UNIVERSIDAD DE OVIEDO

Departamento de Biología de Organismos y Sistemas

Programa de Doctorado: “Biología aplicada a la sostenibilidad de recursos naturales (Mención de Calidad)”

“Evolución de la retención de la puesta y transición al viviparismo en la lagartija de turbera, *Zootoca vivipara*”

TESIS DOCTORAL

Tania Rodríguez Díaz

Oviedo 2012

Financiación

Esta Tesis Doctoral ha sido financiada gracias al proyecto del Plan Nacional de I+D con referencia MEC-07-CGL2007-60187 y a una beca de Formación de Profesorado Universitario (Ref. AP2005-4296) del Ministerio de Educación y Ciencia y el Fondo Social Europeo. La investigación presentada en esta Tesis ha sido realizada en el Área de Zoología del Departamento de Biología de Organismos y Sistemas de la Universidad de Oviedo. Una parte de este estudio se realizó gracias a la disponibilidad de la Unidad de Microscopía Electrónica.

MINISTERIO
DE EDUCACIÓN
Y CIENCIA

FONDO
SOCIAL
EUROPEO

UNIVERSIDAD DE OVIEDO

Agradecimientos

Siempre había pensado que al final del recorrido no habría mucha gente a la que agradecer haber llegado a este punto porque 1) en la sala de becarios somos "cuatro gatos"; 2) no he tenido el gusto de colaborar con una gran cantidad de personas; 3) mi familia y la mayor parte de mis amigos se encuentran allende los mares. Por supuesto, me equivocaba. Es mucha la gente que ha contribuido a que el desarrollo de esta Tesis fuese más llevadero.

Quiero dar las gracias:

A Braña, mi director, por haber confiado en mí y haberme guiado hasta la meta armado con su sabiduría, experiencia, entusiasmo, paciencia y también alguna historieta. A pesar de que en el desarrollo de todo proyecto de tesis debe llegar un punto en que la relación doctorando-director se hace insoportable (Braña, comunicación personal), parece que no nos ha ido tan mal… probablemente porque no he practicado lo suficiente delante del espejo.

A Sara, mi "compañera de lagartijas" y futura compañera de torturas cuando los extraterrestres den con nosotras… ¡Mucho ánimo!

A Félix, por capturar lagartijas en pro de este Proyecto, desde Galicia a Pirineos, y por tus valiosos consejos para aprender a lidiar tanto con las lagartijas como con el jefe.

A Melissa, por quitarme unas cuantas cáscaras de encima.

A los doctores Xiang Ji y Hong Li ("mi chino"), por... dar un toque internacional a nuestro pequeño grupo.

A los doctores Manuel Massot y Jean Clobert, por hacer, probablemente de manera involuntaria, que yo creciera un poco más.

A todas las lagartijas que han pasado por nuestras manos, por permitir, éstas sí, de manera totalmente involuntaria, que *Zootoca vivipara* llegara a ser nuestro organismo modelo.

Al resto de fauna que ocupa o ha ocupado los distintos cubículos del área de Zoología, por hacer más amenas las horas de trabajo, de una manera u otra: el Dr. Outomuro (*lycka till!*), el Dr. Torralba, Saúl y Livingston, María, Izaskun, Andrés, Tania, Höfer, Fernando, Rebeca y Lorena. Tampoco olvido a nuestras mascotas: Braulio, "la gorda" y el puñado de fásmidos.

A Norma, porque nuestros encuentros en los pasillos siempre acaban con una palabra de ánimo.

A Antón, entre otras muchas cosas, por compartir conmigo tantos ratos usualmente entretenidos y brindarme la oportunidad de observar *dráculas*...

A Parrondo, siempre dispuesto a arrojar algo de luz sobre el asunto.

A Bea, por tu paciencia y apoyo; a las otras del *pack*, Isa y Daniela; y al resto de mis compañeros en la Facultad lagunera: Cande, Mónica, Ana, Canarita, Antonio, Miriam, Saday, Acorán, Sergio... por todas las risas que nos hemos echado juntos. ¡Ojalá vengan muchas más!

A mis profesores Pepe y Estrella, que despertaron en mí la curiosidad por la Biología.

A Che, por compartir conmigo tus pequeñas batallas diarias, a Nuria (y su doctorado paralelo), a Mina (mi Laura), por todos los ánimos que me mandas, y a Inma, por llenarme de alegría con la sonrisa de la pequeña Lucía.

A la familia que está, aunque lejos: Fermín, Juany, Rayco, Angélica. Afortunadamente los ánimos no hay que mandarlos por Correos... Y sigo: Armando, Sary, Paqui, Luzma, Solange, Carmelo, Virginia, Miguel, Pepe Juan, Eutimio y Vicenta. Y a la familia que ya no está: Inma, Lela, Mami.

Y a Javi, indudablemente mi mejor compañero de viajes (de todo tipo), que me atrajo a la tierra asturiana... GRACIAS.

A Fermín y Juany, mis padres

ÍNDICE

I. Introducción general

Una de las características esenciales en la definición del ciclo de vida de los animales es el modo de reproducción (Roff, 1992), que puede ser notablemente diverso (Blackburn, 1999a). Tanto entre los invertebrados como entre los vertebrados, son dos las modalidades extremas, de las que ya hablaba Aristóteles en su *Historia Animalium*: oviparismo y viviparismo. El cambio evolutivo entre estos modos de reproducción ha sido objeto de interés (Tinkle y Gibbons, 1977; Wourms y Callard, 1992; Buckley *et al.*, 2007; Touchon y Warkentin, 2008), sobre todo en aquellos grupos con una gran diversidad de estrategias reproductoras, como son los peces condrictios (Wourms *et al.*, 1988) y los reptiles escamosos (Andrews y Mathies, 2000).

La mayor parte de las especies animales ponen huevos con embriones que completan su desarrollo en el ambiente externo al cuerpo de la hembra alimentándose del vitelo, es decir, presentan oviparismo lecitotrófico, aunque muestran diferente nivel de desarrollo en el huevo en el momento de la puesta (Wourms *et al.*, 1988): ovuliparismo (huevos no fecundados), zigoparismo (huevos que contienen cigotos) y embrioparismo (huevos con embriones que pueden alcanzar estadios de desarrollo muy avanzados antes del momento de la puesta). El oviparismo es el único modo de reproducción en algunos taxones (como aves y tortugas, entre los vertebrados), mientras que en otros existen también especies vivíparas, con hembras que retienen los embriones en el interior de su cuerpo durante todo el periodo de desarrollo. Aunque el viviparismo existe en muchos grupos de invertebrados y en cinco de las seis grandes clases de vertebrados (condrictios, osteictios, anfibios, reptiles y mamíferos; la excepción son las aves: Blackburn y Evans, 1986), la transición evolutiva desde el oviparismo hacia el viviparismo ha ocurrido con mayor frecuencia en los reptiles del orden Squamata (lagartos y serpientes), de manera que el modo de reproducción vivíparo ha evolucionado en este grupo más de 100 veces de manera independiente y solo 34 veces en el resto de grupos de vertebrados (Blackburn, 1999b). Además, muchas de estas transiciones evolutivas se han producido en tiempos geológicos recientes y a nivel de género y especie (Blackburn, 1982, 1985, 2006; Shine, 1985), características que convierten a los escamosos en un grupo idóneo para estudiar la evolución del viviparismo.

En los reptiles Squamata, el oviparismo es la condición plesiomórfica y se considera que el viviparismo es virtualmente irreversible (Lee y Shine, 1998; Blackburn, 1999b; ver, sin embargo, De Fraipont *et al.*, 1996; Surget-Groba *et al.*, 2006) y que el proceso de transición hacia el viviparismo es gradual (Packard *et al.*, 1977; Tinkle y Gibbons, 1977; Shine, 1983). Esta condición gradual supone que el viviparismo evoluciona desde el oviparismo con retención hasta completar la embriogénesis en los oviductos maternos mediante la selección de tiempos crecientes de retención de la puesta, lo que implicaría un incremento progresivo en el nivel de desarrollo embrionario intrauterino. Aunque algunos estudios sugieren reversiones desde el viviparismo hacia el oviparismo (De Fraipont *et al.*, 1996; Surget-Groba *et al.*, 2006), estos trabajos no son concluyentes, ya que incurren en importantes errores metodológicos (Blackburn, 1999b; Shine y Lee, 1999).

La distribución de los estadios de desarrollo alcanzados en el momento de la puesta entre los Squamata actuales es bimodal y asimétrica (Blackburn, 1995; Andrews y Mathies, 2000). Por un lado, aunque algunas especies realizan la puesta poco después de la ovulación (e. g., *Chamaeleo chamaeleon*: Andrews *et al.*, 2008), la mayor parte de los reptiles ovíparos retienen los huevos en el oviducto hasta alcanzar un nivel de desarrollo próximo al estadio 30 de la escala de estadios de desarrollo de Dufaure y Hubert (1961), en la que el estadio 1 coincide con el inicio de la segmentación y el estadio 40 con embriones totalmente desarrollados en el momento de la eclosión. Por otro lado, alrededor del 20% de los reptiles escamosos son vivíparos, es decir, paren crías en el estadio 40 (Andrews y Mathies, 2000). Además, aunque no muy numerosas entre los escamosos actuales, unas pocas especies ponen huevos en fases intermedias de desarrollo (estadios 33-39) y algunas de esas especies son incluso capaces de retener los huevos más allá del tiempo normal de puesta hasta alcanzar estadios de desarrollo cercanos al 40 en respuesta a condiciones de sequía (*Sceloporus scalaris*, Mathies y Andrews, 1996). Entre las especies que alcanzan los estadios 33-40 en el momento de la puesta se encuentran los tres únicos amniotas conocidos con bimodalidad reproductora intraespecífica (e interpoblacional). Son especies en las que existen unas poblaciones vivíparas y otras ovíparas, cada una de ellas con un modo de reproducción característico e invariable, y en las poblaciones ovíparas de este tipo de especies también se alcanzan estadios de desarrollo avanzados en el momento de la puesta. Estos organismos con bimodalidad reproductora son modelos ideales para probar las hipótesis de la evolución del viviparismo porque al comparar las formas ovíparas frente a las vivíparas conespecíficas se minimizan las diferencias filogenéticas que podrían conducir a errores de interpretación, ya que tienen un ancestro común reciente. Son el lacértido *Zootoca vivipara* (Braña, 1986) y los escíncidos *Lerista bougainvillii* (Qualls *et al.*, 1995) y *Saiphos equalis* (Smith y Shine, 1997). Algunas poblaciones ovíparas de *L. bougainvillii* sobrepasan el estadio 34 en el momento de la puesta y en *S. equalis* se llega incluso al estadio 39. Los estadios de desarrollo embrionario más frecuentes en el momento de la puesta en las poblaciones ovíparas de *Z. vivipara* son los estadios 32-34 y se han obtenido de manera experimental híbridos entre poblaciones ovíparas y vivíparas que alcanzan los estadios 35-36 en el momento de la puesta (Arrayago *et al.*, 1996). Además, recientemente se ha encontrado una zona de contacto entre poblaciones ovíparas y vivíparas de esta especie donde se han descrito posibles híbridos naturales que alcanzan el estadio 35.5 (Lindtke *et al.*, 2010). El hecho de que estas especies sean capaces de alcanzar estadios avanzados de desarrollo embrionario antes de la puesta apoyaría la idea de que el viviparismo se ha originado desde el oviparismo con retención a través de la selección de tiempos crecientes de desarrollo intrauterino, puesto que evidencian la existencia de una fase de retención prolongada de los huevos, necesaria para que la transición hacia el viviparismo sea gradual.

Los avances progresivos del desarrollo embrionario intrauterino deberían llevar consigo una reducción paralela del grosor o grado de calcificación de la cáscara del huevo y un aumento de la vascularización de las membranas extraembrionarias y del oviducto materno para permitir un adecuado intercambio gaseoso *in utero* cuando el embrión ha adquirido un cierto tamaño y aumenta su demanda de oxígeno (Andrews, 2002; Parker y Andrews, 2006). La cáscara del huevo de la mayor parte de los escamosos está compuesta por una fina capa inorgánica externa formada por carbonato cálcico en forma de calcita (Fig. 1.1A), que es una fuente importante de calcio durante la embriogénesis (Stewart y Ecay, 2010), y una capa interna orgánica bien desarrollada formada por múltiples fibras (Fig. 1.1B; Thompson y Speake, 2004). Se ha determinado que el grosor de la cáscara del huevo en las formas ovíparas de *L. bougainvillii* (Qualls, 1996) y de los híbridos ovíparos x vivíparos de *Z. vivipara* (Heulin *et al.*, 1992), con retención prolongada de la puesta, es intermedio entre el de las típicas cáscaras ovíparas (gruesas y muy calcificadas) y el de las membranas que recubren al embrión en las formas vivíparas (muy finas y poco calcificadas), lo cual apoyaría la idea de que la reducción del grosor de la cáscara y los aumentos en la duración de la retención de los huevos ocurren simultáneamente.

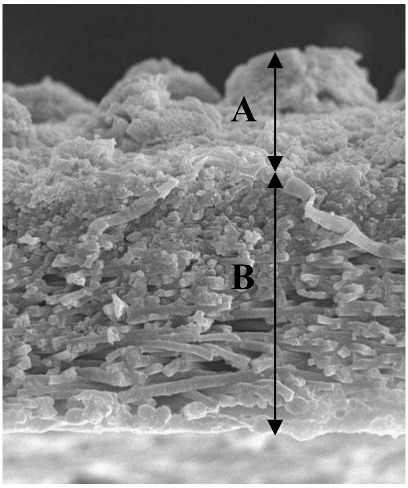


Figura 1.1. Corte transversal de una cáscara de huevo correspondiente a una población ovípara de lagartija de turbera, *Zootoca vivipara*. Desde el exterior hacia el interior, **A:** capa inorgánica de calcita; **B:** capa orgánica formada por múltiples fibrillas.

Principales hipótesis sobre la evolución del viviparismo en reptiles

Existen varias hipótesis para explicar las ventajas adaptativas de prolongar el desarrollo intrauterino en el proceso de evolución del viviparismo y suelen estar relacionadas con factores que afectan a la supervivencia de los huevos o con factores que afectan a la madre (Shine y Bull, 1979). Se ha sugerido, por ejemplo, que este proceso ha sido favorecido por una inadecuada disponibilidad de agua en el sustrato para sustentar la embriogénesis hasta la eclosión o por la especialización de determinadas especies en modos de existencia arbóreos o acuáticos, en los que la falta de lugares adecuados para nidificar podría favorecer el modo de reproducción vivíparo (Packard *et al.*, 1977; Shine, 1985). Sin embargo, son dos las hipótesis más ampliamente aceptadas, ambas relacionadas con la temperatura durante la fase de desarrollo embrionario, factor ambiental con importantes efectos sobre los organismos ectotermos: la hipótesis de “protección ambiental” o de “clima frío” (Tinkle y Gibbons, 1977; Shine, 1985, 2002a; Hodges, 2004) y la hipótesis de “manipulación materna” o de “optimización térmica” de las etapas iniciales de la embriogénesis (Shine, 1995; Webb *et al.*, 2006; Braña y Ji, 2007; Li *et al.*, 2009).

Los reptiles diurnos tienen una gran dependencia de la temperatura, pues este factor afecta a múltiples procesos fisiológicos, como pueden ser los relacionados con la captación de energía destinada a sustentar el crecimiento y la reproducción (Adolph y Porter, 1993, 1996), o el rendimiento en funciones con incidencia inmediata en la supervivencia, como la locomoción (Van Damme *et al.*, 1991). Estos organismos son capaces de controlar su temperatura corporal con bastante precisión gracias a la termorregulación etológica, moviéndose de zonas sombreadas a zonas soleadas o cambiando su orientación con respecto al sol (Bauwens *et al.*, 1990), o mediante mecanismos fisiológicos, produciendo cambios en el flujo sanguíneo y la tasa metabólica, que influyen en las tasas de calentamiento y enfriamiento corporal (Grigg y Seebacher, 1999). La temperatura que experimentan durante el desarrollo embrionario también tiene importantes efectos, ya sea la temperatura a la que transcurre la gestación (Beuchat, 1988; Wapstra, 2000) o la incubación externa (Elphick y Shine, 1998; Qualls y Andrews, 1999a; Braña y Ji, 2007), pues influye en la supervivencia de los embriones (temperaturas muy bajas o muy altas pueden matarles directamente), en la tasa de desarrollo embrionario o en el fenotipo de las crías, de manera que la elección del nido o la termorregulación materna son objeto de fuertes presiones selectivas (Shine y Harlow, 1993; Angilletta *et al.*, 2009; Wapstra *et al.*, 2010).

La hipótesis de “clima frío” sobre la evolución del viviparismo y, por tanto, de los niveles crecientes de desarrollo embrionario intrauterino, sugiere que la retención de la puesta tiene como ventaja la protección de los embriones frente a los riesgos tanto bióticos como abióticos que éstos afrontan en la incubación externa, es decir, en los nidos, y vincula la evolución del viviparismo a altas latitudes o altitudes, caracterizadas por climas fríos. En estas zonas frías los embriones experimentarían temperaturas más altas durante la incubación en los oviductos maternos que en los nidos gracias a la capacidad termorreguladora de la hembra, favoreciendo así la aceleración del desarrollo embrionario. Las bajas temperaturas en los nidos hacen que el desarrollo embrionario progrese más lentamente provocando un retraso del momento de la eclosión, lo cual influiría negativamente en la eficacia biológica de las crías porque éstas tendrían menos tiempo para crecer y almacenar reservas antes del inicio del invierno (Shine y Bull 1979; Warner y Shine, 2007; Wapstra *et al.*, 2010). Además, pueden dañar o matar a los embriones directamente, o pueden afectar negativamente al fenotipo de las crías (Qualls y Andrews, 1999a). También se ha propuesto que, sobre todo en zonas próximas al límite altitudinal superior de la distribución de reptiles ovíparos, la diferencia en la variación térmica diaria entre los nidos y los oviductos maternos, más que la diferencia en la temperatura media, podría favorecer la retención prolongada de la puesta (Shine, 2004). Un hecho que apoya la hipótesis de clima frío sobre la evolución del viviparismo es que la proporción de especies ovíparas de reptiles escamosos disminuye al aumentar la latitud y la altitud (Tinkle y Gibbons, 1977; Shine, 1985).

Una variante de la hipótesis de "clima frío" es que la transición hacia el viviparismo podría haber sido impulsada por un cambio en la localización de los nidos a lugares más superficiales y más cálidos en altas latitudes o altitudes, en vez de por un aumento en la duración de la retención de los huevos en los oviductos maternos, puesto que la mortalidad de los huevos aumenta en los nidos más superficiales, ya sea por un aumento de la depredación o por el estrés fisiológico que sufren en estos emplazamientos

(Andrews, 2000). Pero también es posible que el viviparismo haya evolucionado en zonas cálidas y que simplemente preadaptara a muchos reptiles para habitar en climas fríos (Packard *et al.*, 1977).

La hipótesis de “manipulación materna” o de “optimización térmica” de las etapas iniciales de la embriogénesis propone que la retención ha evolucionado para controlar el ambiente térmico de desarrollo. Las hembras manipularían los regímenes térmicos que experimentan los embriones en desarrollo, mejorando así la eficacia biológica de su descendencia. Se fundamenta en que la temperatura de incubación tiene importantes efectos sobre la supervivencia, el desarrollo y sobre determinados caracteres del fenotipo de las crías, como se ha demostrado en numerosas ocasiones (Van Damme *et al.*, 1992; Shine y Harlow, 1993; Braña y Ji, 2000; Ji *et al.*, 2007) y en que las hembras de algunas especies cambian sus preferencias térmicas durante la gestación, seleccionando temperaturas más bajas o más altas que el resto de individuos de la población, o bien manteniendo temperaturas corporales más estables durante ese periodo (Braña, 1993; Shine, 2006; Angilletta, 2009).

Organismo modelo

La lagartija de turbera *Zootoca vivipara* (Jacquin, 1797) (Fig. 1.2) es la especie que se tomará en esta Tesis como modelo para estudiar la evolución de la retención prolongada de los huevos. Se trata de una lagartija de pequeño tamaño (longitud hocico-cloaca: 34-64 mm), de hábitos terrestres, que presenta una termorregulación heliotérmica y que vive en hábitats húmedos, como turberas y brezales húmedos (Braña y Bea, 2002). Esta especie presenta dimorfismo sexual pues, como ocurre en otras lagartijas, la cabeza y la cola son mayores en los machos (Gvoždík y Van Damme, 2003; Hofmann y Henle, 2006), mientras que el abdomen es mayor en las hembras (Braña, 1996). El periodo reproductor de esta especie coincide con la primavera-verano y sus puestas constan de 2-11 huevos. En la Cordillera Cantábrica la ovulación se produce a principios de Junio y la puesta tiene lugar en Julio (Braña, 1986).

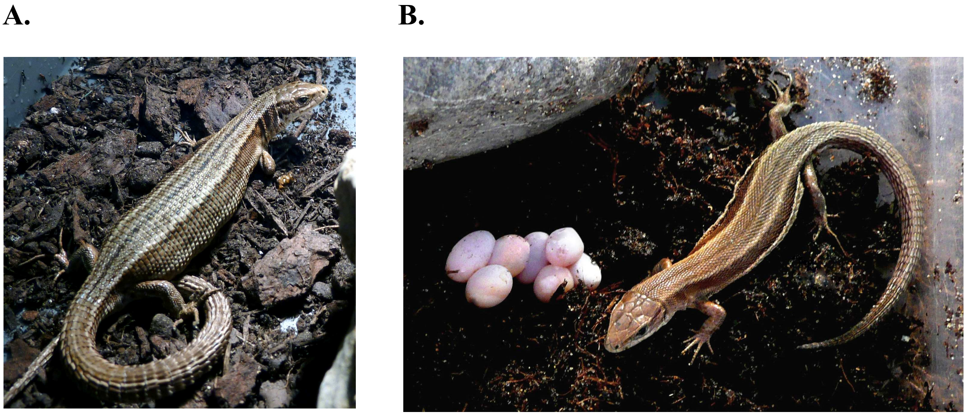


Figura 1.2. Hembras adultas gestante (**A**) y post-puesta (**B**) de *Zootoca vivipara*.

Su área de distribución es la más extensa de entre todos los reptiles vivientes: desde el norte de la Península Ibérica e Irlanda en el oeste, alcanzando Escandinavia en el norte y extendiéndose hasta las islas de Sajalín (Rusia) y Hokkaido (Japón) en el este (Fig. 1.3; Braña y Bea, 2002; Arribas, 2009), siendo además la lagartija que mayores latitudes alcanza. Este hecho se debe probablemente a la precisión de su comportamiento termorregulador, incluso en la región subártica (Herczeg *et al.*, 2003), además de a su tolerancia a la congelación y a su capacidad de subenfriamiento (Grenot *et al.*, 2000).

Aunque durante mucho tiempo se pensó que esta lagartija era exclusivamente vivípara (Panigel, 1956; Packard *et al.*, 1977), a pesar del hallazgo de huevos de esta especie en los Pirineos por parte de Lantz (1927), este lacértido presenta bimodalidad reproductora, como se ha explicado anteriormente. La mayor parte de sus poblaciones son vivíparas, pero son ovíparas las del extremo suroccidental de su distribución (Lantz, 1927; Braña, 1986; Heulin *et al.*, 2000).

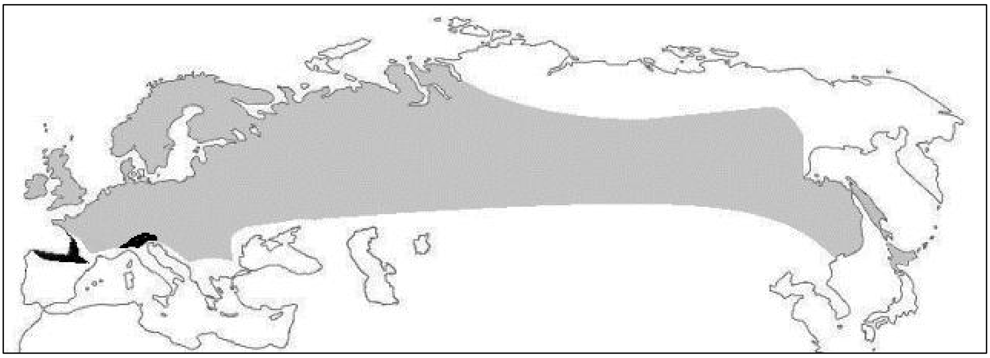


Figura 1.3. Área de distribución de *Z. vivipara.* En gris: poblaciones vivíparas; en negro: poblaciones ovíparas.

