with trait C). 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. 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 \( (n = 417 \text{ clutches}, \text{overall } r^2 = 0.72; \text{effect of lay date } F_{1,411.4} = 13.05, P < 0.0003; \text{effect of summer ambient temperature } F_{1,8.12} = 9.73, P < 0.015; \text{effect of days in captivity prior to oviposition } F_{1,406.6} = 26.94, P < 0.0001; \text{effect of hatchling SVL } F_{1,404.9} = 4.26, P < 0.04) \).

**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. The earlier analyses (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 and Shine 2006; Warner and 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 and 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 and Shine 2007).

Intriguingly, 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 not only with reports of prolonged uterine retention of eggs in other species of oviparous squamates in cool climates (e.g., Neill 1964 for Opheodrys; Huey 1977 for Anolis; Telemeico et al. 2010 for Bassiana). That effect may be due at least partly to phenotypic plasticity in response to low ambient temperatures, as it is in the skink Bassiana duperreyi (Telemeico et al. 210). A role for plasticity is suggested by the observation that incubation periods were reduced following cooler weather in summer. However, adaptation may also fashion canalised 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 five days in uterine retention was associated with a decrease of only about one 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 1986).

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 and Olsson 2003). The same might be true of late-season eggs, 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 versus later-season reproduction in squamates; notably, it is often the largest (oldest) females that are first to breed (Olsson and 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.

Facultative adjustment of incubation durations in response to local conditions is likely to be widespread in many types of animals, 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; Harkonen et al. 2013). Similarly, incubation periods often vary both within and among species (e.g., Ricklefs and 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 widespread (e.g., Henderson 1950 for birds; Sinervo 1990 for lizards; but see Runde and 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 and Andrews 1999; Hepp et al. 2006). Similarly, seasonal shifts in incubation duration have been reported across a broad range of taxa (e.g., seabirds – MacRoberts and 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 and 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). Nonetheless, the pattern is certainly not universal: 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 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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**Literature Cited**


Captions to Figures

Figure 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, the graphs show 5-day categories of the independent variable. Statistical tests in the text use raw data, but analyses based on these grouped categories are as follows: Fig. 1a – ANOVA $F_{5,466} = 4.53$, $P < 0.0005$; Fig. 1b - $F_{5,418} = 176.69$, $P < 0.0001$; Fig. 1d - $F_{5,418} = 4.58$, $P < 0.0004$). Graphs a, b, and d show mean values and associated standard errors.

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