Las poblaciones ovíparas de esta especie pertenecen a dos grupos bien diferenciados (Surget-Groba *et al.*, 2006; ver Fig. 1.3) que presentan diferente grado de retención de la puesta, así como diferencias en el grosor de la cáscara de los huevos. Por un lado, las poblaciones del norte de Italia, Eslovenia, sur de Austria y noroeste de Croacia ponen huevos con embriones que alcanzan el estadio 32 y con cáscaras gruesas y muy calcificadas; por otro lado, las del norte de la Península Ibérica, Pirineos y Aquitania son capaces de retener los huevos hasta el estadio 35 en condiciones forzadas y el grosor de sus cáscaras es intermedio entre el de las ovíparas orientales y el de las finas membranas que recubren a las crías vivíparas en el momento de la puesta (Heulin *et al.*, 2000, 2002). En este estudio se han empleado individuos procedentes de poblaciones ovíparas del área cántabro-pirenaica, que ponen huevos con embriones en estadios de desarrollo muy avanzados (frecuentemente en los estadios 33 y 34; Braña *et al.*, 1991; Fig. 1.4), de modo que representarían un estadio muy avanzado de la transición hacia el modo de reproducción vivíparo.

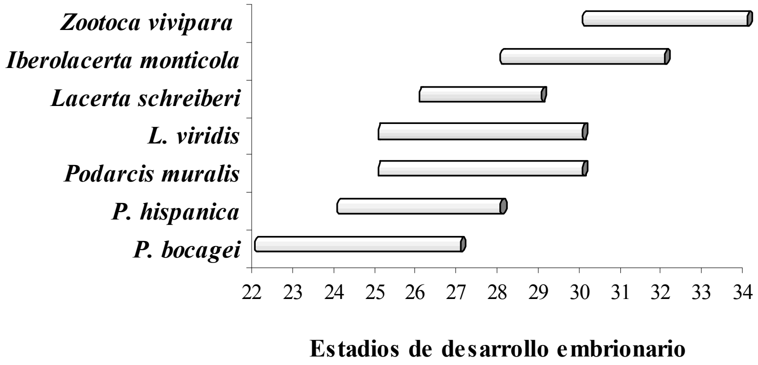


Figura 1.4. Estadios de desarrollo embrionario en el momento de la puesta según la escala de Dufaure y Hubert (1961) de 7 especies de lacértidos. Tomado de Braña *et al.*, 1991.

Objetivos

El objetivo general de esta Tesis consiste en examinar, en un organismo modelo con retención prolongada de la puesta que representa, por tanto, un estadio transicional muy avanzado en la evolución del viviparismo, la variabilidad intra- e interpoblacional en el nivel de desarrollo embrionario intrauterino y analizar esa variabilidad en relación con la variación ambiental. Además, se pretende valorar las principales hipótesis sobre la evolución del viviparismo en reptiles. Estos objetivos generales se desglosan en los siguientes:

Capítulo 1. Analizar la variación en la biología térmica, morfología y nivel de desarrollo embrionario intrauterino, así como en varios aspectos de la reproducción supuestamente relacionados con la transición hacia el viviparismo (Mathies y Andrews, 1995; Calderón-Espinosa *et al.*, 2006), entre poblaciones ovíparas de *Z. vivipara* de los extremos altitudinales de su distribución en el área cantábrica. Se trata principalmente de probar si, como predice la hipótesis de "clima frío" sobre la evolución del viviparismo, las poblaciones de alta montaña, expuestas a temperaturas más bajas, presentan mayor avance en la embriogénesis intrauterina.

Capítulo 2. Examinar la plasticidad en la retención de los huevos en los oviductos y valorar su efecto sobre el desarrollo embrionario provocando la retención forzada de la puesta. Esto se consigue gracias a que las hembras responden a la sequía reteniendo sus huevos, ya que el éxito de la puesta depende de una humedad adecuada del sustrato (Andrews y Rose, 1994; Warner y Andrews, 2003). Así se determinará si la retención durante el desarrollo embrionario tiene variabilidad suficiente para que la especie pueda adaptarse a condiciones adversas y permitir la evolución hacia estados crecientes de retención de la puesta. Asimismo, en este capítulo se pretende examinar la posible selección de rasgos que favorecerían el progreso del desarrollo del embrión durante el avance gradual de la retención de los huevos, como la reducción correlativa del grosor de la cáscara del huevo (Mathies y Andrews, 2000), determinando el grosor de las cáscaras de los huevos de *Z. vivipara* (Heulin *et al.*, 1992) retenidos más allá del tiempo normal de puesta, así como su contenido en minerales (Stewart *et al.*, 2009), tanto en el momento de la puesta como en el de la eclosión.

Capítulo 3. Determinar si existen variaciones en las preferencias térmicas de las hembras gestantes comparándolas con las de otros individuos de la población (Braña, 1993; Carretero *et al.*, 2005) y evaluar el efecto de la temperatura durante la fase de incubación externa sobre el desarrollo embrionario y sobre algunos rasgos del fenotipo de las crías que podrían ser relevantes para su eficacia biológica (Braña y Ji, 2000). De esta forma se evaluará la hipótesis de "optimización térmica" de las etapas iniciales de la embriogénesis (Shine, 1995; Braña y Ji, 2007).

Capítulo 4. Evaluar el efecto del ambiente térmico de gestación sobre el desarrollo embrionario (Beuchat, 1988; Mathies y Andrews, 1997), determinando los efectos de la temperatura seleccionada por hembras no gestantes (más elevada que la seleccionada durante la gestación) sobre el desarrollo de los embriones y el fenotipo de las crías. Esto nos permitirá testar la hipótesis de "optimización térmica" de las etapas iniciales de la embriogénesis, que predice que el cambio en las preferencias térmicas de las hembras durante la gestación está relacionado con la optimización del ambiente térmico de gestación (Shine, 1995; Webb *et al.*, 2006).

II. Capítulo 1.

Altitudinal variation in egg retention and rates of embryonic development in oviparous *Zootoca vivipara* fits predictions from the cold climate model on the evolution of viviparity[*]

---

[*] En revisión en *Journal of Evolutionary Biology*.

Resumen

La evolución del viviparismo en reptiles se ve favorecida, según la hipótesis de “clima frío”, en altas latitudes o altitudes, donde la retención de los huevos implicaría beneficios térmicos para la embriogénesis a causa de la termorregulación materna. Según esta hipótesis, y considerando que el viviparismo habría evolucionado a través de un incremento gradual de la extensión de la retención intrauterina de los huevos, se esperaría que las poblaciones ovíparas de zonas altas exhibieran estadios de desarrollo embrionario más avanzados en el momento de la puesta que las poblaciones de zonas bajas. Evaluamos posibles diferencias en el nivel de retención de los huevos, en el tiempo de desarrollo embrionario y en la biología térmica de poblaciones ovíparas de *Zootoca vivipara* cercanas a los límites altitudinales de la distribución de la especie en el norte de España (altitud media de las poblaciones de zonas bajas, 235 m snm; poblaciones de zonas altas, 1895 m snm). La altitud no influenció ni la temperatura de lagartijas activas en el campo ni la temperatura seleccionada por las lagartijas en un gradiente térmico en el laboratorio y las hembras gestantes seleccionaron temperaturas más bajas en dicho gradiente que los machos y las hembras no gestantes. Los huevos de las poblaciones de zonas altas contenían embriones más desarrollados en el momento de la puesta (estadios 33-35 de Dufaure y Hubert) que los huevos de poblaciones bajas (estadios 30-34) y el tiempo de incubación fue más corto para los embriones de zonas altas. Cuando se analizaron puestas de ambos extremos altitudinales en el mismo estadio de desarrollo en el momento de la puesta (estadio 33), el tiempo de incubación volvió a ser menor en las poblaciones de zonas altas, indicando variación contra gradiente en la tasa de desarrollo. Nuestros resultados indican que la temperatura es un factor ambiental que afecta a la distribución geográfica de

los diferentes niveles de retención de los huevos en *Z. vivipara*, tal y como predice la hipótesis de clima frío sobre la evolución del viviparismo.

Abstract

The evolution of reptilian viviparity is favoured, according to the cold-climate hypothesis, at high latitudes or altitudes, where egg retention would entail thermal benefits for embryogenesis because of maternal thermoregulation. According to this hypothesis, and considering that viviparity would have evolved through a gradual increase in the extent of intrauterine egg retention, highland oviparous populations are expected to exhibit more advanced embryo development at oviposition than lowland populations. We tested for possible differences in the level of egg retention, embryo development time, and thermal biology of oviparous *Zootoca vivipara* near the extreme altitudinal limits of the species distribution in the north of Spain (mean altitude for lowland populations, 235 m asl; for highland populations, 1895 m asl). Altitude influenced neither temperature of active lizards in the field nor temperature selected by lizards in a laboratory thermal gradient, and pregnant females selected lower temperatures in the thermal gradient than did males and non-pregnant females across altitudinal levels. Eggs from highland populations contained embryos more developed at the time of oviposition (Dufaure and Hubert's stages 33-35) than eggs of highland populations (stages 30-34) and partly because of this difference incubation time was shorter for highland embryos. When analysed for clutches from both altitudinal extremes at the same embryonic stage at oviposition (stage 33), again incubation time was shorter for highland populations, indicating genuine countergradient variation in developmental rate. Our results indicate that temperature is an environmental factor affecting the geographical distribution of different levels of egg retention in *Z. vivipara*, as predicted by the cold-climate hypothesis on the evolution of viviparity.

Introduction

Widely distributed species, which are found in a variety of thermal environments, frequently show variation in life-history characteristics (Adolph and Porter, 1993), such as body size (insects: Huey *et al.*, 2000; birds: Ashton, 2002; mammals: Yom-Tov and Geffen, 2006), reproductive traits (reptiles: Forsman and Shine, 1995; amphibians: Liao and Lu, 2012) or annual survival rate (reptiles: Adolph and Porter, 1993). A few widespread species differ in the mode of reproduction along their distribution area, some of them even having both oviparous and viviparous populations (e. g., *Salamandra salamandra,* Buckley *et al.*, 2007; *Lerista bougainvillii*, Qualls *et al.*, 1995). Organisms of the same species showing geographic variation in reproductive modes are ideal to study the evolutionary transition from oviparity to viviparity, as oviparous populations are expected to exhibit intermediate characteristics between both modes of reproduction, such as advanced embryonic stages at oviposition and shorter incubation time (Braña *et al.*, 1991; Smith and Shine, 1997; Oufiero and Angilletta, 2006; Telemeco *et al.*, 2010). In addition, oviparous and viviparous conspecifics can be compared avoiding misleading interpretations, as they share an immediate common ancestor and therefore present similar genetic, physiological and ecological characteristics. Among squamate reptiles, the group of vertebrates in which viviparity has evolved most frequently (Blackburn, 1999b), three species show intraspecific reproductive bimodality: *Lerista bougainvillii* (Qualls *et al.*, 1995), *Saiphos equalis* (Smith and Shine, 1997), and *Zootoca vivipara* (Braña, 1986).

Two main hypotheses try to explain the evolution of viviparity in reptiles, which is generally assumed to occur through selection for progressive increases in the amount of embryonic development in the oviducts (Tinkle and Gibbons, 1977). The maternal manipulation hypothesis

(Shine, 1995; Webb *et al.*, 2006) posits that egg retention evolves because females provide eggs with a more stable and warmer thermal environment than the external one, i. e., with the optimum thermal environment for embryo development. Evidence on females changing their preferred body temperatures during pregnancy (Shine, 2006), as well as on incubation temperatures similar to those preferred by non-pregnant females having detrimental effects on embryonic development supports this hypothesis (Rodríguez-Díaz and Braña, 2011a). Another model, not necessarily incompatible with the maternal manipulation hypothesis (Rodríguez-Díaz *et al.*, 2010), is proposed by the cold climate hypothesis (Shine, 1985, 2002a). According to this model, egg retention is favoured at high altitudes or latitudes, where environmental temperatures are much lower than female body temperatures. At these sites, egg retention would accelerate embryonic development and would reduce external incubation time, thus reducing the amount of time eggs are exposed to low nest temperatures, with negative effects on offspring survival (Qualls and Andrews, 1999a). In support of this hypothesis, viviparous populations of reptiles are frequently found at high latitudes or altitudes (e.g., Qualls and Shine, 1998a). However, this pattern could be explained either because the potential for longer periods of egg retention would have favoured the evolution of viviparity in cool environments or because viviparity, having evolved elsewhere, would have favoured the subsequent invasion of cool enviroments (Packard *et al.*, 1977).

The aim of this study consists of testing the cold-climate hypothesis, taking oviparous populations of the lacertid lizard *Zootoca vivipara* (Jacquin, 1797) from the Cantabro-Pyrenean region (Northern Spain) as a model organism. This species has the largest known distribution range among lizards in the world (Gasc *et al.*, 1997) and populations of the Cantabro-Pyrenean region represent the south-western limit of the distribution area of the species (Fig. 2.1A). In this region, *Z. vivipara* is oviparous, whereas it is viviparous in the rest of its distribution range, and shows a wide altitudinal distribution, being present from areas close to sea level to 2400 m asl, with a relatively discontinuous distribution because of its habitat specificity (peat bogs and wet meadows; Braña and Bea, 2002). Oviparous populations of this species lay eggs containing embryos at more advanced developmental stages than do other sympatric lizards (i. e., exhibit extended egg retention) and show some interpopulation variability at this respect (Braña *et al.*, 1991). These populations represent an advanced situation in the evolutionary transition from common oviparity to viviparity, being considered the ancestral to viviparous populations (Heulin *et al.*, 2002; but see Surget-Groba *et al.*, 2006). If the evolution of viviparity and egg retention is favoured at low environmental temperature, as posits the cold climate hypothesis, high elevation oviparous populations are expected to exhibit more advanced embryo development at oviposition than low elevation populations. In order to test this hypothesis, we assessed altitudinal variation in stage of development at oviposition and incubation time between oviparous populations of the lacertid lizard *Z. vivipara* at the elevational extremes of the species' distribution in the Cantabrian region and the Pyrenees. We also determined whether the thermal environments experienced by low and high altitude populations were indeed different and whether females from both altitudes modify their thermal preferences during pregnancy. Additionaly, thermal preferences of highland and lowland lizards were assessed to identify whether those of high altitudes and therefore cool environments change their thermal preferences, selecting lower body temperatures than lizards of low altitudes, because the temperatures available in the field for their daily activity would be lower and this fact could modify the optimal temperature for their physiological processes (Mathies and Andrews, 1995; Angilletta, 2001; Oufiero and Angilletta, 2006). Another possibility might be that highland lizards adjust their thermoregulatory behaviour or their physiological processes to compensate for low temperatures (Gvoždík, 2002).

Materials and Methods

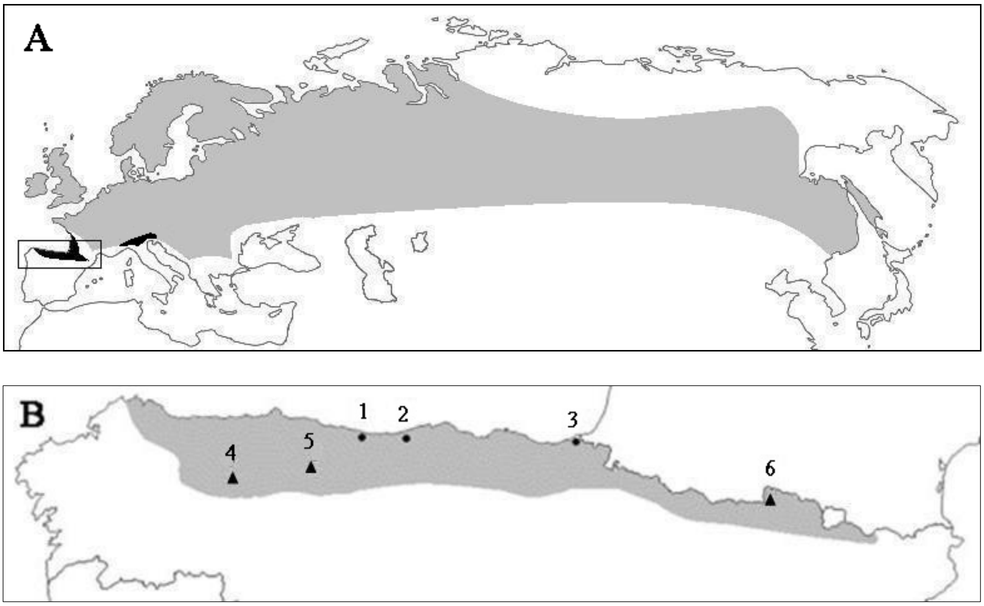


Figure 2.1. A. Distribution area of *Z. vivipara.* Gray area: viviparous populations; black areas: oviparous populations. **B.** Map of the North of the Iberian Peninsula showing the Spanish distribution of *Zootoca vivipara* (grey area) and the collection localities (solid circles). 1 = Buelna (220 m asl); 2 = Treceño (250 m asl); 3 = Irún (230 m asl); 4 = Leitariegos (1670 m asl); 5 = Las Señales (1780 m asl); 6 = Vielha (2230 m asl).

We collected adult male and female *Z. vivipara* in June-July 2008, 2009, and 2010 from six locations of the North of Spain (Fig. 2.1B): three lowland locations, Buelna (Asturias, 220 m asl; *n*=108), Irún (Guipúzcoa, 230 m asl; *n*=24), and Treceño (Cantabria, 250 m asl; *n*=48); and three highland locations, Leitariegos (Asturias, 1670 m asl; *n*=33), Las Señales

(León, 1780 m asl; *n*=122), and Vielha (Central Pyrenees, Lleida, 2230 m asl; *n*=27). These populations, as others in the south-western limit of the species distribution range are oviparous (Braña, 1986; Heulin *et al.*, 2000; Surget-Groba *et al.*, 2006), whereas the species is viviparous over most of its range (see Fig. 2.1A). Lizards were caught by hand or noose and were transported to the Zoology laboratory in the University of Oviedo and housed in plastic terraria (50 x 40 x 30 cm; length x width x height) provisioned with a layer of wet substrate, shelter items, and water *ad libitum*. Mealworms and crickets were provided twice a day. Heating bulbs were placed at one extreme of the terraria and were switched on from 09:00 h. to 13:30 h and from 15:30 to 19:30 h. each day.

Because rates of embryonic development and other developmental characteristics are highly dependent on environmental temperature, we assessed thermal incubation conditions in the field at both altitudinal extremes. We got continuous recordings of temperature in two of the locations studied near both altitudinal limits (Buelna, 220 m asl, and Las Señales, 1780 m asl) from June 2008 to September 2010. Thermal data loggers (Tidbit v2; Onset Computer Corp., Bourne, MA, USA) were placed at 5 cm depth in potential nest sites of *Z. vivipara* in the field, i.e. places similar to those where nests of this species had been previously found.

Body temperatures of adults

In order to determine possible altitudinal variation in thermal preferences among oviparous populations of *Z. vivipara*, we measured the temperature selected by adult lizards of each of the six populations studied in a laboratory thermal gradient with a heating bulb (60W) situated in one extreme of a terrarium (range of substrate temperatures: 21.6-40.6º C). We took one measurement of cloacal temperature for each individual with a

Schultheis quick-reading thermometer (Miller and Weber, Inc., Ridgewood, NY, USA) one hour and a half after having placed it in the thermal gradient. Refuges, water or food were not provided during tests, so that lizards would choose their preferred body temperature without being influenced by other biological needs. We distinguished between cloacal temperature of males, pregnant females, and non-pregnant females, as it has been demonstrated for oviparous *Z. vivipara* that pregnant females select lower temperatures than the other individuals of the same population (Carretero *et al.*, 2005; Rodríguez-Díaz *et al.*, 2010). Preferred body temperatures were compared to body temperatures of active lizards in the field at two locations near the altitudinal extremes, Buelna (220 m asl) and Las Señales (1780 m asl), measured with a *Schultheis* thermometer in a maximum of 10s after capture to avoid changes in lizards' temperature. Temperatures of the substrate and the air (both shaded for measurement; air temperature taken at 10 cm above the ground) were measured in the site where lizards were captured.

Adult size and reproductive output

Because altitudinal variation in body size has been frequently reported for ectotherms (Ashton and Feldman, 2003; Angilletta *et al.*, 2004a; Iraeta *et al.*, 2006; Pincheira-Donoso *et al.*, 2008), we measured body mass (digital balance, to the nearest 0.0001 g), snout-vent length (SVL), and head width (digital caliper Vogel, Germany, to the nearest 0.01 mm) of adult males and females of each population. Body mass was used to calculate robustness (size-corrected body mass) of adults.

Cages were checked for clutches at least twice a day. Clutches and females were removed from the terraria and weighed. Relative clutch mass (RCM) was computed by dividing clutch mass by the mass of the female after egg-laying.

Embryonic stage at oviposition and incubation time

One egg of each clutch was dissected for determination of developmental stage at oviposition according to Dufaure and Hubert (1961); half stages were assigned if embryos had features intermediate between two developmental stages. The remaining eggs within each clutch were weighed and placed individually in plastic containers with distilled water and vermiculite as substrate (proportion 1/1 in mass). Then, they were randomly assigned to one of the three constant incubation temperature treatments (21, 25 and 29º C). These temperatures, although higher than mean temperatures experienced by eggs in the nests, were in the range of the temperatures attained there. Incubation time from oviposition to hatching was determined for each population at each temperature.

Hatchling traits

We measured body mass (digital balance, to the nearest 0.0001 g), SVL, head length, abdomen length, and tail length (digital caliper Vogel, Germany, to the nearest 0.01 mm) of hatchlings from the three thermal incubation regimes. We quantified the locomotor performance of hatchlings within 2 days of hatching, by chasing them with a paintbrush along a 1-meter length racetrack. Hatchlings were maintained at 32º C, a temperature that optimizes locomotor performance in this species (Van Damme *et al.*, 1991), for 90 minutes before the trials. Running trials were recorded with a Nikon video camera at 30 frames per second and locomotor performance was assessed by determining the maximum sprint speed (speed in the fastest five consecutive frames), and the number of stops during trials (see Braña and Ji, 2000).

Statistical analyses

We first assessed whether our data fulfilled the assumptions of normality (Kolmogorov – Smirnov test) and homocedasticity (Bartlett's test). Clutch size and incubation time were log-transformed to meet these assumptions. Embryo stage at oviposition did not fulfil the above mentioned conditions even after transformation and is an ordinal variable, so a Kruskal-Wallis test was used to analyse this variable. After the Kruskal-Wallis test, Mann-Whitney U-tests were performed *a posteriori* with a signification level set at $\alpha=0.01$. Altitude effect on body temperatures of active lizards in the field was analysed by a one-factor (elevation) analysis of variance (ANOVA) and an analysis of covariance (ANCOVA) with air and substrate temperatures as covariates. Nested mixed model ANOVAs or ANCOVAs were performed to analyse the remaining variables. Population of origin (nested within the elevation) was included as a random effect in the following analyses: one-factor (elevation) ANOVA for RCM and ANCOVA for clutch size and clutch mass, with female SVL as a covariate; two-factor ANOVA for preferred body temperature of adults (fixed factors: elevation and reproductive condition); and, in the analyses of the phenotype of adult lizards, two-factor (elevation and sex) ANOVA (for SVL) or ANCOVAs (for head width and body mass, with SVL as covariate). As three eggs from the same clutch were distributed among the three incubation treatments, clutch (nested within the population of origin) was included as a random factor along with the population of origin in the analyses of incubation time: two-factor ANOVA (elevation and incubation temperature); and the phenotype of hatchlings: two-factor ANOVAs (for SVL, head length, abdomen length, and number of stops) or ANCOVAs (for body mass and tail length with SVL as a covariate, and sprint speed, with SVL and number of stops as covariates). Each of the variables measured is known to affect reproductive success or other traits likely influencing fitness (head length: Gvoždík and Van Damme, 2003; abdomen length: Braña, 1996; tail length: Hofmann and Henle, 2006; sprint speed: Garland *et al.*, 1990), and for this reason we were interested in assessing each variable separately and we did not carry out multivariate analyses of variance or covariance. Fisher LSD tests were used as tests *a posteriori*. Signification level was set at α=0.05 for all the analyses.

Results

Nest and body temperatures

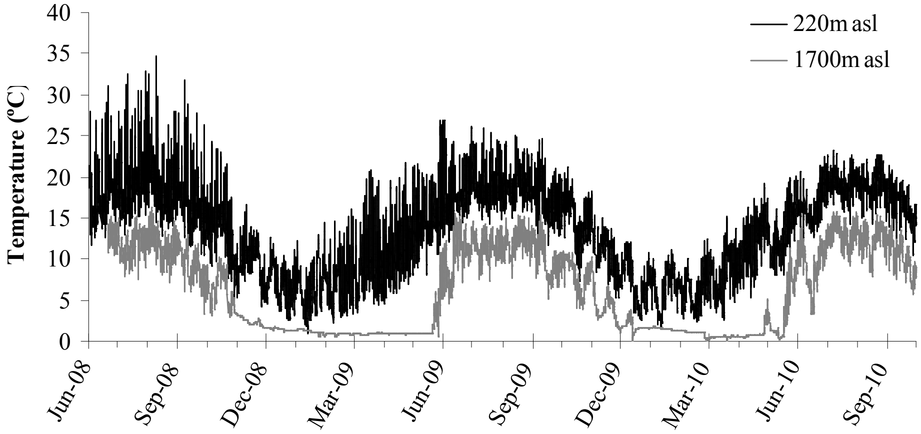


Figure 2.2. Temperatures in potential nests sites of *Z. vivipara* located near the altitudinal extremes in the Cantabrian region (Northern Spain), from June 2008 to September 2010.

Recordings of temperatures at potential nests sites in the field revealed that soil temperatures are consistently higher at the low altitude (220 m asl; mean temperature ± sd, during the reproductive period, i.e. from June to September: 18.04 ± 2.77° C), even occasionally exceeding 30° C in

2008 (see Fig. 2.2); on the contrary, maximum temperature hardly reached 15º C at the high location (1780 m asl; mean temperature from June to September ± sd: 11.09 ± 2.26º C).

We found neither significant altitude effect (ANOVA $F_{1,174} = 0.065$; $P = 0.81$; Table 1) nor between-factor interaction (altitude x reproductive condition: $F_{2,174} = 1.269$; $P = 0.28$) when analysing preferred body temperatures of adult lizards. Overall, pregnant females selected lower body temperatures (mean ± sd; 32.3 ± 1.2º C) than males and non-pregnant females (33.4 ± 1.5º C; ANOVA $F_{2,174} = 7.745$, $P = 0.0006$). Field body temperatures of lizards active in summer were not affected by altitude (lowland population: 31.0 ± 1.8º C, n=21; highland population: 31.2 ± 1.6º C, n=66; ANOVA: $F_{1,85} = 0.321$; $P = 0.57$; ANCOVA with environment temperatures as covariates: $F_{1,82} = 0.291$; $P = 0.59$; Fig. 2.3).

TABLE 1.- Preferred Tb temperatures in a laboratory thermal gradient (mean ± sd; ºC).

	Reproductive condition		
Locality	M	Non-gravid F	Gravid F
Llanes (220 m asl)	33.7±0.8 (8)	32.1±2.1 (9)	31.8±1.4 (5)
Irún (230 m asl)	33.4±1.6 (10)	33.3±0.8 (5)	31.8±1.7 (8)
Treceño (250 m asl)	33.6±1.2 (24)	34.3±1.2 (7)	32.7±1.3 (16)
Leitariegos (1670 m asl)	33.5±0.7 (8)	34.0±1.3 (22)	31.9±0.5 (3)
Señales (1780 m asl)	32.6±1.8 (11)	33.3±1.3 (15)	33.3±0.9 (7)
Vielha (2230 m asl)	33.2±2.0 (7)	32.3±2.6 (3)	31.9±0.8 (16)

M = Males; F = Females. Sample sizes are in brackets.

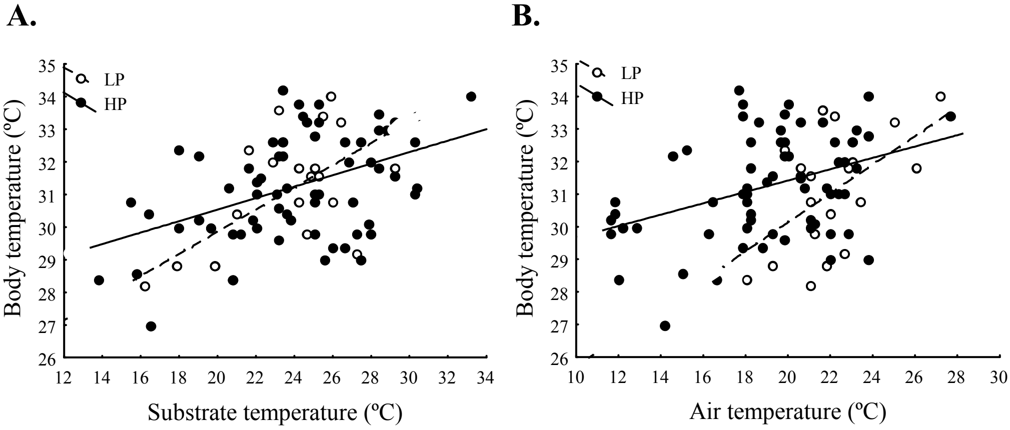


Figure 2.3. Relationship between field body temperatures of active *Z. vivipara* at low and high elevation localities (Buelna and Las Señales, respectively) and substrate (A; R^2=0.37 for the lowland population; R^2=0.20 for the highland population) and air temperatures (B; R^2=0.27 for the lowland population; R^2=0.15 for the highland population). LP = lowland population; HP = highland population.

Adult size and reproductive output

Sexual dimorphism was found in all of the three adult traits measured: males are more robust and have wider head, whereas females have larger SVL. Lizards from low elevations are smaller (SVL) than high elevation lizards. Altitude did not influence robustness or head width. However, there were elevation x sex interactions for head width and robustness: whereas males from low elevations have wider heads and are less robust than males from high elevations, females from low elevations have a similar head width and are more robust than those from high elevations (Table 2).

Clutch size and mass do not differ with altitude when corrected for female SVL (ANCOVAs, for clutch mass: $F_{1,87} = 0.346$, $P = 0.58$, Fig. 2.4; for clutch size: $F_{1,87} = 0.339$, $P = 0.59$). Clutch sizes for the sampled populations were: Buelna, 3-7 (*n*=17); Irún, 4-7 (*n*=8); Treceño, 2-7 (*n*=11); Leitariegos, 3-8 (*n*=6); Las Señales, 4-9 (*n*=37); and Vielha, 2-6 (*n*=17).

RCM was not influenced by altitude either (ANOVA, $F_{1,82}$ = 2.902, P = 0.16).

TABLE 2.- Morphology of *Zootoca vivipara* adults according to the elevation of the population of origin and sex[1]

	Effects		
Adult traits	Elevation	Sex	Interaction
Body mass (g)	$F_{1,4.3}$ = 0.012 ns	**$F_{1,353}$ = 224.77*** M>F**	**$F_{1,353}$ = 33.913*****
SVL (mm)	**$F_{1,4.2}$ = 7.438***	**$F_{1,354}$ = 49.417*** M<F**	$F_{1,354}$ = 0.149 ns
Head width (mm)	$F_{1,4.1}$ = 0.103 ns	**$F_{1,353}$ = 352.68*** M>F**	**$F_{1,353}$ = 5.620***

[1]Statistical tests correspond to single effects and between-factor interactions in ANCOVAs (body mass, and head width with SVL as covariate) and ANOVA (SVL). Bold face shows statistically significant values. ns P>0.05, *P<0.05, ***P<0.001. M=Male; F=Female.

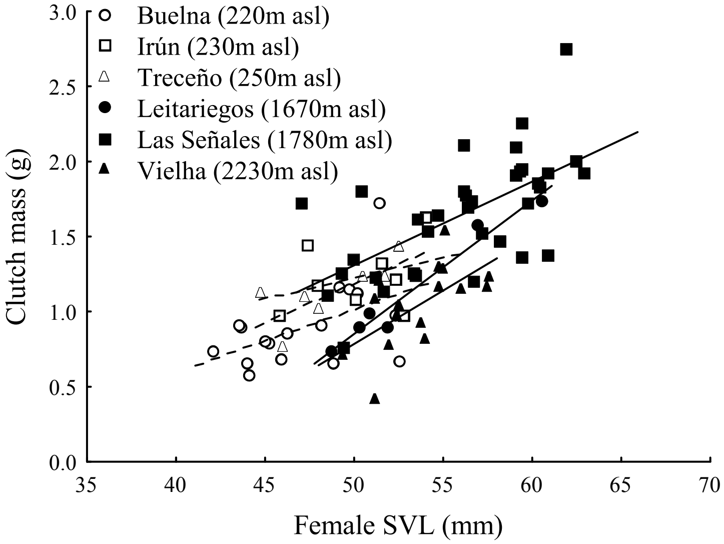


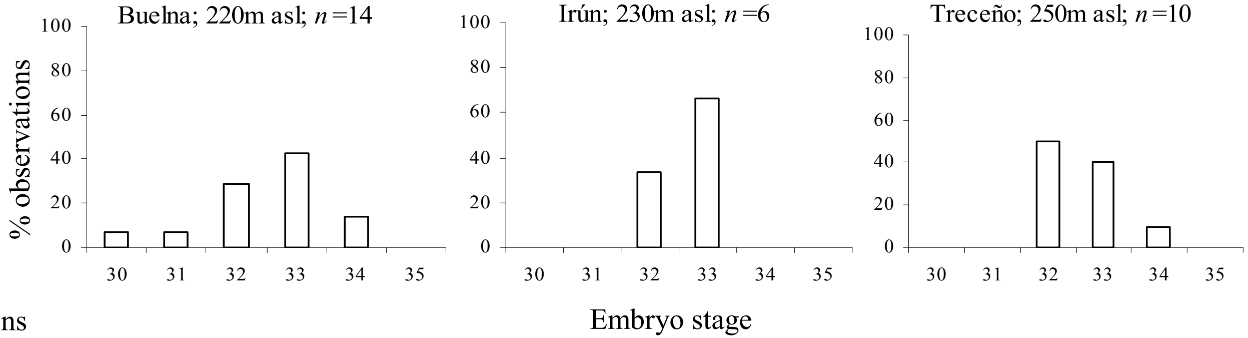
Fig. 2.4. Relationship between clutch mass and female SVL for the six sampled populations: three lowland populations (open symbols; dashed lines) and three highland populations (solid lines and symbols).

Embryonic development at oviposition and incubation time

Embryo stage at oviposition varied with elevation (Kruskal-Wallis test: H (5, n=94) = 58.089, P = 0.000). Mann-Whitney U tests *a posteriori* revealed that the females from the two highest populations laid eggs with embryos at more advanced developmental stages (Las Señales, and Vielha, stages 33-35) than the females from the other populations (Buelna, Irún, and Treceño, Leitariegos; stages 30.5-34; Fig. 2.5).

Eggs incubated at higher temperatures hatched sooner (ANOVA, $F_{2,106}$ = 13551.000, P = 0.000). Furthermore, embryos from high-elevation populations hatched earlier at any of the three incubation treatments (ANOVA, $F_{1,106}$ = 11.560, P = 0.027; Fig. 2.6A). In order to assess whether this difference in external incubation time was caused only by the difference in embryo stage at oviposition among lowland and highland populations, we analysed incubation time only for clutches with embryos at stage 33 at oviposition because it was the only stage present in both highland and lowland populations. We included in this analysis one lowland population and one highland population having enough clutches at stage 33 to allow the analysis (nested mixed model ANOVA with the female as a random factor nested within the population of origin, and population and incubation temperature as fixed factors). Again, eggs incubated at higher temperatures hatched sooner ($F_{2,7}$ = 3956.5, P = 0.000) and incubation time was shorter for embryos of the highland population ($F_{1,7}$ = 48.6, P = 0.000; Fig. 2.6B). The altitude x incubation temperature interaction effect was not significant ($F_{1,7}$ = 0.2, P = 0.69).

A. Lowland populations



B. Highland populations

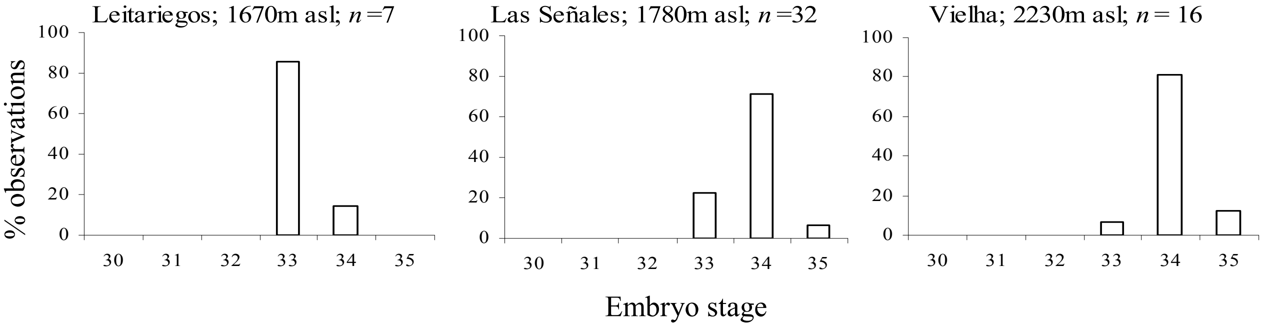
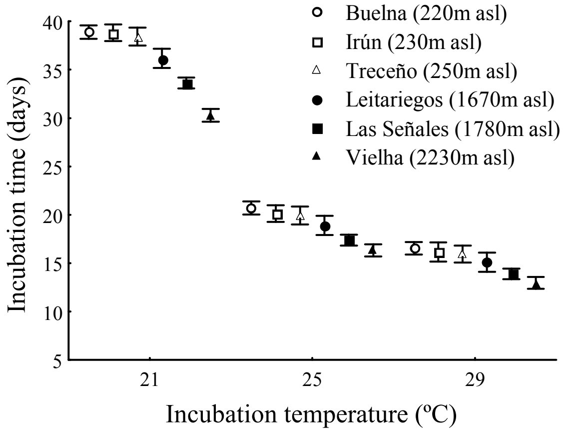


Figure 2.5. Developmental stages at oviposition of embryos of oviparous *Z. vivipara* for each location studied, according to the stages described by Dufaure and Hubert (1961). Half stages were not depicted: they were assigned to the closer stage when possible. Number of clutches for each population is in brackets.

A. All clutches.



B. Clutches at stage 33 only.

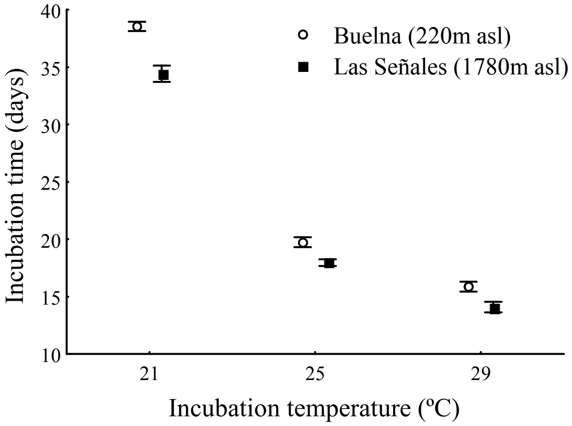


Figure 2.6. Incubation time, from oviposition to hatching, at the three constant incubation regimes (21, 25 and 29° C) for the six sampled populations (A); and for one lowland and one highland population, considering only clutches at stage 33 at oviposition (B). Open and closed symbols represent lowland and highland populations, respectively.

Hatchling traits

Most hatchling traits differed between populations: SVL, $F_{4,106}$ = 5.14; $P = 0.001$; head length, $F_{4,102} = 3.470$, $P = 0.011$; tail length, $F_{4,105}$ = 8.888, $P = 0.000$; maximum sprint speed, $F_{4,100} = 5.957$, $P = 0.000$; and number of stops, $F_{4,103} = 15.803$, $P = 0.000$; and some of them between clutches: SVL, $F_{87,106}$ = 2.56; P = 0.000; body mass, $F_{87,105}$ = 6.207, P = 0.000; abdomen length, $F_{87,102}$ = 1.53; P = 0.019; tail length, $F_{87,105}$ = 3.038; P = 0.000. Altitude did not influence any of the hatchling traits measured (Table 3) and the thermal regime experienced by embryos affected SVL, tail length, and locomotor performance traits: hatchlings from 21º C were larger (SVL), had shorter tails, ran slower, and stopped less often than hatchlings from the other incubation treatments (Table 3).

TABLE 3.- Morphology and locomotor performance of *Zootoca vivipara* hatchlings according to the elevation of the population of origin (L=low elevation; H=high elevation) and incubation temperature[1]

Hatchling traits	Effects		
Morphology	Elevation	Incubation Temperature	Interaction
SVL (mm)	$F_{1,4.1}$ = 2.184 ns	$F_{2,194}$ = 1.892 ns	$F_{2,194}$ = 0.640 ns
Head length (mm)	$F_{1,4.2}$ = 0.037 ns	$F_{2,190}$ = 1.745 ns	$F_{2,190}$ = 0.097 ns
Abdomen length (mm)	**$F_{1,4.4}$ =7.567* L<H**	$F_{2,190}$ = 0.704 ns	$F_{2,190}$ = 0.559 ns
Body mass (g)	$F_{1,4.4}$ = 0.655 ns	$F_{2,193}$ = 0.157 ns	$F_{2,193}$ = 0.529 ns
Tail length (mm)	$F_{1,4.1}$ = 0.205 ns	**$F_{2,193}$ = 26.480*** 21 < (25, 29)**	$F_{2,193}$ = 0.195 ns
Locomotor performance			
Sprint speed (cm·s^{-1})	$F_{1,4.1}$ = 1.228 ns	**$F_{2,188}$ = 10.364*** 21 < 29**	$F_{2,188}$ = 0.977 ns
Number of stops	$F_{1,4.1}$ = 0.029 ns	**$F_{2,194}$ = 9.182*** 21 < (25, 29)**	$F_{2,194}$ = 1.090 ns
Distance	$F_{1,4.3}$ = 0.904 ns	**$F_{2,190}$ = 7.144** 21 > (25, 29)**	$F_{2,190}$ = 1.205 ns

[1]Statistical tests correspond to single effects and between-factor interactions in ANCOVAs (tail length, hatchling mass, and distance with SVL as covariate; sprint speed, with SVL and number of stops as covariates) and ANOVAs (SVL, head length, abdomen length, and number of stops). Parentheses below F values show the results revealed by Fisher LSD tests *a posteriori* for the effects of incubation temperatures. Bold face shows statistically significant values. ns P>0.05; *P<0.05; **P<0.01; ***P<0.001.

Discussion

Oviparous *Zootoca vivipara* lays eggs at very advanced embryonic stages compared to other lacertid lizards (Braña *et al.*, 1991), and our present results show that highland females lay eggs with embryos at more advanced developmental stages (stages 33-35) than lowland females (30-34), as expected from predictions of the cold climate hypothesis on the evolution of viviparity. A similar pattern of intraspecific altitudinal variation in the level of egg retention has also been found in other lizards (*Sceloporus scalaris*: Mathies and Andrews, 1995; *Bassiana duperreyi*: Telemeco *et al.*, 2010). According to the cold climate hypothesis, prolonged egg retention is most advantageous in cold environments because the mean difference in temperature between eggs in nests and in the female is the greatest at high altitudes. As a consequence, eggs retained by females at high altitudes will be exposed to warmer temperatures on average than they would be in nests and thus egg retention will increase the rate of development and potentially enhance survival (Andrews, 2000; Shine, 2002a).

Congruent with differences in developmental stages at oviposition, embryos from higher populations completed external incubation faster than those from lowland populations at any temperature. The same pattern was found when comparing incubation time of one highland and one lowland Mediterranean species (Monasterio *et al.*, 2011), as well as highland and lowland populations of the same species (e.g. *Sceloporus undulatus*: Oufiero and Angilletta, 2006; Du *et al.*, 2010), presumably due to faster developmental rates of highland embryos, which would be a physiological adaptation to low temperatures (Olsson *et al.*, 1996; Andrews *et al.*, 1999; Angilletta *et al.*, 2004b; Oufiero and Angilletta, 2006). However, most studies that have reported physiological adaptation of embryonic development to cold climates in lizards (Olsson *et al.*, 1996; Qualls and

Shine, 1998b; Oufiero and Angilletta, 2006) have not properly determined the developmental stage of embryos at oviposition for each of the studied populations, and therefore cannot appropriately separate the initial development stage of the embryo from genuine differences in developmental rates as the cause of the reported differences in incubation time. The shorter external incubation period for our highland populations might be partly influenced by the embryonic stage at oviposition (see Results) but embryos from high altitudes oviposited at stage 33 still had shorter incubation times and faster developmental rates than embryos from low altitudes oviposited at stage 33. This pattern of counter-gradient variation (Conover and Schultz, 1995; Conover *et al.*, 2009) entails that high developmental rates compensate for, to some extent at least, low incubation temperature.

The relative discontinuity between populations throughout the Cantabrian Mountains and the Pyrenees due to the discontinuous habitat distribution suggests that increased egg retention at high elevations might have evolved several times independently, although genetic analyses are needed to confirm this idea. However, considering population clusters resulting from analyses of geographic variation of the female MPI sex-linked alleles (Guillaume *et al.*, 2000) and mtDNA (Heulin *et al.*, 2011), at least two different origins of prolonged egg retention at high elevations are likely, as the highland population of Vielha (Central Pyrenees) would belong to the eastern sub-group of the SW oviparous clade of *Z. vivipara*, whereas the remaining sampled populations from the Cantabrian region would be included in the western sub-group.

Despite the differences in environmental temperatures experienced by lowland and highland populations in their respective locations of origin, there is no evidence of adaptation of thermal preferendum to local conditions, as adults from different altitudes selected similar body temperatures in the laboratory thermal gradient. The same has been found for several lizard species (Mathies and Andrews, 1995) including viviparous *Z. vivipara* (Van Damme *et al.*, 1990; Gvoždík and Castilla, 2001). Field body temperatures did not differ between lowland and highland populations, which could point out either behavioural adaptation of lizards to compensate for cold temperatures at high altitudes (Gvoždík, 2002) or similar thermoregulation opportunities provided by summer thermal environments at both altitudinal extremes, even though activity period is shorter at higher elevations. Field body temperatures were lower than preferred body temperatures both at high elevation locations and at low elevation locations, likely reflecting the difficulty for lizards to thermoregulate accurately in their moist habitat with dense vegetation cover. We have also found for all populations the expected pattern that pregnant females select lower body temperatures than males and non-pregnant females, which has also been reported for other populations of the same species, both viviparous (Van Damme *et al.*, 1986) and oviparous (Carretero *et al.*, 2005). Change in thermal preferences of gravid females toward lower temperatures is probably linked to the maintenance of temperatures suitable for a successful embryonic development, in accordance with the maternal manipulation hypothesis. Therefore, this hypothesis is not incompatible with the cold-climate hypothesis (see Rodríguez-Díaz *et al.*, 2010), as the low temperatures selected by pregnant females would not entail an important increase in the potential incubation time (Rodríguez-Díaz and Braña, 2011a).

Adult *Z. vivipara* from high-elevation populations are larger, despite having a shorter activity period in such a cool environment and therefore a shorter growing season. The same pattern has been found in other lizard species, such as *Sceloporus undulatus* (Angilletta *et al.*, 2004a; Sears and Angilletta, 2004) or *Niveoscincus ocellatus* (Wapstra and Swain, 2001), which delay maturation in colder environments, reaching a larger body size at maturity (Adolph and Porter, 1996; Wapstra *et al.*, 2001). In spite that having a large body in cool climates would have a number of benefits, (e.g. higher survival over the hibernation period, Ashton, 2001), most lizards reverse Bergmann's rule. This rule states that species or populations in colder environments tend to have larger body sizes (Blackburn *et al.*, 1999) and the reason why this rule is not valid for most lizards would be that, in cool environments, small lizards gain heat more rapidly than large lizards (Ashton and Feldman, 2003; Pincheira-Donoso *et al.*, 2008). However, different environments at low and high elevation sites with respect to food availability or temperature might have influenced the growth of lizards (Niewiarowski and Roosenburg, 1993), and the altitudinal size difference found in *Z. vivipara* may have been the result of delayed maturation in colder environments, instead of genetic divergence.

Our results showed that hatchlings from the lowest thermal treatment (21º C) were larger (SVL) than those from 25º and 29º C, which would be advantageous because large individuals have higher overwinter survival (Civantos *et al.*, 1999). However, the lowest thermal incubation treatment had effects on traits relevant to the survival of hatchlings, such as tail length and locomotor performance (Husak, 2006; Parker and Andrews, 2007). Tail length can influence reproductive success in *Z. vivipara* (Hofmann and Henle, 2006), and locomotor performance has been proved to affect social interactions in several lizard species (*Sceloporus occidentalis*: Garland *et al.*, 1990; *Urosaurus ornatus*: Robson and Miles, 2000). Therefore, according to our results, there is not direct adaptation of highland *Z. vivipara* to enhance hatchling phenotypes at low temperatures, unlike some cold climate species (e. g. *Nannoscincus maccoyi*, Shine, 1999). Instead, highland *Z. vivipara* seems to avoid low nest temperatures by increasing intrauterine egg retention and developmental rates.

Our finding of different levels of intrauterine egg retention in oviparous *Z. vivipara* depending on the altitude concurs with the view that the evolution of viviparity in reptiles occurs gradually through increasingly developed embryo stages at oviposition (Packard *et al.*, 1977; Qualls *et al.*, 1997). The fact that highland populations attain more advanced embryo stages than lowland populations, together with the geographic distribution of the reproductive modes of *Z. vivipara* (oviparity in several areas at the southernmost limit and viviparity in most of its distribution area) agrees with the expected under the cold-climate hypothesis on the evolution of viviparity in reptiles (Tinkle and Gibbons, 1977; Shine, 1985; Hodges, 2004), which links the evolutionary transition towards viviparity to high altitudes or latitudes, i.e. to cold climates.

III. Capítulo 2.

Plasticity and limitations of extended egg retention in oviparous *Zootoca vivipara* (Reptilia: Lacertidae)[*]

---

[*] Este capítulo está basado en *Rodríguez-Díaz T., Braña F. 2011. Plasticity and limitations of extended egg retention in oviparous* Zootoca vivipara *(Reptilia: Lacertidae).* Biological Journal of the Linnean Society, *102: 75-82.*

Resumen

En general, se acepta que la transición entre el oviparismo y el viviparismo en reptiles es un proceso gradual, resultado de la selección de una retención cada vez más prolongada de los huevos en el oviducto. Hemos examinado la plasticidad en la retención de los huevos en una población ovípara de la lagartija *Zootoca vivipara*, una especie con poblaciones ovíparas y vivíparas. Se forzó a un grupo de hembras de esta especie a retener su puesta *in utero* manteniéndolas en sustrato seco y se evaluó el efecto sobre el desarrollo embrionario, el éxito de eclosión y el fenotipo y desarrollo locomotor de la descendencia. La retención forzada de los huevos durante una semana afectó al estadio de desarrollo de los embriones en el momento de la puesta, así como a la robustez y desarrollo locomotor de las crías. Los embriones del tratamiento de retención forzada de los huevos alcanzaron, en el momento de la puesta, estadios de desarrollo 33-35 en la escala de estadios de desarrollo de Dufaure y Hubert (1961), más avanzados que los alcanzados por los embriones control (estadios 32-24). Sin embargo, los embriones de los huevos control alcanzaron un mayor nivel de desarrollo que los embriones de los huevos experimentales después de aproximadamente el mismo período de tiempo (una semana), sugiriendo que la retención forzada produjo un retraso en el desarrollo embrionario a pesar de que la temperatura de incubación era más baja que la temperatura en el cuerpo de la madre. Solo se encontraron diferencias significativas en el tiempo de incubación externa en uno de los dos años de estudio. El éxito de eclosión fue mucho más bajo en el grupo experimental con retención forzada de los huevos (21,1%) que en el grupo control (95,4%). Las cáscaras son más gruesas y contienen más calcio en el momento de la puesta que en la eclosión, lo que sugiere que la cáscara es una fuente de este mineral para los embriones durante el desarrollo. Sin embargo, no hay diferencias entre las

cáscaras control y las sometidas a retención forzada. Concluimos que hay limitaciones que dificultan el avance del desarrollo embrionario intrauterino más allá del momento normal de la puesta y que la retención prolongada de los huevos no representa claras ventajas para esta población de *Z. vivipara*. Sin embargo, el hecho de que algunos huevos tengan éxito después de la retención forzada podría ser ventajoso para aquellas hembras capaces de retener su puesta bajo condiciones climáticas desfavorables.

Abstract

The transition between oviparity and viviparity in reptiles is generally accepted to be a gradual process, the result of selection for increasingly prolonged egg retention within the oviduct. We examined egg retention plasticity in an oviparous strain of the lacertid lizard *Zootoca vivipara*, a species having both oviparous and viviparous populations. We forced a group of females of this species to retain their clutch *in utero* by keeping them in dry substrata, and assessed the effect on embryonic development, hatching success along with offspring phenotype and locomotor performance. Forced egg retention for one additional week affected the developmental stage of embryos at oviposition, as well as hatchling robustness and locomotor performance. Embryos from forced clutch retention treatment reached developmental stages 33-35 of the developmental scale of Dufaure and Hubert (1961) at oviposition time, more advanced than the stages reached by control embryos (stages 32-34). However, embryos from control eggs were more developed than embryos from experimental eggs after approximately the same period of time (one week), showing that embryonic development is retarded during the period of extended egg retention despite that external incubation temperature was lower than the temperature inside the mother's body. Significant differences in external incubation time were only found in one of the two years of study. Hatching success was much lower in the experimental group with forced egg retention (21.1%) than in the control group (95.4%). Eggshells were thicker and contained more calcium at oviposition than at hatching time, suggesting that the eggshell is a source of this mineral for embryos during development. No differences were found between control and treatment eggshells. We conclude that there are limitations which hinder the advance of intrauterine embryonic development beyond the normal time of oviposition and extended

egg retention does not represent clear advantages in this population of *Z. vivipara*. Nevertheless, the fact that some eggs are successful after forced egg retention could be advantageous for the females which are able to retain their clutch under unfavourable climatic conditions.

Introduction

It is generally accepted that viviparity in reptiles has evolved from oviparity and that such an evolutionary transition has come about gradually through selection for increasingly prolonged egg retention within the oviducts, with its implied progressive increase in the degree of intrauterine embryonic development (Tinkle and Gibbons, 1977; Shine, 1983; Andrews, 2002). For this reason, egg retention is considered to be a key component of the evolutionary progression to viviparity in reptiles and several studies have focused on the constraints and selective pressures that operate on the time that eggs are retained *in utero* before oviposition (Andrews and Rose, 1994; Mathies and Andrews, 1996; Warner and Andrews, 2003; Braña and Ji, 2007). Consequently, extended egg retention has achieved great importance in current models of the evolution of viviparity, particularly in the "cold-climate" model (Shine, 1985; Rodríguez-Díaz *et al.,* 2010). In this model, retaining eggs in cold environments is thought to be selectively advantageous as retained eggs are generally subjected to warmer temperatures than those in nests and, therefore, embryonic development proceeds faster *in utero*.

Considerable variation in the stage of development of the embryo at oviposition has been documented among Squamate reptiles, from chamaleonid lizards in which embryos are diapausing gastrulae at the time of oviposition (Andrews *et al.*, 2008) to viviparous lizards and snakes that bear fully developed hatchlings (Blackburn, 1995; Lourdais *et al.*, 2004). Between these extremes, the majority of oviparous squamates oviposit eggs with embryos at early limb bud stages (Shine, 1983; Andrews and Mathies, 2000). In addition to some intraspecific variability in the duration of egg retention under normal conditions (see, e.g., Braña *et al.,* 1991; Calderón-Espinosa *et al.,* 2006), many species of squamates are able to extend the period that eggs are retained in the oviduct beyond the normal time of oviposition in response to unfavourable (e.g., dry) environmental conditions (Stamps, 1976; Warner and Andrews, 2003). The consequences of extending egg retention vary greatly between species: in some species, embryonic development continues almost to the point of hatching during the extended time of retention (e.g. *Sceloporus scalaris*; Mathies and Andrews, 1996), whereas in other species development progresses by only one or two additional stages (e.g. *Sceloporus undulatus*; Warner and Andrews, 2003) or is completely arrested (e.g. *Urosaurus ornatus*: Mathies and Andrews, 1999; *Calotes versicolor*; Shanbhag *et al.,* 2003).

A potential limitation in the evolutionary transition to viviparity could be the increased demand of oxygen *in utero* as the embryo grows, which could not be entirely satisfied due to the existence of the eggshell, a barrier among maternal and foetal circulation. One of the predictions coming from the gradualistic model of the evolution of viviparity is that the progressive advance in the intrauterine embryonic development would lead to a parallel reduction in eggshell thickness and/or an increase in vascular density in the oviduct and in the extraembryonic membranes (Mathies and Andrews, 2000). Thus, the evolution of viviparity might be precluded in those organisms whose eggs require the protection of a thick, heavily calcified eggshell, and favoured in organisms whose eggs do not (Qualls, 1996). Eggshell thickness has been measured for oviparous and viviparous *Z. vivipara* (Heulin, 1990), a lacertid lizard being one of the very few amniote species in which there are viviparous and oviparous populations, and it has been determined that eggshells of oviparous populations of this species are much thicker and much more calcified than eggshells of viviparous populations. It has also been found out that eggshells of oviparous x viviparous hybrids of this species obtained under laboratory conditions, which lay eggs with embryos at developmental stages intermediate between those of oviparous and viviparous populations, show also intermediate thickness and level of calcification (Heulin *et al.,* 1992; Arrayago *et al.*, 1996). A similar pattern is found when comparing oviparous populations of *Z. vivipara* with different lengths of intrauterine egg retention (Heulin *et al.,* 2002), supporting the idea of a negative correlation between the length of intrauterine clutch retention and eggshell thickness.

The aim of this study was to determine egg retention plasticity and its consequences on incubation time, hatching success of eggs, thickness and mineral content of the eggshell, hatchling locomotor performance, and several hatchling phenotype traits which probably influence fitness, in order to evaluate the implications for the evolution of viviparity. To do this, we used *Zootoca vivipara* (Jacquin, 1797) (Squamata: Lacertidae) as the model organism. This organism, showing reproductive bimodality with each population exhibiting a characteristic and stable mode of reproduction (Braña, 1986), is an ideal model in which to study the evolution of viviparity, and hence variation in egg retention: phylogenetic differences that could lead to misinterpretations are minimized as oviparous and viviparous individuals are the same species, with otherwise similar morphological, physiological and ecological adaptations. In this study, we worked with individuals from a Cantabrian (North Spain) population of *Z. vivipara*, which lay eggs with embryos at quite advanced developmental stages (31-34 according to Dufaure and Hubert's 1961 developmental scale; see Braña, 1986; Braña *et al.*, 1991). Most oviparous Squamate reptiles lay eggs with embryos at around stage 30 (Shine, 1983), few species oviposit at stages 31-34, and very few species at stages 35-39 (Dufaure and Hubert's stage 40 represents full development as attained in live-bearing species). This suggests that some constraints must exist that make it difficult for embryonic development to progress inside the mother's body much beyond stage 30 (Andrews and Mathies, 2000).

Materials and Methods

We collected gravid females from an oviparous population located in Las Señales (León, northern Spain), between 1650 and 1750 m asl, during the spring-summers of 2006 and 2007. These individuals were transported to the Zoology laboratory of the University of Oviedo and were housed in terraria exposed to natural photoperiod. Terraria were also illuminated for 7 hours a day with 60W light bulbs which allowed behavioral thermoregulation. Refuges, dishes of water, and food (mealworms) were also provided. After egg-laying, all the adults collected, as well as hatchlings from experimental incubations were released into the wild at their places of capture.

Plasticity of egg retention was assessed by maintaining a group of females (*n*=29; experimental group) in terraria with dry soil as a substrate, which forces them to delay oviposition in response to drought conditions (Andrews and Rose, 1994). Another group of gravid females (*n*=29; control group) was kept in terraria with moist soil, an appropriate substrate for oviposition to occur at the normal time. Both groups were balanced as regards female size and reproductive state.

One week after females from the control group started to lay their eggs, we placed moist peat in the experimental terraria and experimental females started oviposition at that moment. Immediately following oviposition, clutches were collected from terraria and weighed. Then, one egg was dissected to determine the embryonic stage at oviposition according to the developmental scale of Dufaure and Hubert (1961). Embryos with traits intermediate between two stages of this scale were designed as half stages. The remaining eggs of each clutch were weighed and incubated individually at 25º C in plastic containers with vermiculite and distilled water (proportion 1/1 by mass) as substrate. Containers were periodically checked to verify that the vermiculite stayed moist and they were rotated daily inside the incubators to minimize possible effects of temperature gradients on development.

At the same time, we determined developmental stage and dry mass of a set of control embryos sampled at four-day intervals from oviposition to hatching, for use as a reference to compare with embryo stage after extended egg retention. These eggs were incubated at 25º C, a temperature which is considered to be close to the mean temperature experienced by eggs inside their mother's body, as the temperature selected in a laboratory thermal gradient by gravid females from the population used in this study is 32.33 ± 1.27º C (Rodríguez-Díaz *et al.*, 2010) and their body temperature is much lower than this temperature during the period of time (17 hours a day) in which they do not thermoregulate (laboratory temperature, mean minimum temperature ± sd: 18.56 ± 2.75º C, *n*=25; mean maximum temperature ± sd: 26.68 ± 4.53º C, *n*=25). In order to assess the relationship between embryo dry mass and incubation time, the curve was fit to an exponential function by log-transforming the dependent variable (see Andrews, 2004).

We studied the effect of forced egg retention on clutch mass, eggshell thickness and mineral content (percent by weight of calcium, magnesium, and potassium), stage of development at oviposition, incubation time, hatching success, and hatchling morphology (head, abdomen, and tail lengths, and robustness). A number of clutches (*n*=8) from the experimental group were not included in the final analyses (except for clutch size and developmental stage of embryos at oviposition) because they were laid in dry substrate and thus became highly dehydrated, so that no embryo from those clutches survived until hatching. We used one egg per clutch to analyse the thickness of the internal protein layer and mineral content of the eggshells at oviposition and at hatching time. Eggshells were cleaned with distilled water, cut using PASCHEFF-WOLFF's microsurgery scissors, air-dried at room temperature, and mounted on brass stubs using double-face Scotch tape. For each eggshell, cross sections and a sample showing the outer surface were coated with gold, using a coater Sputtering Balzers SCD 004, and photographed with a scanning electron miscroscope (JEOL-6100).

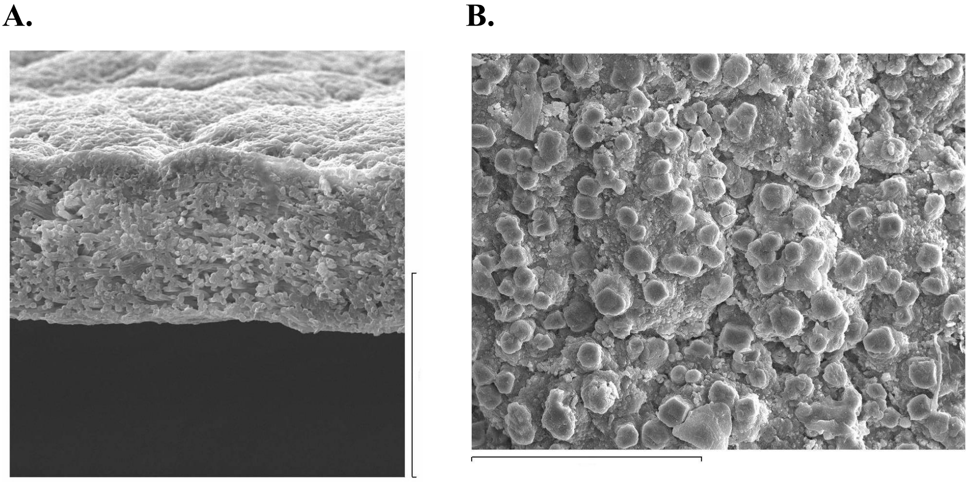


Figure 3.1. Cross section (**A**; scale bar = 50 μm.) and external mineral layer (**B**; scale bar = 100 μm.) of two eggshells of oviparous *Z. vivipara.*

We took an image of the cross section (Fig. 3.1A) and another one of the external mineral layer of each eggshell (Fig.3.1B) from control and forced retention groups and averaged the amount of calcium, magnesium, and potassium (percent by weight) of 8 areas of each image with an Oxford INCA Energy 200 Energy Dispersive Spectrometry (EDS) system. The thickness of the internal protein layer of each eggshell was estimated by averaging five different measurements taken in different sites along the cross section by using the Image-Pro plus image processing software (Version 1.1, Media Cybernetics Inc., Maryland, USA).

The morphological traits of hatchlings were measured *in vivo,* using a digital caliper (Vogel, Germany). We took only one hatchling from each clutch to avoid pseudoreplication problems and balanced the number of males and females to minimize the effects of sexual dimorphism in the analyses. Sex determination by eversion of hemipenes in males (Harlow, 1996) was carried out after locomotor performance tests to avoid excessive manipulation of hatchlings before conducting these trials.

The locomotor performance of hatchlings was evaluated as it provides data on a complex, integrative and ecologically meaningful variable likely influencing fitness (Van Damme *et al.*, 1992; Garland and Losos, 1994; Braña and Ji, 2000; but see Le Galliard *et al.,* 2004). Maximal sprint speed is thought to be selectively important in Squamates because, although this feature is seldom used in the lizard's everyday activities, it is of great importance, for example, in avoiding predators, and hence the faster the lizard runs, the higher its probability of surviving to breed (Braña, 2003). Sprint speed was tested within 48h of hatching and hatchlings were not fed until after the test. They were kept at 32º C for 30 minutes prior to testing as a way to ensure that all hatchlings were at the same temperature prior to running. The test was carried out in a one-meter-length corridor where the hatchling lizards were encouraged to run by tapping them lightly with a paintbrush. Races were recorded with a video camera at 30 frames per second. From the recording, we calculated: a) the speed in the fastest five consecutive frames (sprint speed); b) the number of stops; and c) the maximum distance covered between stops.

Statistical analyses

All data were checked for normality and homocedasticity (through Kolgomorov-Smirnov and Levene's tests, respectively) prior to further statistical analysis. A Mann-Whitney *U*-test was carried out to analyse stage of embryonic development at oviposition and a Generalized Linear Model (GLM) was performed to analyse the amount of calcium in the external mineral layer because these variables did not meet the assumptions needed to conduct parametric tests. The amount of potassium was log-transformed to meet these assumptions. Two-factor ANOVAs were used to analyse eggshell thickness and the amounts of magnesium and potassium of the eggshells. χ^2 tests between control and experimental groups were conducted to assess hatching success. Some clutches were not included in these analyses because they were extremely dehydrated at oviposition due to the fact that they had been laid in dry substrate. Incubation time, abdomen length and number of stops were log-transformed to approximate to normality.

One-factor analyses of variance (ANOVA) were carried out to analyse incubation time, head and abdomen lengths, and number of stops during trials. We also performed a number of ANCOVAs using the following covariates: female snout-vent length or SVL (analysis of clutch size); female mass (analysis of clutch mass); and hatchling SVL (analyses of tail length, hatchling mass, sprint speed, and the longest distance covered between stops). Significance level for all tests was set at $\alpha = 0.05$.

Results

Significant differences in embryos' stage of development at oviposition were found between groups (Mann-Whitney *U*-tests, 2006: *Z* adjusted = -3.869, $P < 0.001$; 2007: Z adjusted = -3.300, $P < 0.001$; Fig. 3.2).

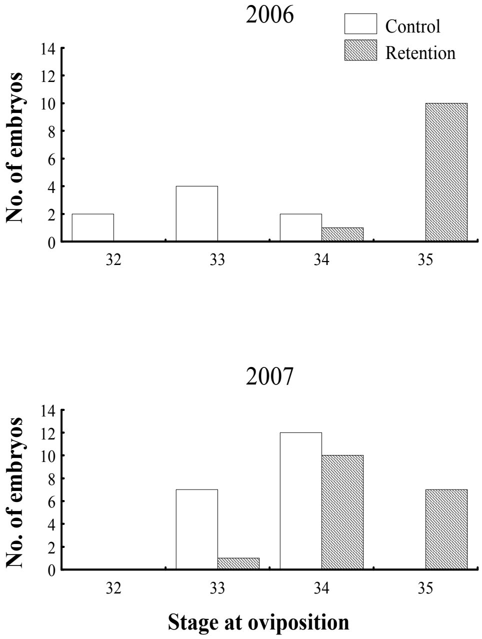


Figure 3.2. Development of embryos *Z. vivipara* from control and forced retention groups at oviposition in 2006 and 2007, according to the stages described by Dufaure and Hubert (1961).

Females in the control group laid eggs containing embryos at stages 32-34, according to Dufaure and Hubert's (1961) staging system, as previously found in the same population (Rodríguez-Díaz *et al.*, 2010), whereas the maximum stage attained by embryos from the experimental group was 35. In spite of this difference, we did not find a significant effect of forced egg retention on incubation time from oviposition to hatching (ANOVA, $F_{1,45}$ = 1.727, P = 0.195). We did, however, find significant differences in incubation time between years (ANOVA, $F_{1,45}$ = 31.946, $P < 0.001$) and for this reason, we analysed the effects of treatment on incubation time separately in 2006 and 2007. Forced egg retention affected incubation time in 2006 (control group: 20.44 ± 1.04 d, n=9; forced egg retention group:

17.80 ± 0.97 d, n=5; ANOVA, $F_{1,12} = 22.83$, $P < 0.001$) but not in 2007 (ANOVA, $F_{1,31} = 0.777$, $P > 0.05$), possibly due to the larger overlap of embryo stages between groups in 2007 (see Fig. 3.2).

Hatching success was much lower in the experimental group (32.39%) than in the control group (95.37%; $\chi^2 = 81.53$; $P < 0.001$). This result was not only explained by the differences in developmental stage at oviposition: although hatching success was particularly low in clutches at stage 35 (17.78%), differences for clutches at stage 34 in both treatments were also significant (experimental group: 58.33%; control group: 94.12%; $\chi^2 = 14.58$; $P < 0.001$). We found differences between years regarding clutch size (ANCOVA, $F_{1,42} = 31.680$, $P < 0.001$), but not in relation to clutch mass (ANCOVA, $F_{1,38} = 1.456$, $P > 0.05$). Clutch sizes were larger in 2007 than in 2006, which presumably reflects differences in the field conditions between years and different reproductive investment by females. However, differences between treatments were not found for any of these variables (for clutch size: ANCOVA 2006, $F_{1,18} = 0.518$, $P > 0.05$; 2007, $F_{1,21} = 2.222$, $P > 0.05$; for clutch mass: ANCOVA, $F_{1,38} = 0.477$, $P > 0.05$).

TABLE 1.-. Thickness of the internal protein layer (µm; mean±sd) and percent by weight (mean±sd) of calcium, magnesium, potassium, carbon and oxygen in the external mineral layer of eggshells of *Zootoca vivipara* at oviposition and hatching time. C=Control group; T=Treatment group: forced egg retention. Sample sizes are in brackets.

		Oviposition	Hatching			Oviposition	Hatching
Thickness	C	38.82±5.77 (14)	31.63±5.98 (14)	Mg	C	0.49±0.12 (14)	0.38±0.18 (14)
	T	43.74±7.84 (17)	28.90±6.00 (12)		T	0.47±0.21 (17)	0.43±0.19 (12)
Ca	C	38.72±2.16 (14)	27.35±10.09 (14)	K	C	0.23±0.08 (14)	0.28±0.23 (14)
	T	35.14±2.57 (17)	32.67±7.38 (12)		T	0.21±0.10 (17)	0.15±0.07 (12)

Eggshells were thicker at oviposition than at hatching time ($F_{1,53}$ = 39.626, $P < 0.001$; Table 1; Fig. 3.3A) and eggshell thickness was not influenced by treatment ($F_{1,53}$ = 0.391, P = 0.534). A between-factor interaction was also found ($F_{1,53}$ = 4.770, $P < 0.05$).

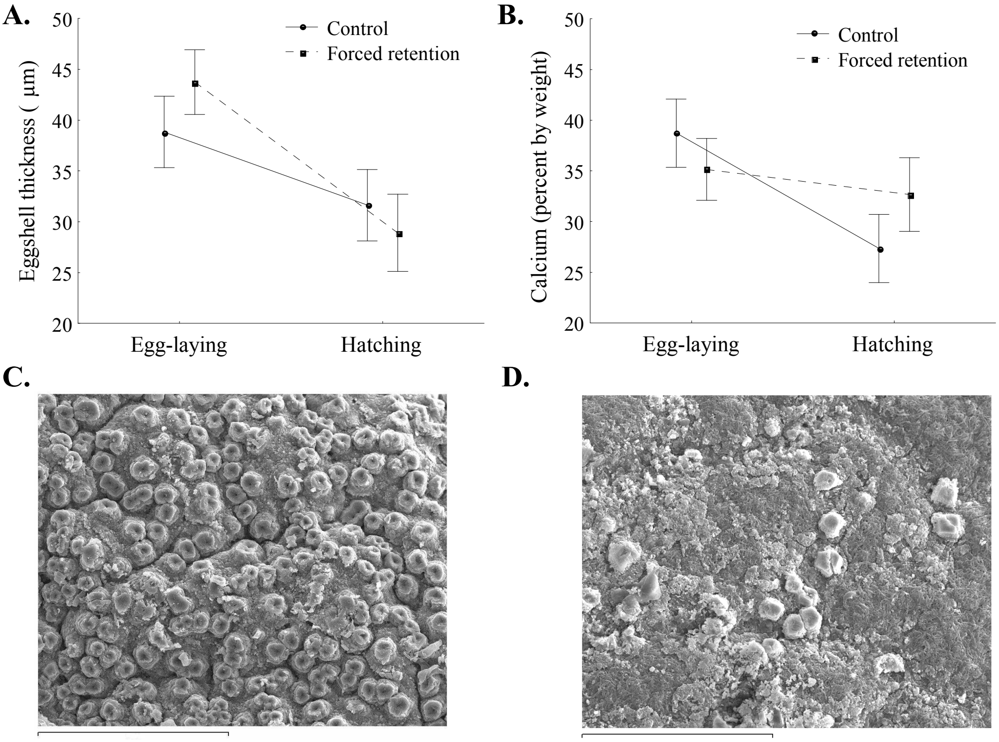


Figure 3.3. Eggshell thickness (**A**) and amount of calcium (**B**) at egg-laying and hatching time for control and forced egg retention groups and scanning electron micrographs of the external mineral layer of eggshells from control female *Z. vivipara* at oviposition (**C**) and at hatching time (**D**). Scale bars = 100 µm.

As regards eggshell mineral content (see Table 1), calcium was more abundant at oviposition than at hatching time (*Wald* $\chi^2(1)$ = 18.315, $P <$ 0.001; Fig. 3.3B, C, D), and this difference was more clear for control eggshells (between-factor interaction: *Wald* $\chi^2(1)$ = 7.579, $P < 0.01$). The amount of magnesium and potassium did not vary during the external incubation (Mg: $F_{1,53}$ = 2.665, $P > 0.05$; K: $F_{1,53}$ = 1.506, $P > 0.05$), and none of the studied elements were influenced by treatment (Ca: *Wald* $\chi^2(1) = 0.287$, $P = 0.592$; Mg: $F_{1,53} = 0.090$, $P = 0.765$; K: $F_{1,53} = 3.511$, $P = 0.066$).

Preliminary analyses showed that the morphology and locomotor performance of hatchlings did not differ between years. For this reason, only treatment was used as a factor in the analyses of these variables. Experimental manipulation had no significant effects on most morphological traits (head, abdomen and tail length), but hatchlings from the control group were more robust (size corrected mass). With regard to locomotor performance, control hatchlings stopped less often during trials than those from the retention group (Table 2) and no significant differences were found in sprint speed or maximum distance covered without stopping during trials.

TABLE 2.- Effects of forced egg retention on morphology and locomotor performance of hatchling *Zootoca vivipara* (mean ± sd)[1]

Hatchling traits		Control group	Retention group	Treatment effects
Head (mm)	M	7.16±0.49	7.44±0.45	$F_{1,43}$=0.592 ns
	F	7.24±0.53	6.79±0.40	
Abdomen (mm)	M	13.41±0.39	13.36±0.72	$F_{1,44}$=1.512 ns
	F	14.96±0.74	15.00±0.58	
Tail (mm)	M	26.85±1.25	26.63±1.65	$F_{1,43}$=2.133 ns
	F	26.52±1.82	25.89±0.82	
Robustness (g)	M	0.229±0.019	0.216±0.010	**$F_{1,43}= 7.505{**}$** **C>T**
	F	0.231±0.023	0.222±0.013	
Sprint speed ($cm \cdot s^{-1}$)	M	40.96±6.50	36.00±7.75	$F_{1,43}$=1.963 ns
	F	36.60±7.19	36.33±5.07	
Number of stops	M	0.088±0.048	0.151±0.096	**$F_{1,45}=3.927{*}$** **C<T**
	F	0.118±0.070	0.138±0.072	
Distance (cm)	M	18.58±5.57	17.43±10.42	$F_{1,42}$=0.046 ns
	F	16.27±8.46	16.89±7.43	

[1] *F* ratios correspond to single effects of treatment in ANOVAs (head and abdomen length, number of stops) or ANCOVAs (tail, robustness, sprint speed and maximum distance covered during trials with SVL as covariate). Descriptive statistics for abdomen length and number of stops are presented as direct values but analyses were carried out on log transformed variables. M = Male, F = Female. C=Control, T=Treatment. Symbols immediately after *F* values represent significant levels: ns P>0.05, *P<0.05 **P<0.01.

Embryos of the subsample of eggs used to assess variation of embryo dry mass and developmental stage along the external incubation, were at stages 33-34 (*n*=14) at oviposition, and reached stages 34.5-36 (*n*=9) after 4 days and 37-37.5 (*n*=9) after 8 days of external incubation at 25º C. After that, embryonic development reached stages 38.5-39.5 (*n*=9) the day 12 and stage 40 (*n*=9) the day 16. On the other hand, after seven days of forced egg retention, eggs contained embryos at stages 33-35 (*n*=29), less developed than those from control eggs after 4 days (Mann-Whitney *U*-test adjusted $Z = 3.317$; $P < 0.001$) and after 8 days of external incubation (adjusted $Z = 4.783$; $P < 0.001$). Dry mass of embryos increased exponentially with incubation time (Fig. 3.4). In the first four days following oviposition embryo mass increased slowly, after which increase in mass proceeded at a much faster rate.

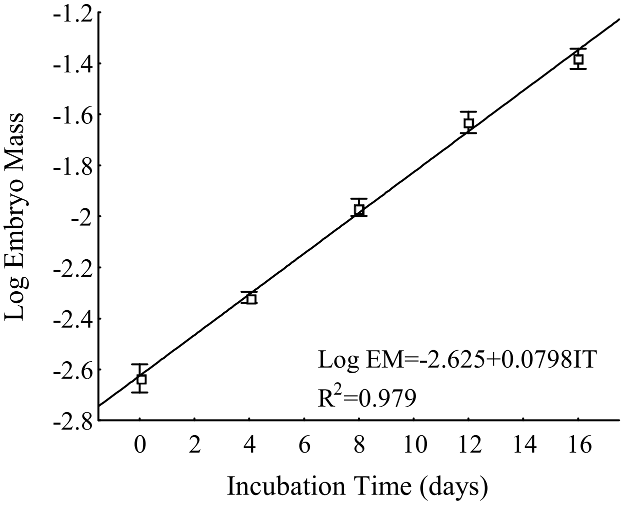


Figure 3.4. Increase in embryo dry mass during external incubation at 25º C in eggs of *Z. vivipara*. EM=Embryo mass; IT=Incubation time.

Discussion

After one week of forced egg retention, most female *Z. vivipara* laid eggs containing embryos at stages 34 and 35, i.e. one stage unit beyond the maximum stage reached by embryos from females allowed to lay their eggs at the normal time of oviposition (stages 32-34 according to Dufaure and Hubert developmental scale, 1961; this study, Rodríguez-Díaz *et al.*, 2010), with this upper limit in embryonic stage at oviposition being very marked, as no female produced eggs with embryos beyond stage 35.

In *Z. vivipara,* as in many other lizard species, embryonic development proceeds faster at higher incubation temperatures (*Z. vivipara*, this population: Rodríguez-Díaz *et al.*, 2010). However, in spite of the fact that thermoregulating pregnant females maintain a body temperature higher than the environmental temperature and higher than our experimental incubation temperature (25º C), intrauterine embryogenesis is retarded during the retention period in this species. This is evidenced by the high degree of development attained during the first week of external incubation in control eggs (stages 37-37.5), which contrasts with the maximal embryo stages attained *in utero* by experimental embryos (stages 33-35). A similar retardation in embryogenesis during extended egg retention occurs in the sceloporine lizard *Sceloporus undulatus* (Mathies, 1998, Warner and Andrews, 2003).

A shortage of oxygen supply inside the oviducts, as a consequence of increasing demands of growing embryos (Andrews and Mathies, 2000; Parker and Andrews, 2006) could be a reason why embryonic development does not continue beyond stage 35 inside the mother’s body, as it has been demonstrated that low oxygen concentrations *in utero* have negative effects on differentiation and growth (Parker *et al.,* 2004). Moreover, eggshell should be thin enough to permit gas exchange during prolonged egg retention (Guillette, 1991; Andrews and Rose, 1994). In fact, experimental hybridizations between oviparous and viviparous strains of *Z. vivipara*, in which embryos reach stages 35-36, produce eggs with intermediate eggshell thickness and scarcely mineralized eggshells (Heulin *et al.*, 1992; Arrayago *et al.,* 1996). However, eggshells of the forced retention treatment were not thinner or less calcified than control eggshells, maybe because embryonic development only attained stage 35 and the uptake of calcium from the eggshell takes place during the final phases of the incubation period (Shadrix *et al.*, 1994). Another limitation for intrauterine embryogenesis beyond the normal time of oviposition could be that females might not be able to bear the increase in mass and volume that eggs would undergo if development progressed normally: embryos experience a significant increase in dry mass during the first week of external incubation (see Fig. 3.4) and also dramatically increases water uptake during development (Mathies and Andrews, 1996; Andrews and Mathies, 2000).

The percentage of calcium in the eggshell at hatching time is much smaller than it is at oviposition time, which would mean that embryos *Z. vivipara* take this mineral from the eggshell during development, agreeing with previous studies on the same species in which embryos have been reported to obtain 81% of their calcium from the eggshell (Stewart *et al.*, 2009). Embryos of other lacertid lizard, *Podarcis muralis,* take 53% calcium and 20% magnesium from the eggshell (Ji and Braña, 1999), and minerals are also taken from the eggshell in other oviparous organisms (Tompa, 1975; Packard *et al.,* 1984; Thompson *et al.,* 2001; Osborne and Thompson, 2005). Dependence on eggshell calcium could be a constraint for the evolution of viviparity, as a reduction in eggshell thickness would reduce the amount of calcium available for embryonic metabolism, unless the amount of minerals increases in the yolk while the eggshell thickness is progressively reduced (Packard *et al.,* 1977). However, it has been recently found for *Z. vivipara* that the mechanism of calcium transport is conserved during the transition to viviparity, which would explain the large number of independent origins of this mode of reproduction among squamates (Stewart *et al.,* 2011).

Females of some lizard species can delay oviposition until the appearance of favourable conditions if nest-sites are too dry to lay the eggs (Andrews and Rose, 1994; Mathies and Andrews, 1996) and this plasticity could favour the selection of more advanced developmental stages at oviposition in those species in which embryonic development advances during egg retention. Some females of our study population of *Z. vivipara* (*n*=11) laid their eggs after a few days of forced clutch retention even when wet substrate was not available, showing that an advance in embryonic development beyond the normal time of oviposition involves some difficulties, as has been previously found in other lizard species (Mathies, 1998). This behaviour decreases a female's reproductive success because clutches laid under drought conditions become dehydrated and are thus lost (Packard, 1991). Hatching success of normal (i.e., non dehydrated) clutches was in any case very low for eggs hatched after the forced egg retention time (32.39% vs. 95.37% for control clutches), further reinforcing the idea that there is a viability limitation at Dufaure and Hubert's stage 35 and beyond.

Despite the difference in intrauterine incubation time (one week longer in the experimental group) and the corresponding difference in embryo developmental stage at oviposition (more advanced in embryos from the forced retention treatment), significant between-treatment differences were not found in external incubation time in 2007. A possible reason explaining this result is the high level of overlap of embryonic stages at oviposition between control and forced retention groups that year: although all experimental females were maintained in dry substrates for one additional week after the control females started to lay eggs, it is possible that some of them might have not actually experienced extended egg retention, being the experimental period part of the normal retention time. Moreover, hatching success was particularly low in 2007 in clutches with embryos at stage 35 at oviposition, which makes the overlap wider for the remaining clutches over which incubation time was measured.

In addition to the above mentioned effects on embryonic development, forced egg retention has some direct effects on aspects of offspring phenotype which are likely to affect fitness. Hatchlings from the forced retention treatment did not exhibit morphological differences with respect to control individuals, but were less robust, which might make them more vulnerable as prey, less able to get food and less resistant to prolonged fasting (Ferguson and Bohlen, 1978). Furthermore, although no significant differences were found with respect to sprint speed, experimental hatchlings showed a higher frequency of pauses during the tests. Pausing while fleeing could be connected to a lizard’s inclination to run (Sorci *et al.*, 1995; Braña, 2003) such that a high number of pauses would be a behavioural trait with negative consequences on hatchling fitness as regards, for example, their ability to avoid predators.

Extended egg retention is an important component of the evolution of viviparity in Squamate reptiles because the oviparity-viviparity transition is considered to be the result of a progressive increase in the level of intrauterine embryonic development (Tinkle and Gibbons, 1977; Shine, 1985). According to our findings, there is some plasticity in egg retention in the studied population of *Z. vivipara* but also limitations that reduce the amount of intrauterine embryonic development beyond the normal time of oviposition in this species. However, the fact that some eggs are successful after forced egg retention could be advantageous for the females that, under unfavourable climatic conditions, are able to retain their eggs until more suitable conditions. We have not studied long-term effects of egg retention, and it remains unknown whether, in spite of the low hatching success, retention beyond the normal time of oviposition could have some kind of beneficial effects on survival and reproduction of hatchling *Z. vivipara*, as has been found in other lizard species (Warner and Andrews, 2003).

IV. Capítulo 3.

Effects of incubation temperature on hatchling phenotypes in an oviparous lizard with prolonged egg retention: are the two main hypotheses on the evolution of viviparity compatible?*

---

*Este capítulo está basado en *Rodríguez-Díaz T., González F., Ji X., Braña F. 2010. Effects of incubation temperature on hatchling phenotypes in an oviparous lizard with prolonged egg retention: are the two main hypotheses on the evolution ofviviparity compatible?* Zoology, *113: 33-38.*

Resumen

Las hembras de varias especies de lagartos modifican su temperatura corporal durante la gestación, probablemente en relación con la optimización de los fenotipos de las crías. Estudiamos las variaciones en la temperatura seleccionada por las hembras gestantes comparadas con las seleccionadas por los machos y las hembras no gestantes en una población ovípara de *Zootoca vivipara* (Jacquin, 1797; Squamata: Lacertidae) del norte de España y examinamos los efectos de la temperatura de incubación sobre la variación fenotípica de las crías. Las temperaturas cloacales de las hembras grávidas activas en el campo fueron más bajas que las de machos y hembras no gestantes, al igual que las temperaturas seleccionadas en un gradiente térmico creado en el laboratorio (media ± DE: 32,33 ± 1,27º C para hembras gestantes; 34,05 ± 1,07º C para machos y hembras no gestantes). Los efectos de la temperatura se evaluaron incubando los huevos a 5 temperaturas constantes (21º, 25º, 29º, 32º y 34º C). El tiempo de incubación disminuyó al aumentar la temperatura siguiendo una función exponencial negativa. Las temperaturas de incubación también afectaron a la morfología de las crías: las crías incubadas a 34º C tenían cabezas más cortas que las de otras temperaturas. La supervivencia a 34º C (58%) fue significativamente más baja que a las otras temperaturas (media, 93%). Las hembras gestantes seleccionan temperaturas corporales más bajas, aproximándose a las temperaturas que optimizan los fenotipos de las crías, según las predicciones de la hipótesis de “manipulación materna” sobre la evolución del viviparismo. El cambio en la temperatura preferida de las hembras gestantes resultaría sólo en un retraso muy corto, en caso de haberlo, del momento de la eclosión y, como la temperatura seleccionada por las hembras gestantes es mucho más alta que las temperaturas medias registradas en nidos naturales de *Z. vivipara*, la retención de los huevos acorta considerablemente el tiempo

de incubación, según las predicciones de la hipótesis de "clima frío". Nuestros resultados experimentales indican que las dos principales hipótesis sobre la evolución del viviparismo son compatibles en nuestro modelo de estudio.

Abstract

Females of several lizard species modify their body temperature during pregnancy, probably in connection with the optimization of hatchling phenotypes. We studied variations in the temperature selected by gravid females compared with those selected by males and non-gravid females in an oviparous population of *Zootoca vivipara* (Jacquin, 1797; Squamata: Lacertidae) of Northern Spain and examined the effects of incubation temperature on phenotypic variation of hatchlings. Cloacal temperatures of gravid females active in the field were lower than those of males and non-gravid females, as well as the temperatures selected in a thermal gradient created in the laboratory (mean ± sd: 32.33 ± 1.27º C for gravid females; 34.05 ± 1.07º C for males and non-gravid females). Effects of temperature were assessed by incubating eggs at five constant temperatures (21º, 25º, 29º, 32º and 34º C). Incubation time decreased as temperature increased following a negative exponential function. Incubation temperatures also affected hatchling's morphology: hatchlings incubated at 34º C had shorter heads than those from other temperatures. Survival at 34º C (58%) was significantly lower than at the other temperatures (mean 93%). Pregnant females select lower body temperature, approaching the temperatures that optimise hatchling phenotypes, according to predictions of the "maternal manipulation" hypothesis on the evolution of viviparity. The shift in preferred temperature by pregnant females would result in only a very short delay, if any, of hatching time and, because the temperature selected by pregnant females is much higher than average temperatures recorded in natural nests of *Z. vivipara*, egg retention considerably shortens incubation time, according to predictions of the "cold-climate" hypothesis. Our experimental results indicate that the two main hypotheses on the evolution of viviparity are compatible in our study model.

Introduction

Maternal effects and environmental conditions during embryonic development have a great influence on an organism's phenotype and can affect fitness in reptiles (Shine and Harlow, 1996; Deeming, 2004). Effects induced by developmental environment have special importance in oviparous species because eggs are exposed to a wide variation of environmental factors, which may have detrimental effects on embryo phenotypes if certain limits are reached (Flatt *et al.,* 2001). Temperature is one of the most relevant environmental factors inducing phenotypic variation in ectothermic vertebrates (Johnston and Bennett, 1996). In squamate reptiles (lizards and snakes), incubation temperature influences incubation time, hatching success and several hatchling traits (e.g. Birchard, 2004; Deeming, 2004; Booth, 2006). There are, however, interespecific differences in the way that hatchlings are affected by incubation temperature. High incubation temperatures, within the range that produces viable offspring, are detrimental to hatchlings of some lizard species (e.g. *Podarcis muralis*: Van Damme *et al.,* 1992; Braña and Ji, 2000), whereas low incubation temperatures produce "poor-quality" hatchlings in other species (e.g. *Sceloporus virgatus*: Qualls and Andrews, 1999a). Furthermore, pregnant females of several lizard species modify their body temperature by increasing or decreasing it in comparison to non-pregnant females (Beuchat, 1986; Braña, 1993; Shine, 2006). The change in preferred temperature during pregnancy could be selected to provide suitable temperatures either for embryonic development or for physiological processes of the female (Beuchat and Ellner, 1987; Beuchat, 1988; Mathies and Andrews, 1997).

In addition to the choice of nest-site or the season in which incubation takes place (Shine, 2002b; Birchard, 2004), maternal thermoregulation during the intrauterine phase can importantly affect egg temperature. This could be especially relevant in viviparous lizards or in oviparous species such as *Zootoca vivipara*, in which females retain eggs for an important fraction of development (Braña *et al.,* 1991; this article). *Zootoca vivipara* (Jacquin, 1797) (Squamata: Lacertidae), is the only lacertid lizard which exhibits intraspecific reproductive bimodality, being viviparous in most populations of its wide Eurasian distribution but oviparous in the southernmost limits of its range (Surget-Groba *et al.,* 2001). Oviparous populations exhibit prolonged egg retention (Braña *et al.,* 1991; Heulin *et al.,* 1991) and likely represent the ancestral stage to current viviparous populations (but see Surget-Groba *et al.,* 2006). While pregnant, females of some populations of *Z. vivipara* select lower body temperatures than do non-pregnant females (see, for viviparous populations, Van Damme *et al.,* 1986; Heulin, 1987; for oviparous populations, Carretero *et al.,* 2005; this article). Therefore, it is likely that temperature plays a relevant role on embryonic development in this species.

The aim of this study was to assess the influence of reproductive status on maternal thermoregulation and to evaluate the effect of incubation temperature on the time of embryonic development as well as on some traits of hatchling phenotype that could be relevant for offspring fitness. There are two outstanding hypotheses on the evolution of viviparity in reptiles. On the one hand, the classical cold-climate hypothesis proposes that prolonged uterine egg retention is favoured in cold climates because eggs develop faster at maternal body temperatures than at soil temperatures and retention enhances survivorship in unfavourable climates (Tinkle and Gibbons, 1977). On the other hand, the maternal manipulation view proposes that thermal conditions during embryogenesis *in utero* enhance relevant phenotypic traits of the offspring directly, not through shortening development time (Shine, 1995). Actually, the cold-climate hypothesis could be considered in some instances as just a special case of a broader maternal manipulation hypothesis (Webb *et al.,* 2006). However, both views can be conflicting if, for example, temperatures that enhance offspring phenotypes inside the mother's oviduct prolong incubation. The present study aims to offer an insight into the compatibility of both hypotheses as well as into the selective forces acting in a critical phase of development in oviparous populations of one of the very few amniote species that exhibit intraspecific reproductive bimodality.

Materials and Methods

Zootoca vivipara is a small (adult snout-vent length, in our sample, 40-64 mm) ground-dwelling lizard inhabiting a variety of vegetated and wet habitats, such as peat bogs, meadows and humid heathlands. The reproductive phenology of oviparous populations of *Z. vivipara* in the Cantabrian mountain range has been described by Braña (1986): females reproduce once a year and lay clutches of 2-11 eggs; the ovulation takes place generally in early June and the oviposition occurs generally in July. Gravid females of *Z. vivipara* were captured by hand from a highland population in Las Señales (León, Northern Spain; elevation: 1650 to 1750 m asl) and were transported to the Zoology laboratory in the University of Oviedo. Lizards were housed in terraria exposed to natural photoperiod in which suspended bulbs provided lizards opportunity for behavioural thermoregulation. Dishes of water, food (mealworms and crickets) and an appropriate substrate to lay eggs were also provided. After egg-laying, eggs were individually weighed and assigned to one of the incubation treatments. Although we couldn't get a balanced design to analyse family effects (as regards female parent), due to the reduced clutch size (3-9 eggs per clutch in the used sample), we distributed the eggs as equally as possible among treatments. Anyway, family effects can be considered irrelevant to the overall results because of the high number of females and the multiple paternity within clutches in this species (Laloi *et al.,* 2004). One egg of each clutch was dissected to ascertain the embryonic stage at oviposition, according to the development table by Dufaure and Hubert (1961). The remaining eggs were individually incubated in 50-ml covered plastic containers at one of five constant temperatures: 21º, 25º, 29º, 32º and 34º C. We kept a constant moisture level at all incubation temperatures (2g distilled water/1 g vermiculite). In addition, a small number of eggs (*n*= 4) were incubated at 17º C in order to extend the range and improve the fitting of the relationship between incubation time and temperature.

In order to have a general reference about conditions in which external incubation takes place in the field, we carried out continuous recordings of temperature by placing miniature thermal data-loggers (TidbiT v2, Onset Computer Corporation, Massachusetts, USA) in nest locations of clutches of *Z. vivipara* from May to October in 2006 and 2007, including retention (May-June) and external incubation (July-September) periods. Data from two actual and three potential nest locations, that is, close places similar to those where clutches of *Z. vivipara* had been found, were recorded. The two lowest temperatures utilised in our experimental incubations (21º and 25º C) are in the range of temperatures registered in natural nests of *Z. vivipara* during the external incubation period (Fig. 4.1). 29º and 32º C are over this range, but they are temperatures frequently reached during the early embryonic development inside the mother's body. Finally, 34º C is an incubation temperature higher than that selected by gravid females in a thermal gradient in the laboratory but is roughly the temperature "preferred" by males and non-gravid females (see Results).

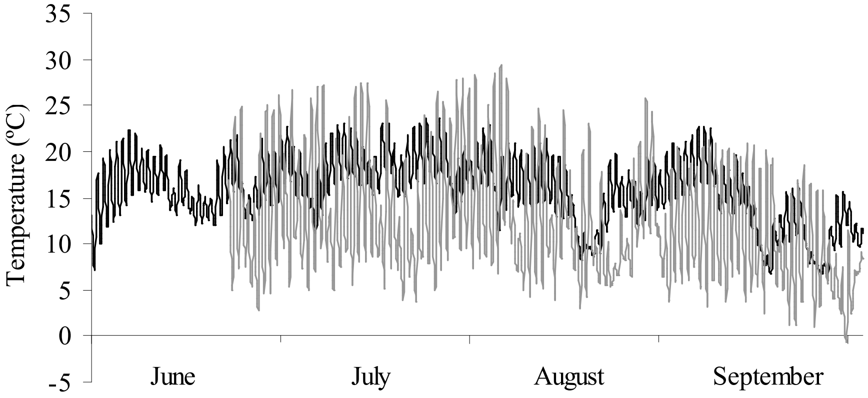


Figure 4.1. Temperatures registered in potential nests of *Z. vivipara* during the incubation period (one record per hour from June to September). Two representative series were depicted: 1650 m asl-2006 (black) and 1750 m asl-2007 (grey).

1. Modification of body temperature during gestation

According to the model which connects the evolution of viviparity with thermal optimization in early stages of embryogenesis (Shine, 1995), females modify their body temperature during pregnancy in several lizard species. To check this pattern in our study population, we measured the body temperature of 143 adult lizards active in the field in the reproductive period, using a quick-reading cloacal *Schultheis* thermometer (Miller and Weber, Inc.). Further, we measured the substrate temperature at the point where individual was first observed and the air temperature at 10 cm above that point. These data were compared with the temperatures "selected" by the individuals, which behave as heliothermal organisms in a laboratory thermal gradient, where they can regulate its body temperature with a minimum of associated costs (Braña, 1993). Temperature measurements of gravid females were taken in the week before oviposition, so that the embryonic development stage was roughly the same for all gravid females at that time.

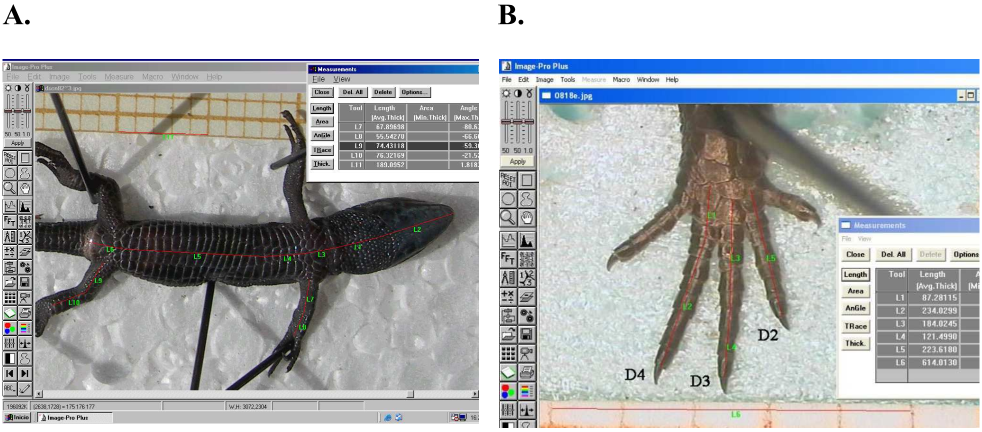
A heat source (60W light bulb) was placed in one extreme of a terrarium to establish a thermal gradient between the coldest zone (substrate temperature, $T_s = 22.72 \pm 1.70º$ C; air temperature, $T_a = 22.84 \pm 0.85º$ C) and the warmest one ($T_s = 37.34 \pm 3.62º$ C; $T_a = 28.36 \pm 1.09º$ C). An hour and a half after locating the lizards in the thermal gradient, we measured their body temperatures with a *Schultheis* quick-reading thermometer. We placed no more than 7 individuals in the gradient to avoid competitive interference for basking places and we took just one measurement of body temperature from each individual (Fig. 4.2).

Figura 4.2. Male *Zootoca vivipara* thermorregulating under the bulb in the thermal gradient created in the laboratory.

One-factor analyses of variance were carried out to elucidate whether there were differences between the body temperature of males, non-gravid females and gravid females in the thermal gradient as well as in the field. Differences in field body temperatures among males, non-gravid females and gravid females, were further assessed by analysis of covariance with substrate and air temperatures as covariates. *A posteriori* comparisons were made with Fisher LSD tests.

2. Effects of incubation temperature on incubation time and hatchling phenotype.

Hatchlings were weighed immediately following birth, sexed by eversion of hemipenes in males (Harlow, 1996), and measured using the Image-Pro plus image processing software. The following measurements were taken: head, abdomen, forelimb, hind limb (Fig. 4.3A) and tail lengths. These size measurements were taken to assess how incubation temperature affected each morphological trait separately since each of these traits have influence on hatchling fitness and most of them are sexually dimorphic. For example, large abdomen might favour female reproductive success by rising clutch size or clutch volume (Braña, 1996), whereas large head size favours male reproductive success through enhanced fighting and grasping ability (Gvoždík and Van Damme, 2003). Besides, head length, as well as body size, has influence on bite capacity, an important ecological attribute in adult *Z. vivipara* because of its influence on prey selection (Herrel *et al.,* 2001). Furthermore, males have longer tails than females and Hoffmann and Henle (2006) have found that tail length of male *Z. vivipara* is related to their reproductive success, since the longer the tail length of males, the higher the number of offspring sired. We also measured the length of digits 2, 3 and 4 (D2, D3 and D4 respectively, numbered from the inner to the outer side; Fig. 4.3B) of the right forelimb because sexual dimorphism in digit length ratios has been reported for three species of lizards (Chang *et al.,* 2006; Rubolini *et al.,* 2006).



A.

Tool	Length (Avg.Thick)	Area (Min.Thick)	Angle (Max.Th
L7	67.89698		-80.6
L8	55.54278		-66.6
L9	74.43118		-59.3
L10	76.32169		-21.5
L11	189.0952		1.818

B.

Tool	Length (Avg.Thick)
L1	87.28115
L2	234.0299
L3	184.0245
L4	121.4990
L5	223.6180
L6	614.0130

Figure 4.3. Screen captures of the Image Pro Plus software while measuring **A.** Head, abdomen, forelimb, and hindlimb lengths of a hatchling *Z. vivipara*. **B.** The length of digits 2, 3, and 4 (D2, D3, and D4 respectively).

All data were checked for normality (Kolgomorov-Smirnov test) and for homocedasticity (Bartlett's test), prior to further statistical analysis. Some variables (body mass, egg mass, head, abdomen and forelimb lengths) were log-transformed to approximate to these assumptions for using parametric tests in the analyses. Incubation time did not meet the above assumptions and did not improve with simple transformations; for this reason, it was analysed by the nonparametric Kruskal-Wallis test. Two-factor analyses of covariance with snout-vent length (SVL) as the covariate were used to determine whether incubation temperature and sex influenced body mass, tail, forelimb, hind limb and digits lengths. In the case of head and abdomen lengths we used log egg mass instead of SVL as the covariate, because these measures are parts of SVL. Hatchling mass was not used as the covariate because males are more robust than females (see Results). Significance level for all tests was set at $\alpha = 0.05$. *A posteriori* comparisons among means were assessed with Fisher LSD tests.

Results

1. Modification of body temperature during gestation

Mean body temperature “preferred” by gravid females is lower than that “preferred” by males and non-gravid females in the thermal gradient (Table 1; ANOVA: $F_{2,216} = 54.449$, $P < 0.001$; Fisher LSD test *a posteriori*). Temperatures of lizards active in the field were considerably lower than temperatures selected in the thermal gradient. Mean cloacal temperature of gravid females active in the field was also lower than that of the other two groups (Table 1; ANOVA: $F_{2,140} = 4.977$, $P < 0.01$; and ANCOVA: $F_{2,119} = 3.823$; $P < 0.05$; Fisher LSD test). In addition, gravid females were found in the field on cooler substrates than the other individuals (Table 1; ANOVA: $F_{2,124} = 3.712$; $P < 0.05$; Fisher LSD test). Air temperatures at the lizard’s location in the field did not differ among groups (ANOVA: $F_{2,122} = 1.439$, $P > 0.05$).

TABLE 1.- Body temperatures of adult *Z. vivipara* according to their reproductive condition (in thermal gradient and in the field) and substrate and air temperatures in those places where they were in the field (ºC, mean ± sd). Sample sizes corresponding to field and laboratory measurements are in brackets. M = Males; F = Females.

	Reproductive condition		
	M	Non-gravid F	Gravid F
Tb gradient	34.25±0.96 (58)	33.94±1.12 (97)	32.33±1.27 (64)
Tb field	29.77±2.70 (39)	29.20±2.89 (41)	27.91±3.33 (63)
T substrate	24.39±3.60 (31)	23.97±3.57 (40)	22.31±4.14 (56)
T air	20.48±3.15 (30)	20.13±2.77 (39)	19.29±3.83 (56)

2. *Effects of incubation temperature on incubation time and hatchling phenotype*

Eggs dissected at oviposition contained embryos at stages 31 to 34, according to Dufaure and Hubert's (1961) developmental series. Incubation temperature clearly influenced the duration of embryonic development (Kruskal-Wallis test: H (5, 168) = 157.58; $P < 0.001$) and the function that relates both variables is a negative exponential one (Fig. 4.4). Incubating eggs at 21º C reduced incubation time to half (mean ± sd: 40.51 ± 2.21 days) compared with incubating eggs at 17º C (86.00 ± 2.83 d), whereas there were no important differences in incubation time between 32º C and 34º C (12.57 ± 0.65 d and 12.39 ± 0.50 d).

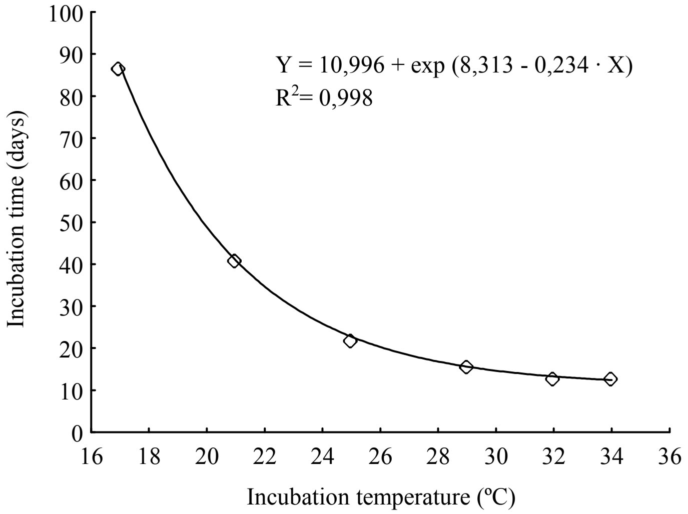


Figure 4.4. Relationship between incubation temperature (constant) and duration of the incubation (mean ± sd) of common lizard eggs.

There were marked between-sex differences in several hatchling traits: males are heavier (relative to SVL) and have larger tail and legs than females, whereas females have longer abdomen (Table 2). Besides, males have larger digits (D3 and D4) than females (ANCOVAs: $F_{1,121} = 6.854$; $P <$

0.01 for D3 and $F_{1,121} = 6.228$; $P < 0.05$ for D4). No influence of sex was found on D2 length ($F_{1,121} = 1.623$; $P > 0.05$).

Incubation temperature significantly influenced survival and morphology of the survivors. Survival at 34° C (58%, n=31) was much lower than at the other incubation temperatures (93%, n=156; χ^2 test: $\chi^2 = 42.203$, degrees of freedom = 4, $P < 0.001$). Most dead embryos were full-term ones with deformed heads and unable to hatch (Fig. 4.5). Abdomen and tail lengths were larger in hatchlings from higher incubation temperatures whereas head was much shorter in hatchlings from the highest incubation temperature (Fig. 4.6; Table 2). Hatchlings from the lowest and the highest incubation temperatures had shorter forelimbs.

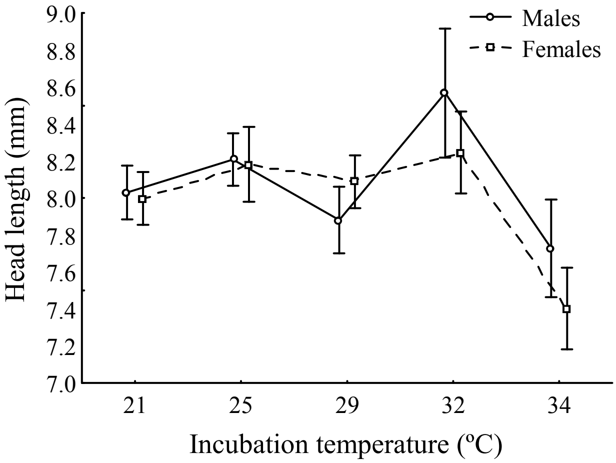


Figure 4.5. Effects of incubation temperature and sex on head length (mean and 95% confidence intervals were represented for each measurement).

TABLE 2.- Phenotype of hatchling common lizards (*Zootoca vivipara*) according to sex and incubation temperature (mean ± sd).

Hatchling trait		Incubation temperature					Effects		
		21ºC	25ºC	29ºC	32ºC	34ºC	Sex	Temperature	Interaction
Body mass (g)	M	0.219±0.02	0.221±0.01	0.216±0.02	0.234±0.02	0.231±0.03	**$F_{1,142}$=14.00** M>F**	$F_{4,142}$=1.38 ns	$F_{4,142}$=1.13 ns
	F	0.223±0.01	0.230±0.03	0.226±0.02	0.222±0.02	0.228±0.04			
Head (mm)	M	8.00±0.29	8.23±0.39	7.88±0.36	8.59±0.37	7.70±0.20	$F_{1,142}$=2.23 ns	**$F_{4,149}$=11.82*** (25,32)>(21,29)>34**	$F_{4,142}$=2.31 ns
	F	7.99±0.33	8.22±0.50	8.12±0.44	8.21±0.31	7.38±0.40			
Abdomen (mm)	M	12.15±0.64	12.86±0.98	12.12±0.57	13.15±1.18	13.76±1.70	**$F_{1,142}$=90.15*** F>M**	**$F_{4,142}$=14.10*** 21<(25,29)<(32,34)**	$F_{4,142}$=2.40 P=0.053
	F	13.40±0.62	14.12±1.17	13.98±0.90	14.98±0.55	14.30±0.55			
Forelimb (mm)	M	3.83±0.32	4.04±0.52	3.74±0.37	3.99±0.29	3.56±0.52	**$F_{1,139}$=7.77** M>F**	**$F_{4,139}$=4.24** (21,34) < (25,29,32)**	$F_{4,139}$=1.25 ns
	F	3.73±0.34	3.97±0.38	4.10±0.43	4.18±0.29	3.57±0.38			
Hind limb (mm)	M	4.83±0.55	5.06±0.37	4.90±0.40	5.18±0.32	4.82±0.64	**$F_{1,142}$=18.94*** M>F**	$F_{4,142}$=1.85 ns	$F_{4,142}$=0.84 ns
	F	4.88±0.38	4.86±0.46	4.85±0.46	5.03±0.20	4.60±0.41			
Tail (mm)	M	23.59±2.70	25.81±1.49	25.60±2.27	26.35±2.39	26.27±2.41	**$F_{1,141}$=28.50*** M>F**	**$F_{4,141}$=6.74*** 21< (25,29,32,34)**	$F_{4,141}$=0.46 ns
	F	23.05±1.03	25.37±1.54	25.02±1.22	25.89±1.50	24.20±2.48			

F ratios correspond to single effects and between-factor interactions in two-factor ANCOVAs with SVL (for body mass, tail, forelimb and hind limb lengths) or egg mass (for head and abdomen lengths) as the covariate. Parentheses below F values for the effects of incubation temperature group together treatments that did not differ as revealed by Fisher LSD tests *a posteriori*. M = Male; F = Female. Symbols immediately after *F* values represent significant levels: ns $P>0.05$; *$P<0.05$; **$P<0.01$; ***$P<0.001$.

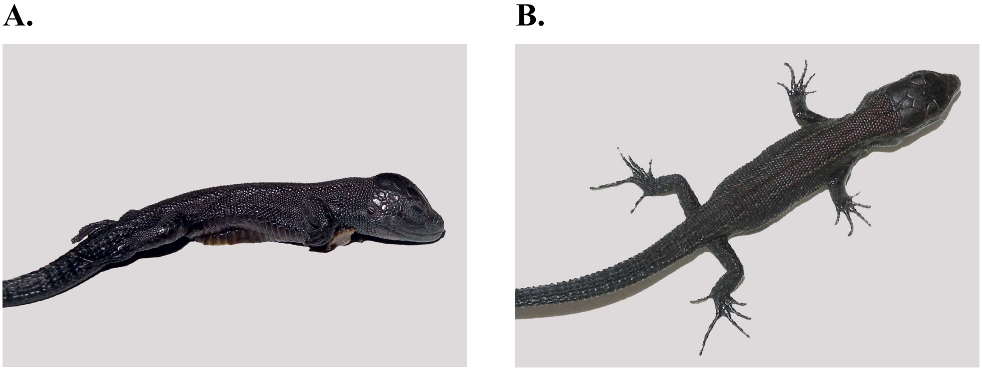


Figure 4.6. Hatchling *Zootoca vivipara* **A**) from the 34º C incubation treatment, died in the egg without having been able to hatch. Note de deformed head and short limbs; **B**) from the 25º C incubation treatment, normal phenotype.

There was a nearly significant interaction between sex and temperature treatments, corresponding to abdomen length (*P* = 0.053; see Table 2): Sexual dimorphism in abdomen length was not significant in hatchlings from the highest incubation temperature (34º C) in spite of the fact that it is significantly different in hatchlings from the other temperatures.

Discussion

Results of the present study indicate that incubation temperature influences development time, survival and a number of phenotypic traits of hatchling *Zootoca vivipara*. In several aspects, and outstandingly in survival along the incubation time, high temperatures produced negative effects on development. Experimental incubations in the lacertid *Podarcis muralis* have also shown that high incubation temperatures produce detrimental effects on hatchling morphology and fitness (Ji and Braña, 1999; Braña and Ji, 2000), even if they are applied only during the earliest phase of the extrauterine incubation (Braña and Ji, 2007). *P. muralis* lay eggs containing embryos at developmental stage 25-29 (according to the development table by Dufaure and Hubert, 1961), and therefore, the early extrauterine incubation phase in this species corresponds to retention phases in *Z. vivipara*, a species in which embryos are at more advanced stages at oviposition. For this reason, although effects of thermal conditions on egg development and hatchling traits were tested during external incubation in the present study, we assume that these effects can also apply to the intrauterine incubation period and thus our results are useful to explain modifications of basking behaviour of females during egg retention. Gravid females *Z. vivipara* selected lower temperatures than did males and non-gravid females in the laboratory thermal gradient, just like in other closely related lacertid lizard species (*P. muralis*: Braña, 1993) and in other viviparous (Van Damme *et al.,* 1986; Heulin, 1987) and oviparous (Carretero *et al.,* 2005) populations of the same species. The fact that there were neither costs of predation nor food supply in the laboratory thermal gradient, supports the idea that low temperatures of gravid females were not a consequence of its restricted opportunities of thermoregulating because of its limited mobility (Le Galliard *et al.,* 2003). The pattern could be related to the fact that the optimal temperature for embryonic development would be lower than the optimal one to most physiologic functions of females and, therefore, gravid females would select lower temperatures to optimize reproductive success (Braña, 1993).

Field body temperatures were considerably lower than temperatures selected in the laboratory thermal gradient, and this would indicate that the thermal environment in our highland study population makes difficult an accurate thermoregulation. Anyway, field body temperatures of pregnant females were also lower than that of other adult animals and, in addition, gravid females occupied places with lower substrate temperatures than males and non-gravid females. The fact that pregnant females occupy places with lower substrate temperatures could reflect that they are active even at environmental temperatures below the normal activity range but allowing some increase of body temperatures via behavioural thermoregulation, because this accelerates embryonic development *in utero*.

Shine (2004) proposed that thermal variation instead of high temperature could be in some instances the main feature enhanced by maternal retention, and some other studies support this conclusion (Webb *et al.,* 2006). Our data apparently support this possibility, as temperatures registered in lizard nests were much more variable (mean ± sd temperature in actual nests: 16.74 ± 2.68º C; 14.98 ± 3.44º C) than those maintained by pregnant females when active. However, activity and thermoregulatory time occupies only approximately one third of the day, and pregnant females probably expend the remaining time at or near soil temperature; this would imply, in fact, higher thermal diel variation, as the temperature during activity should be much higher than during inactivity. On the other hand, there is no evidence in our data of important differences in thermoregulatory precision of pregnant vs. non-pregnant females either in the field or in the laboratory gradient, as expected if extended egg retention had evolved to provide low thermal variation to developing embryos. In fact, pregnant females had lower mean temperatures but also lower minimal activity temperatures as they are active in cooler substrates than non-pregnant females.

Our data support findings of previous studies in lacertid lizards and show that incubation time decreases with temperature (Braña and Ji, 2007). Low incubation temperatures within the range analysed in this study could affect hatchling survival because the longer the hatching is delayed, the longer the eggs are exposed to adverse biotic or abiotic factors in the nest. Moreover, considering that egg laying takes place most frequently by late June in our study population, hatchlings from eggs incubated at low temperatures would have a short growth period before the onset of winter dormancy. Delayed hatching would imply overwintering with lower fat reserves (Elphick and Shine, 1998; Qualls and Andrews, 1999a) and, through reduced growth prior to winter, can also delay reproductive maturity (Warner and Shine, 2007) likely affecting life-time fitness.

Two main hypotheses have been proposed to explain the evolution of viviparity through progressive egg retention: 1) the “cold-climate hypothesis” posits that egg retention has evolved to accelerate development in cold environments where low nest temperatures would retard developmental progress (Packard *et al.,* 1977; Shine, 1985); 2) the “maternal manipulation hypothesis” (Shine, 1995; Webb *et al.,* 2006) posits that females retain eggs to keep suitable conditions for embryonic development. One critical assumption of the maternal manipulation hypothesis is that the phenotypic changes induced by maternal thermoregulation should enhance offspring characteristics, thereby influencing fitness. Results of our experimental incubations indicate that incubation at 34º C (near the temperature selected by non-gravid females) influences hatchling survival: about 42% of eggs incubated at this temperature gave way to unviable offspring (as reported for *Podarcis muralis* by Ji and Braña, 1999). Additionally, survivors from this incubation temperature had smaller heads and fore limbs, which could imply having a lower fitness because: 1) head size has influence on bite capacity,_an important ecological attribute in adult *Z. vivipara* because of its influence on prey selection (Herrel *et al*., 2001) and in particular, head size favours male reproductive success through enhanced fighting and grasping ability (Gvoždík and Van Damme, 2003); 2) forelimb length can influence locomotor performance (Braña, 2003), which has a relevant role in the ability of escaping from predators, obtaining food, and while interespecific interaction. Assuming that high temperatures would have the same effect on oviductal eggs as on oviposited eggs, our results indicate that the temperature selected by males and non-gravid females would be detrimental to survival and phenotypes of hatchlings, and would explain why females select lower body temperatures when gravid. In addition, this provides a plausible evolutionary scenario for the increase of egg retention, since any advance in retention would extend the time of exposition of the embryos to suitable temperatures (Braña and Ji, 2007). Therefore, our results are consistent with the expected under the "maternal manipulation hypothesis", as pregnant females modify their body temperature and avoid keeping eggs at temperatures that influence negatively hatchling characteristics. On the other hand, the fact that gravid common lizards "select" lower body temperatures than similarly sized males and non-pregnant females would seemingly contradict the expected under the "cold-climate hypothesis", because the decrease in temperature during pregnancy would retard the embryonic development. However, the shift of thermal preference of pregnant females is not incompatible with the acceleration of development proposed by the cold climate model. Because of the nonlinearity of the relationship between temperature and incubation time, the temperature selected by females during the time of egg retention would result in only a very short delay, if any, of hatching time. If eggs are kept during the external incubation period at 34º C (close to the temperature selected by non-gravid females), there is only a slight decrease in incubation time with respect to those incubated at 32º C (close to the temperature selected by gravid females). If a similar rate of thermal dependence occurs during the phase of intrauterine development, egg retention would considerably shorten incubation time, as the temperature selected by pregnant females, although lower than the one selected by non-pregnant females, is much higher than average temperatures recorded in natural nests of *Z. vivipara*.

V. Capítulo 4.

Shift in thermal preferences of female oviparous common lizards during egg retention: insights into the evolution of reptilian viviparity[*]

---

[*] Este capítulo está basado en *Rodríguez-Díaz T., Braña F. 2011. Shift in thermal preferences of female oviparous common lizards during egg retention: insights into the evolution of reptilian viviparity.* Evolutionary Biology, *38: 352-359.*

Resumen

Las hembras gestantes de *Zootoca vivipara* seleccionan temperaturas corporales más bajas que los machos o las hembras no gestantes y se cree que este cambio en sus preferencias térmicas está relacionado con la optimización de las condiciones para la embriogénesis. Por tanto, someter a los embriones a las altas temperaturas seleccionadas por machos y hembras no gestantes podría tener efectos sobre el desarrollo embrionario y sobre la eficacia biológica de las crías, según las predicciones de la hipótesis de "manipulación materna" sobre la evolución del viviparismo. Para probar el papel del ambiente de gestación sobre el desarrollo embrionario en poblaciones ovíparas de *Z. vivipara*, mantuvimos un grupo de hembras gestantes a la temperatura seleccionada por hembras no gestantes en un gradiente térmico en el laboratorio, mientras que a las hembras control se les permitió regular su temperatura corporal sin restricciones. El estadio de desarrollo en el momento de la puesta fue más avanzado para los embriones de las puestas experimentales, que pesaron más que las del grupo control. La temperatura de gestación forzada también afectó al éxito de eclosión (58,62% en el tratamiento experimental vs. 97,37% en el grupo control). Las cáscaras eran más gruesas y contenían más calcio y magnesio en el momento de la puesta que en el de la eclosión, sugiriendo que los embriones toman minerales de la cáscara durante la incubación externa. Además, las crías de las hembras sometidas a altas temperaturas durante la gestación eran más pequeñas, tenían cabezas más cortas y se desenvolvían peor en las carreras. Nuestros resultados concuerdan con la predicción de la hipótesis de "manipulación materna" y sugieren que el cambio en la temperatura corporal de la hembra durante la gestación optimiza la embriogénesis y el fenotipo de las crías evitando los efectos negativos de las altas temperaturas de incubación preferidas por las hembras no gestantes.

Abstract

Pregnant female *Zootoca vivipara* select lower body temperatures than males or non-pregnant females, and this shift in the thermal preferendum is believed to be related to optimising the conditions for embryogenesis. Thus, subjecting embryos to the higher temperature selected by males and non-gravid females might have detrimental effects on embryonic development and on hatchling fitness, according to predictions of the "maternal manipulation" hypothesis on the evolution of viviparity. To test the role of gestation environment on embryonic development in oviparous *Z. vivipara*, we kept a number of gravid females at the temperature selected by non-gravid females in a laboratory thermal gradient, whereas control females were allowed to regulate their body temperature without restrictions. Developmental stage at oviposition was more advanced for embryos of the experimental clutches, which were heavier than those of the control group. Forced gestation temperature also affected hatching success (58.62% in the experimental treatment vs. 97.37% in the control group). Eggshells were thicker and contained more calcium and magnesium at oviposition than at hatching time, suggesting that embryos take minerals from the eggshell during the external incubation. In addition, hatchlings from females subjected to high temperatures during pregnancy were smaller, had shorter head length and performed worse in running trials. Our results fulfil the prediction of the "maternal manipulation" hypothesis, and suggest that the shift in female body temperature during pregnancy optimizes embryogenesis and hatchling phenotype by avoiding the negative effects of the high incubation temperatures preferred by non-gravid females.

Introduction

Environmental maternal effects, such as mother's feeding rate or exposition to predators, and several other aspects of the maternal gestation environment have been proved to influence the phenotype and behaviour of neonatal lizards (e.g. Sorci and Clobert, 1997; Shine and Downes, 1999). For example, maternal thermoregulation can affect egg temperature throughout embryonic development, and maternal temperature has been demonstrated to have a great influence on offspring fitness (Rock and Cree, 2003; Birchard, 2004; Ji *et al.,* 2006). Research on this topic has mainly focused on viviparous species, in which the contact between mother and embryo exists over a long period from conception to hatching (Beuchat, 1988; Swain and Jones, 2000), but most oviparous squamates retain eggs *in utero* for a considerable proportion of the embryogenesis (Shine, 1983; Braña *et al.,* 1991; Blackburn, 1995), so offspring phenotypes can also be influenced by variation in the preoviposition maternal environment.

Females of some squamate species prefer lower body temperatures during pregnancy (e.g. *Podarcis muralis*: Braña, 1993; *Sceloporus virgatus*: Andrews and Rose, 1994; *Zootoca vivipara*: Van Damme *et al.,* 1986). In other species, gravid females prefer higher body temperatures than other individuals in the same population (e.g. *Chalcides ocellatus*: Daut and Andrews, 1993; *Hoplodactylus maculatus*: Rock *et al.,* 2000), and this increase in body temperature would accelerate embryonic development inside the mother's body (Mathies and Andrews, 1997; Shine, 2006). Shift in thermal preferendum during pregnancy, especially toward lower body temperatures that retard development (but see Rodríguez-Díaz *et al.,* 2010), is assumed to be related to optimising the conditions for embryogenesis (Beuchat, 1988; Mathies and Andrews, 1997; Rodríguez-Díaz *et al.,* 2010) and could favour the evolution of extended egg retention since a longer intrauterine stay would extend the time of the developing embryos' exposure to suitable conditions (Braña and Ji, 2007).

The aim of the present study was to determine whether the shift of preferred temperature in pregnant females of an oviparous lizard with prolonged egg retention avoids the negative effects that high temperatures could have on embryogenesis, as predicted by the maternal manipulation hypothesis on the evolution of viviparity (Shine, 1995; Webb *et al.,* 2006). This hypothesis posits that egg retention has evolved to control the thermal environment during embryonic development; accordingly, the shift in body temperature during pregnancy would occur because gravid females adjust thermoregulation to provide optimal thermal conditions for embryogenesis. To test this prediction, we evaluated the role of gestation environment on embryonic development, hatching success, and hatchling phenotype in oviparous *Zootoca vivipara* (Jacquin, 1797), by keeping a group of gravid females at a higher temperature than they would normally select, i.e., similar to that preferred by non-gravid females of the same population, as determined in a laboratory thermal gradient (Rodríguez-Díaz *et al.,* 2010). We chose this experimental approach rather than a possible alternative consisting of inducing oviposition (see Mathies and Andrews, 1995) and then incubating the eggs under the selected thermal regime because our model more closely resembles the natural conditions of the incubation environment inside the mother's oviduct with the exception of the thermal regime applied. We also determined thickness and mineral content of eggshells to elucidate whether embryos take minerals from the eggshell and whether the thermal treatment influences this process. Our study organism is an ideal model for these tasks not only because females shift their preferred temperature during pregnancy, but also because they lay eggs with the embryo at quite an advanced stage of development (31-34 according to

Dufaure and Hubert's 1961 developmental table; Braña *et al.,* 1991, Rodríguez-Díaz *et al.,* 2010). The species *Z. vivipara* also includes viviparous populations that lay soft-shelled eggs with fully developed hatchlings that emerge a few hours after oviposition (Panigel, 1956). Due to the significant degree of development attained before oviposition in oviparous *Z. vivipara*, maternal thermoregulation during the pre-oviposition phase could be expected to affect developing embryos over a longer period than in most other oviparous species.

Materials and Methods

Gravid females of *Zootoca vivipara* (snout-vent length: mean ± sd, 52.71 ± 5.18 mm) were collected from an oviparous population in Las Señales (León, northern Spain), between 1650 and 1750 m asl, during late June of 2007 and were kept until oviposition in plastic terraria (50 x 40 x 30 cm; length x width x height) in the Zoology laboratory of the University of Oviedo. A maximum of 6 lizards was housed in each terrarium. Cages contained refuges and moist peat, a substrate suitable for egg laying. All lizards were provided with water *ad libitum* and mealworms twice a day.

Female *Z. vivipara* prefer a lower body temperature during pregnancy (mean body temperature ± s.d.: 32.33 ± 1.27° C; *n*=64) than do adult males or non pregnant adult females (34.05 ± 1.07° C; *n*=155; data from this population: Rodríguez-Díaz *et al.,* 2010). If pregnant females prefer lower body temperatures to optimize embryonic development, even at the cost of some increase in incubation time, then incubating eggs *in utero* at the higher temperatures preferred by non-gravid females can be expected to have some detrimental effects on embryogenesis, and thus on hatchling phenotypes. To test this hypothesis, we kept a group of 27 gravid females in incubators at a constant temperature of 34º C, which is close to the mean temperature preferred by males and non-gravid females, for 6 hours a day (11:00-17:00 h). This period of 6 hours is roughly the average daily time they spend thermoregulating in the field. Because of the uniformly high air temperature and the lack of a radiant heat source, females are unable to perform behavioural thermoregulation, and their body temperature should approach air temperature inside the incubator; in fact, a sample of cloacal temperatures of these females, measured with a quick-reading cloacal *Schultheis* thermometer (to the nearest 0.1º C; Miller and Weber, Inc.) at the end of their daily period in the incubator, verified that their mean body temperature was indeed close to that sought (mean body temperature ± sd; 33.70 ± 0.30º C, *n*=19). A fluorescent light was placed inside the incubator so that the photoperiod was the same for both groups of lizards. Before and after the daily period in the incubator, we let the lizards thermoregulate in a terrarium with a 60W bulb from 9:00 to 11:00 h, and from 17:00 to 19:00 h. Another group of gravid females (*n*=19) was allowed to thermoregulate without restrictions under these conditions from 9:00 to 19:00 h. At other times of the day all lizards were maintained at room temperature (mean laboratory temperature ± sd: minimum, 18.56 ± 2.75º C, *n*=25; maximum, 26.68 ± 4.53º C, *n*=25). In this study, lizards that oviposited at least one week after the experimental treatment had started were used in order to guarantee that the experimental conditions were able to affect embryonic development.

Clutches were collected from the terraria and weighed within a few hours of the time of oviposition, and embryonic stage at oviposition was determined for one egg from each clutch according to the developmental scale of Dufaure and Hubert (1961). We used plastic containers with moistened vermiculite as substrate (proportion distilled water/vermiculite 1/1 by weight) to incubate the eggs individually. Incubation temperature was set at 25º C, a temperature which has resulted in high hatching success and normal hatchling phenotypes in previous studies (Rodríguez-Díaz *et al.,* 2010). Containers were rotated inside the incubator daily to minimize possible effects of temperature gradients on development and were periodically checked to verify that the vermiculite was still moist. Protocols for the incubation of control eggs were the same as those described for experimental eggs.

We assessed the effect of the gestation environment on clutch mass, embryonic stage at oviposition, length of incubation time (from oviposition to hatching), and percentage of eggs that hatched successfully. We also analysed the thickness and mineral content of eggshells at oviposition and at hatching time by using one egg per clutch and (following the procedure previously described in the Materials and Methods section of Chapter 2) and measured several hatchling phenotype traits: head length, abdomen length, snout-vent length (SVL), tail length, robustness (size-corrected hatchling mass), and locomotor performance. The morphological traits were measured *in vivo* using a digital caliper (to the nearest 0.01 mm; Vogel, Germany). Sex determination was carried out by eversion of hemipenes in males by applying gentle forceps pressure at the base of the tail (Harlow, 1996). Hatchling locomotor performance was tested 2 days after hatching. Before the running trials, hatchlings were kept for 30 minutes in an incubator at a high temperature (32º C), thus ensuring the same conditions for all hatchlings, i.e., a body temperature that maximizes locomotor performance in *Z. vivipara* (Van Damme *et al.,* 1991). They were then made to run along a 1-meter-long corridor and videofilmed laterally with a Nikon videocamera. Whenever they stopped, we gently pushed them with a paintbrush. Three variables were measured on video recordings at 30 frames per second: a) maximum sprint speed: speed in the fastest five consecutive frames; b) number of stops during the entire run (two or more consecutive frames without moving); and c) maximum distance covered between stops.

Statistical analyses

All data were checked for normality (Kolgomorov-Smirnov test) and homocedasticity (Levene's test) prior to further statistical analysis. A Mann-Whitney *U*-test was carried out to analyse stage of embryonic development at oviposition and a Generalized Linear Model (GLM) was performed to analyse eggshell calcium content because these variables did not meet the assumptions needed to conduct parametric tests, even after logarithmic transformation. Two-factor ANOVAs were used to analyse thickness and content of magnesium and potassium in the eggshells. A Generalized Linear Model using binomial errors and logit link function was carried out to assess differences in hatching success between the control and experimental group.

A one-factor analysis of variance was carried out to analyse between group differences in the mean incubation time for each clutch and a one-factor analysis of covariance (covariate: female mass) was carried out to examine clutch mass. Nested mixed model ANOVAs or ANCOVAs were performed to analyse the remaining variables. Treatment, sex, and their interaction were used as fixed effects and clutch as a random effect nested within the treatment, in order to control family effect. We included SVL as covariate in the analyses of several size related variables: tail length, body mass, sprint speed, and the longest distance done without pauses during trials. In the analyses of SVL, head length, and abdomen length, egg mass was used as covariate. The significance level for all tests was set at $\alpha = 0.05$. All statistical analyses were conducted with Statistica software (StatSoft, Inc., version 6.0, 2001).

Results

Control females oviposited eggs with embryos at Dufaure and Hubert's (1961) stages 33 and 34, whereas embryos from females kept at 34° C during the experimental period were more advanced, some even reaching stage 35 (Mann-Whitney *U*-test, *Z* adjusted = -2.66; $P < 0.01$; Fig. 5.1A). This advance in embryonic development at oviposition did not significantly affect incubation time at 25° C (ANOVA, $F_{1,37} = 2.878$; $P = 0.098$).

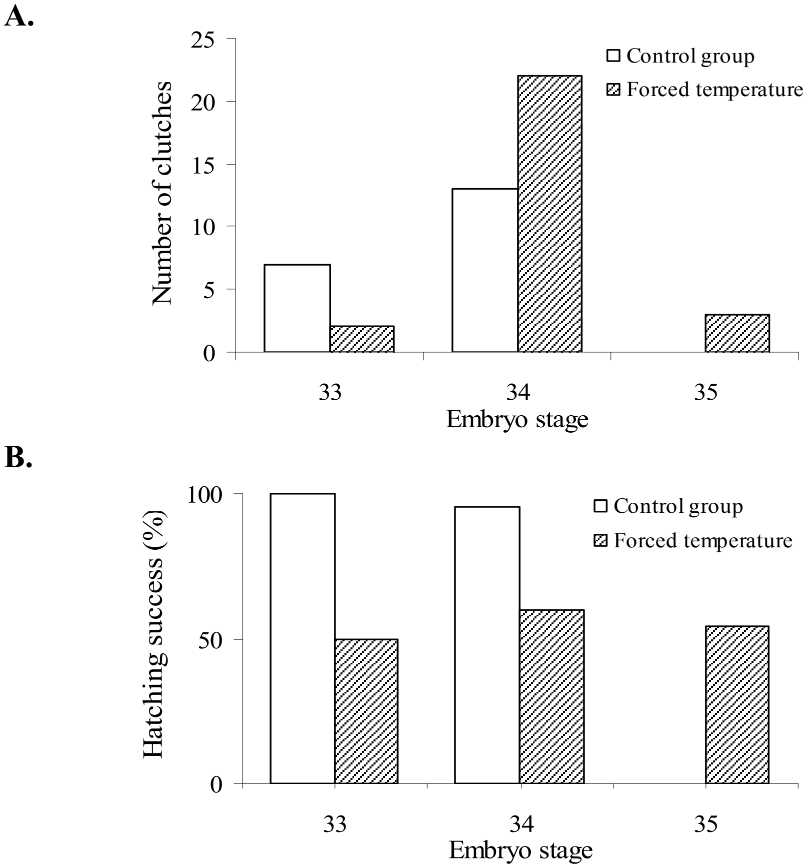


Figure 5.1. A. Development stages at oviposition of embryos of oviparous *Z. vivipara* in control clutches and in clutches oviposited by females subjected to forced temperature while pregnant, according to the stages described by Dufaure and Hubert (1961). **B.** Percentage of hatching success for each of the embryo stages in control and experimental groups.

Hatching success was lower in the experimental group (58.62%, n=145; Control group: 97.37%, n=76; Wald χ^2 (1) = 19.434, $P < 0.001$). This difference in hatching success is not connected with the between-treatment differences in developmental stage at oviposition, as differences in hatching success were still found for stage 34, which is the most frequent embryo stage in both experimental groups (Wald $\chi^2(1) = 12.024$, $P < 0.001$; Fig. 5.1B). Clutch size did not differ between treatments (ANCOVA with female SVL as covariate: $F_{1,31} = 2.438$; $P = 0.129$), but clutches of the experimental group were on average heavier than those of the control group (ANCOVA with female mass: $F_{1,29} = 5.306$; $P = 0.029$).

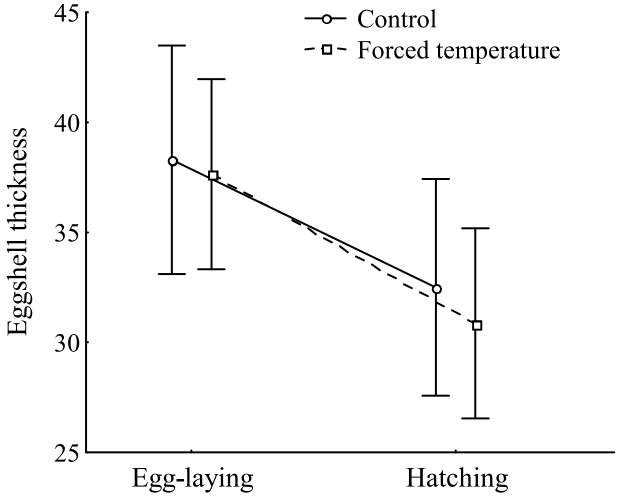


Figure 5.2. Eggshell thickness (µm) at egg-laying and hatching time for control and forced temperature groups.

Eggshells were thicker at oviposition than at hatching time ($F_{1,41}$ = 7.307, $P < 0.01$; Fig. 5.2; Table 1). Treatment or interaction effects were not found for this variable ($F_{1,41} = 0.242$, $P > 0.05$ for treatment; $F_{1,41} = 0.045$, $P > 0.05$ for between-factor interaction). As regards eggshell mineral content, whereas calcium and magnesium are more abundant at oviposition than at hatching time (calcium: *Wald* $\chi^2(1) = 40.491$, $P < 0.001$; magnesium: $F_{1,41}$ =

9.739, $P < 0.01$), the amount of potassium did not vary during the external incubation ($F_{1,41} = 0.317$, $P > 0.05$). Treatment did not affect calcium (*Wald* $\chi^2(1) = 0.357$, $P > 0.05$), magnesium ($F_{1,41} = 3.338$, $P > 0.05$) or potassium ($F_{1,41} = 0.352$, $P > 0.05$). There were not between-factor interactions for any of the minerals studied: *Wald* $\chi^2(1) = 0.032$, $P > 0.05$ for calcium, $F_{1,41} = 0.023$, $P > 0.05$ for magnesium; and $F_{1,41} = 2.296$, $P > 0.05$ for potassium).

TABLE 1.-. Thickness of the internal protein layer (µm; mean±sd) and percent by weight (mean±sd) of calcium, magnesium, and potassium in the external mineral layer of eggshells of *Zootoca vivipara* at oviposition and hatching time. C=Control group; T=Treatment group: forced temperature. Sample sizes are in brackets.

		Oviposition	Hatching			Oviposition	Hatching
Thickness	C	38.30±6.27 (9)	32.50±6.72 (10)	Mg	C	0.50±0.13 (9)	0.34±0.13 (10)
	T	37.65±9.15 (13)	30.86±7.70 (13)		T	0.58±0.12 (13)	0.44±0.22 (13)
Ca	C	39.69±1.90 (9)	27.14±9.74 (13)	K	C	0.21±0.09 (9)	0.27±0.27 (10)
	T	38.20±4.52 (13)	26.33±7.57 (13)		T	0.26±0.21 (13)	0.15±0.09 (13)

Family effects were significant for all of the hatchling traits analysed ($P<0.05$ for all cases). As has been previously found in the same population (Braña, 2008; Rodríguez-Díaz *et al.,* 2010), SVL, abdomen, and tail length of hatchlings varied according to sex, with the SVL and abdomen being longer in females (SVL: $F_{1,117} = 68.69$, $P < 0.001$; abdomen length: Table 2), and the tail being longer in males. Furthermore, hatchling males were more robust than females (body mass with SVL as covariate). There were no differences between sexes as regards head length or locomotor performance (Table 2).

TABLE 2.- Effects of treatment (forced body temperature during intrauterine incubation) and sex on morphology and locomotor performance of *Zootoca vivipara* hatchlings[a].

	Effects		
Hatchling traits	Treatment	Sex	Interaction
Morphology			
Head length	**$F_{1,117}$=5.820* C>T**	$F_{1,117}$=0.398 ns	$F_{1,117}$=1.512 ns
Abdomen length	$F_{1,117}$=0.170 ns	**$F_{1,117}$=110.75*** M<F**	$F_{1,117}$=2.920 ns
Tail length	$F_{1,116}$=1.039 ns	**$F_{1,116}$=6.741* M>F**	$F_{1,116}$=42.618 ns
Body mass	$F_{1,117}$=0.007 ns	**$F_{1,117}$=5.760* M>F**	$F_{1,117}$=0.304 ns
Locomotor performance			
Sprint speed	**$F_{1,117}$=21.164*** C>T**	$F_{1,117}$=0.035 ns	$F_{1,117}$=0.325 ns
Distance	**$F_{1,117}$=6.329* C>T**	$F_{1,117}$=0.005 ns	$F_{1,117}$=0.898 ns
Number of stops	**$F_{1,115}$=4.309* C<T**	$F_{1,115}$=0.758 ns	$F_{1,115}$=0.157 ns

[a] Statistical tests correspond to single effects and between-factor interactions in nested ANOVAs (for number of stops) or ANCOVAs (for tail length, hatchling mass, sprint speed, and distance with SVL as covariate; and head and abdomen length with egg mass as covariates). Although not shown, random factor (clutch) effects were significant for all traits analyzed. Symbols immediately after *F* values represent significant levels: ns $P>0.05$, *$P<0.01$, ***$P<0.001$. M=Male, F=Female. C=Control, T=Treatment. Significant effects are in bold type.

Control hatchlings were marginally larger (ANCOVA with SVL as the dependent variable and egg mass as the covariate: $F_{1,117} = 4.04$, $P = 0.050$) and had larger heads. The other morphological traits analysed do not differ between treatments. Hatchlings from the control group performed better in the running tests: control hatchlings ran faster (Fig. 5.3), paused less frequently, and covered larger distances without stopping than those from the forced temperature treatment (Table 2).

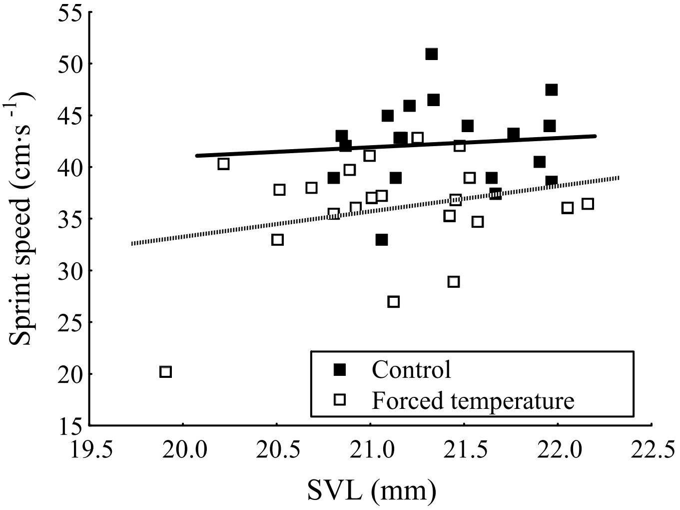


Figure 5.3. Relationship between maximal sprint speed and snout-vent length (SVL) of hatchling *Z. vivipara* from control and forced temperature groups. Squares represent the mean values for each clutch.

Discussion

Maternal temperature has been widely reported to affect traits influencing offspring fitness (Beuchat, 1988; Shine and Harlow, 1993; Mathies and Andrews, 1997; Wapstra, 2000) and females are expected to evolve a more accurate control of body temperature during pregnancy. In fact, gravid females of several lizard species shift their preferred body temperatures (Braña, 1993; Govždík and Castilla, 2001), which are generally less variable (Mathies and Andrews, 1997; Shine, 2006). Our results show that maintaining the comparatively high temperature preferred by non-pregnant females during pregnancy has detrimental effects on development and could ultimately influence hatchling fitness. These results concur with the predictions of the “maternal manipulation” hypothesis (Shine, 1995; Webb *et al.,* 2006), which sets out the adaptive advantages of egg retention in the evolutionary transition from oviparity to viviparity.

Results from incubations at 34º C in the postovipositional phase reported in a previous paper on the same oviparous population used in the present study (Rodríguez-Díaz *et al.,* 2010) suggested that the shift in thermal preference of pregnant females towards lower body temperatures might be explained by the fact that the temperature preferred by females when non-gravid has detrimental effects on developing embryos. In the present study, new data support this same conclusion. Although the two studies differ in the phase in which the experimental thermal conditions were applied (before or after oviposition), there is an overlap with regard to the embryonic development stages affected by the two thermal incubation regimes. This is because, in the current study, females from the forced temperature treatment retained their eggs until a more advanced embryo stage. The effects on hatchling phenotype of gestation temperature at 34º C, even if applied for just 6 hours a day during the later stages of egg retention, were similar to the effects of external incubation at constant 34º C from oviposition to hatching (see Rodríguez-Díaz *et al.,* 2010). This result parallels the findings reported by Braña and Ji (2007) in the lacertid *Podarcis muralis*, in which high temperature experienced by embryos during the early external incubation period only, produced similar phenotypic responses to those produced by the same constant temperature applied during the whole incubation period (Braña and Ji, 2000). Interestingly, the embryonic stages affected by high temperatures were probably very similar in the experiment by Braña and Ji (2007) and in the present study, with the affected developmental stages ranging from 26-28 to 34: *P. muralis* lay eggs containing embryos at stages 26-28 (Braña *et al.,* 1991), and it takes 14-15 days to attain stage 34 at 26º C (Dhouailly and Saxod, 1974), thus the 13 days of treatment at a higher temperature of 32º C in the Braña and Ji (2007) study, will certainly have affected the range of development stages between 26-28 and 34; in turn, *Z. vivipara* were under the forced temperature regime in the present study for 7-10 days, and most eggs were laid at stage 34 (see results), hence, according to the development timetable of Hubert (1985), development would have been at stages 26-28 when the treatment was initiated.

Our results show that intrauterine incubation temperature can influence embryonic development in an oviparous species, as has been previously found in viviparous species (Beuchat, 1988; Mathies and Andrews, 1997). Some of the females forced to maintain high temperature during pregnancy oviposited eggs with embryos at stage 35, one stage more advanced than the maximal reached by the embryos of eggs laid by control females. There seems to be an almost absolute upper limit around this stage in the extent of development attainable *in utero* for oviparous populations of *Z. vivipara*: a previous study on the same population dealing with the limitations of egg retention beyond the normal time of oviposition also showed that stage 35 was the maximal reached *in utero* before egg-laying (Rodríguez-Díaz and Braña, 2011a). F1 hybrids between oviparous and viviparous populations lay eggs containing embryos that can surpass this stage of intrauterine development and reach stage 36 (Arrayago *et al.,* 1996), but these eggs, unlike the eggs of the present study, have thin and incompletely calcified eggshells (Heulin *et al.,* 1992), thereby probably favouring respiratory exchanges. We have found that eggshell thickness and calcium and magnesium content decreased during the external incubation phase, suggesting that embryos take minerals from the eggshell during this period, as demonstrated for several lizard species, including *Z. vivipara* (*Eumeces fasciatus*: Shadrix *et al.*, 1994; *Podarcis muralis*: Ji and Braña, 1999; *Z. vivipara*: Stewart *et al.*, 2009). Therefore, a reduction in eggshell thickness, which could favour the evolution of extended egg retention through enhanced gas exchange, could difficult for the uptake of the calcium necessary for the embryo nutrition (Packard *et al.,* 1977).

In our study, the main effect of high temperature during the intrauterine incubation is reduced hatching success, which would significantly lower the reproductive success of females. The experimental treatment also influenced hatchling phenotypes: hatchlings that experienced higher temperature inside their mother's oviduct at the end of the retention phase were smaller, had shorter heads, and performed worse during running trials. Even though clutches of the forced temperature treatment were heavier, hatchlings from this experimental treatment were smaller in length, which seems to be contradictory. This pattern could be explained by, on the one hand, the between-treatments difference in egg hydration at oviposition. Eggs of lizards, with flexible eggshells, absorb water and swell as embryonic development proceeds (Packard, 1991; Qualls and Andrews, 1999b) and the eggs of the forced temperature group contained more developed embryos than the control eggs. On the other hand, higher incubation temperatures usually produce smaller hatchlings (Atkinson, 1994; Mathies and Andrews, 1997; Braña and Ji, 2000), which may be linked to the acceleration of metabolic rates and reduction of metabolic efficiency as a result of high temperatures (Packard and Packard, 1988; but see Angilletta *et al.,* 2000). The head length difference is important to note as it, along with body size, has an influence on bite capacity, an important ecological attribute in adult *Z. vivipara* because of its influence on prey selection (Herrel *et al.,* 2001; Verwaijen *et al.,* 2002) and also probably on male reproductive success (Braña, 1996; Gvoždík and Van Damme, 2003). The final point, that experimental hatchlings exhibit slower sprint speed, stop more frequently, and cover shorter distances without stopping, could be detrimental to their fitness. Although evidence for positive correlation of locomotor performance with Darwinian fitness is somewhat contradictory (see, e.g. Garland and Losos, 1994; Clobert *et al.,* 2000), it is generally thought that individuals with outstanding locomotor capacity may be better able to capture prey and escape from predators or have advantages in social hierarchies (e.g. see Garland *et al.,* 1990; Robson and Miles, 2000). For a viviparous population of our study species, Le Galliard *et al.* (2004) demonstrated that under dietary restriction, differences in locomotor performance (endurance) at hatching were retained one year later and positively influenced fitness (survival).

The effects resulting from our experimental conditions arise from the exposition of developing embryos to high temperatures for just a few hours per day. Females from treatment and control groups were exposed to the same temperatures for 18 h per day, including during the night, when they experienced the same minimum temperatures (mean ± sd, 18.56 ± 2.75º C). For only 6 hours per day both groups experienced slight differences in body temperature: control females could freely thermoregulate, keeping a narrow range of body temperatures (mean, 32.33º C; see Rodríguez-Díaz *et al.,* 2010), and experimental females maintained a mean temperature of 33.70º C (see Methods). Experimental studies have shown effects of mean of (Braña and Ji, 2000; Andrews *et al.,* 2000) as well as variance in (Shine and Harlow, 1996; Du and Ji, 2006) incubation temperatures on development. In this study, overall mean temperature and variance were very similar in both experimental groups and hence they would not explain the differences obtained between groups. It is therefore reasonable to assume that the most likely explanation to account for the differences observed is in fact that females were kept for a certain period at temperatures which were above an upper thermal limit suitable for development.

Taken together, our findings show that temperatures similar to those preferred by non-gravid females applied during the later phase of egg retention considerably reduce hatching success and produce hatchling phenotypes exhibiting characteristics that are likely to be connected with low fitness, a finding previously reported by Mathies and Andrews (1997) in the viviparous lizard *Sceloporus jarrovi*. In conclusion, the shift in preferred body temperature during pregnancy would avoid the negative effects of high incubation temperatures on hatchling fitness and, as predicted by the maternal manipulation hypothesis on the evolution of viviparity, would provide the thermal environment that optimises hatchling traits, suggesting that this shift in the thermoregulatory pattern could have been an important component in the evolution of extended egg retention.

VI. Discusión integradora

En la mayor parte de los grupos animales se admite que el oviparismo es la condición reproductora primitiva (e. g., moluscos: Köhler *et al.*, 2004; peces: Wourms *et al.*, 1988; anfibios: Wake, 1993; reptiles: Shine, 1985) y que el viviparismo, una vez alcanzado, representa un estado irreversible. No obstante, a pesar de que las transiciones del oviparismo al viviparismo son mucho más frecuentes y están más fuertemente apoyadas (Lee y Shine, 1998), existe un debate en torno a la posible reversibilidad del viviparismo, centrado principalmente en los reptiles, al tratarse del grupo con más orígenes independientes de este modo de reproducción (De Fraipont *et al.*, 1996; Lee y Shine, 1998; Blackburn, 1999b; Surget-Groba *et al.*, 2006).

El objetivo principal de esta Tesis consiste en explorar el significado adaptativo de la retención de la puesta y el avance del desarrollo embrionario intrauterino que constituye el camino obligado en la evolución del viviparismo. Para ello, se ha analizado la variación en el grado de retención de la puesta en los oviductos, tanto entre poblaciones como entre individuos, así como la plasticidad fenotípica inducida por diferentes condiciones del ambiente de incubación relevantes en la transición hacia el viviparismo, en un organismo cuyo nivel de retención de la puesta representa una fase muy avanzada en la transición hacia el viviparismo.

La retención de los huevos en los oviductos maternos juega un papel clave en la evolución del viviparismo en reptiles, ya que la transición evolutiva desde el oviparismo hacia el viviparismo es un proceso que ocurre de forma gradual, mediante estados crecientes de retención de la puesta (Packard *et al.*, 1977; Shine, 1983, 2002a). Sin embargo, aunque muchos reptiles Squamata ponen huevos conteniendo embriones en fases relativamente avanzadas del desarrollo, muy pocas especies ovíparas alcanzan los estadios 33-39 de la escala de estadios de desarrollo de Dufaure y Hubert (1961) (D&H, en adelante) en el oviducto materno. En esto se ha apoyado Blackburn (1995, 1998) para criticar el modelo gradualista sobre la evolución del viviparismo en reptiles, favoreciendo un debate en torno al mismo. Qualls *et al.* (1997) atribuyen la escasez de estados intermedios a que la transición gradual podría ocurrir bien por cladogénesis con una subsiguiente extinción de los estados intermedios, o bien por anagénesis, en cuyo caso los estados intermedios habrían sido reemplazados por los sucesores. Sin embargo, ninguno de estos dos escenarios propuestos resuelve el problema, puesto que no impiden la existencia en la actualidad de especies que representen esos estados intermedios.

La escasez de especies que realizan la puesta conteniendo embriones en estadios de desarrollo 33-39 de D&H en el momento de la puesta entre los reptiles podría deberse a que los estadios intermedios de la transición no sean evolutivamente estables, de manera que la etapa final de la evolución hacia el viviparismo transcurriría muy rápidamente y bajo una fuerte presión direccional (Blackburn, 1995), lo cual podría estar relacionado con algún límite fisiológico que dificultaría la retención prolongada de la puesta más allá del estadio 30. Una limitación fisiológica podría ser el aumento de los requerimientos de oxígeno por parte del embrión debido al inicio de la etapa de crecimiento exponencial durante su desarrollo (Shine, 1983; Andrews y Mathies, 2000; Andrews, 2002). Esto originaría un posible conflicto entre las adaptaciones para controlar el intercambio gaseoso y el ambiente hídrico ya que, por un lado, se requeriría un adelgazamiento de la cáscara para evitar la hipoxia y favorecer el intercambio gaseoso y, por otro lado, una cáscara más fina dificultaría el mantenimiento de las condiciones hídricas adecuadas para el desarrollo del embrión en el nido (Shine y Thompson, 2006). Además, el aumento de peso y volumen de los huevos durante la incubación intrauterina debido al crecimiento del embrión y a la absorción de agua (Vleck, 1991) reduciría la movilidad de la hembra, haciendo que disminuya su habilidad para alimentarse y que aumente su vulnerabilidad a los depredadores (Shine, 1980; Sinervo *et al.*, 1991).

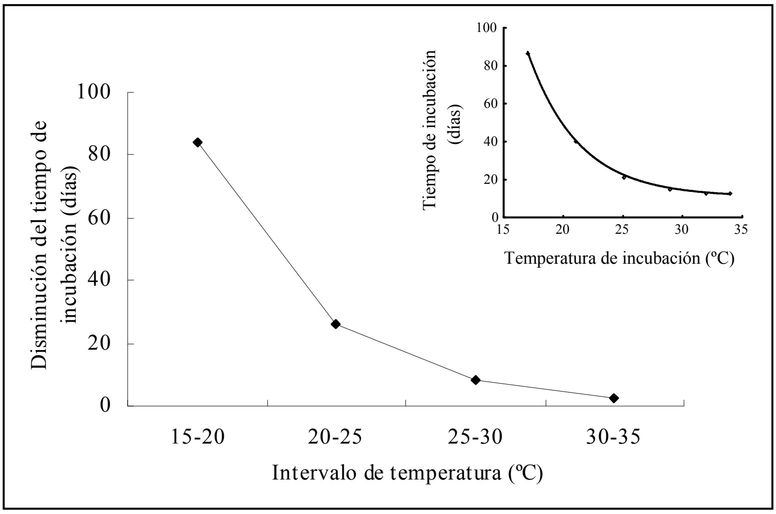


Figura 6.1. Número de días que disminuye el tiempo de incubación cada 5ºC de aumento de temperatura. En la esquina superior derecha, relación entre el tiempo de incubación y la temperatura de incubación en *Z. vivipara* (ver capítulo III).

En el proceso de evolución del viviparismo, la retención de la puesta supondría una serie de ventajas adaptativas. La hipótesis de “clima frío” sobre la evolución del viviparismo (Shine, 1985, 2002a) predice que la retención de la puesta es adaptativa en climas fríos donde las temperaturas que experimentan los huevos en los oviductos maternos es más elevada que la que experimentarían en los nidos. Debido a que el tiempo de incubación externa disminuye al aumentar la temperatura (especialmente a temperaturas bajas; ver Fig. 6.1), la retención de la puesta provocaría el adelanto del momento de la eclosión de los huevos con respecto a los huevos incubados en los nidos y, por lo tanto, estarían menos tiempo expuestos a los riesgos bióticos y abióticos asociados a la incubación externa. Además, las crías tendrían más tiempo para crecer y acumular reservas energéticas antes de iniciar la hibernación (Elphick y Shine, 1998; Qualls y Andrews, 1999a), lo cual puede influir en su supervivencia (Civantos *et al.*, 1999) o en la edad de adquisición de la madurez reproductora (Warner y Shine, 2007).

La hipótesis de clima frío predice que las especies (o las poblaciones) de latitudes elevadas presentarían niveles más avanzados de retención (incluyendo por tanto mayor proporción de formas vivíparas) que las poblaciones de latitudes medias. Lo mismo podría decirse sobre la variación en poblaciones asentadas en altitudes extremas: cabe esperar mayor nivel de retención de la puesta en altitudes elevadas. Estas predicciones se han contrastado en diferentes clados de Squamata (ver Shine, 1985; Smith *et al.*, 2001) y también en nuestra especie modelo a escala de distribución general en Eurasia, ya que las poblaciones ovíparas de *Zootoca vivipara* ocupan el extremo sur del extensa área de distribución (Braña, 1986; Surget-Groba *et al.*, 2006). En lo que respecta a la variación a escala local hemos comprobado que las hembras de las poblaciones ovíparas de zonas altas del área cántabro-pirenaica de *Z. vivipara* retienen los huevos hasta alcanzar estadios de desarrollo embrionario más avanzados (estadios 33-35 de D&H) que las hembras de zonas bajas (30-34) (ver Capítulo I de esta Memoria). Además, el ritmo de desarrollo de los embriones es más rápido en zonas altas, de modo que el efecto que las bajas temperaturas producen sobre el tiempo de incubación queda parcialmente contrarrestado, es decir, existe variación contra gradiente en la tasa de desarrollo embrionario (Conover y Schultz, 1995; Conover *et al.*, 2009). Debido al avance en la retención de los huevos y a una tasa de desarrollo mayor, el periodo de incubación externa se ve reducido en las poblaciones de montaña y esto, junto con la distribución geográfica de los modos de reproducción de *Z. vivipara*, vivípara en la mayor parte de su distribución y ovípara solo en el límite meridional, concuerda con la hipótesis sobre la evolución del viviparismo en climas fríos. El hecho de que las hembras de las poblaciones ovíparas de esta especie pongan huevos con embriones en estadios de desarrollo más avanzados que la mayoría de reptiles ovíparos apoyaría además la idea de que la transición oviparismo – viviparismo es un proceso gradual, en el que estas poblaciones representarían una fase de transición entre ambos modos de reproducción.

La retención prolongada de los huevos también presenta desventajas relacionadas, sobre todo, con la supervivencia de la hembra. Durante la gestación se reduce la movilidad y la habilidad para alimentarse de las hembras (Lourdais *et al.*, 2002; Shine, 2003; Lin *et al.*, 2008), que también se vuelven más vulnerables frente a los depredadores (Shine, 1980). Además, la retención de la puesta puede afectar directamente al éxito reproductor de las hembras, pues retrasa el momento en que la hembra puede ovular y producir su siguiente puesta (Tinkle y Gibbons, 1977; Braña *et al.*, 1991), de manera que en zonas donde el período reproductor es corto las hembras pueden ver imposibilitada su capacidad para realizar una segunda puesta. Las hembras de *Z. vivipara* de zonas altas realizan una sola puesta en cada estación reproductora, en lugar de las dos que son capaces de hacer las lagartijas de zonas bajas, que retienen los huevos durante menos tiempo y cuya estación reproductora es más larga. Lo mismo se ha comprobado en otro lacértido del norte ibérico, *Iberolacerta monticola*, que presenta niveles de retención solo ligeramente inferiores a *Z. vivipara* (Braña *et al.*, 1990; Braña *et al.*, 1991).

Una de las condiciones necesarias para la variación adaptativa en características del ciclo vital es que exista (o se genere) en la población una cierta variación heredable en el rasgo de interés que pueda ser objeto de selección. Es importante, por tanto, probar la variabilidad entre individuos en el nivel de retención y comprobar los efectos de los niveles extremos de retención de la puesta que pueden alcanzarse en condiciones ambientales límite, como temperaturas extremas o periodos prolongados de sequía. En ese contexto, hemos estudiado la plasticidad fenotípica en la retención de los huevos y sus consecuencias en una población ovípara de *Z. vivipara* de la Cordillera Cantábrica con retención prolongada de la puesta, obligando a las hembras gestantes a retener los huevos en los oviductos en respuesta frente a la sequía (manteniéndolas en sustratos de puesta secos), y se encontró cierta plasticidad en este rasgo. A diferencia de lo que ocurre en otras especies en las que el desarrollo embrionario se detiene completamente durante la retención forzada de los huevos (Mathies y Andrews, 1999) o continúa avanzando hasta producir juveniles viables, en estadios próximos al 40 de D&H (Mathies y Andrews, 1996), el desarrollo embrionario continúa avanzando en *Z. vivipara* durante la retención forzada de los huevos, aunque solo hasta el estadio 35, es decir, se alcanza únicamente un estadio de desarrollo más que en condiciones normales. Es posible que exista un límite superior del nivel de desarrollo alcanzable en los oviductos antes de la puesta en torno a este estadio de desarrollo, pues no solo no se sobrepasa el estadio 35 al forzar a las hembras gestantes a retener la puesta más allá del tiempo normal, sino que además aumenta considerablemente la mortalidad de los embriones, tanto por el hecho de que al llegar a ese límite algunas hembras realizan la puesta incluso en condiciones desfavorables (sustrato seco), como por el efecto que tiene la retención forzada de la puesta sobre el desarrollo embrionario en huevos puestos en sustratos adecuados. Por otra parte, la retención forzada de los huevos ocasiona también un retraso en el desarrollo embrionario con respecto al desarrollo potencial a la misma temperatura, de forma similar a lo encontrado en *Sceloporus virgatu*s (Andrews y Rose, 1994). El nivel de desarrollo alcanzado por los embriones control tras una semana de incubación externa a 25ºC llega al estadio 37 de D&H, lo que supone un avance de 2-4 estadios con respecto al desarrollo alcanzado en el mismo tiempo *in utero* por los embriones retenidos experimentalmente, a pesar de que la temperatura de la hembra es más alta que la temperatura de incubación externa. Todo esto hace pensar que existen limitaciones que dificultan el avance del desarrollo embrionario intrauterino más allá del momento normal de la puesta y que la retención prolongada de la puesta no representa claras ventajas en la población estudiada, salvo en lo que respecta a evitar el riesgo de deshidratación al depositar la puesta en sustratos secos. Sin embargo, Lindtke *et al.*, (2010) han encontrado en Austria puestas de *Z. vivipara* en que el estadio de desarrollo alcanzado por los embriones (estadio 35,5, D&H) y el grosor de la cáscara, intermedio entre el de las ovíparas y el de las vivíparas, hacen pensar en posibles híbridos naturales, ya que se trata de una zona de contacto entre poblaciones ovíparas y vivíparas de esta especie. En todo caso, este estudio plantea bastantes dudas, ya que el criterio para definir los híbridos (solamente dos hembras) fue precisamente el grado de desarrollo de los embriones en el momento de la puesta, y no se aporta ninguna información adicional sobre la viabilidad de esas puestas. Existen también híbridos experimentales, resultado de cruzar individuos vivíparos con ovíparos, que alcanzan el estadio 36 en el momento de la puesta y cuya cáscara es de grosor y grado de calcificación intermedios entre los de las cáscaras de ambos modos de reproducción (Heulin *et al.*, 1992; Arrayago *et al.*, 1996). Sin embargo, la viabilidad y el éxito reproductor de esos híbridos parecen ser menores que los de los individuos resultantes de cruces normales y, en cualquier caso, es muy probable que su viabilidad en el campo sea baja, puesto que la cáscara fina de los huevos híbridos haría disminuir su supervivencia. En nuestro estudio se comprobó que no hay reducción del grosor de la cáscara de los huevos tras una semana de retención forzada de la puesta y esto puede estar limitando el intercambio gaseoso entre el embrión y la hembra y, por tanto, probablemente también el progreso del desarrollo embrionario intrauterino más allá del estadio 35. El estudio de la variación altitudinal en el grosor de la cáscara de los huevos en *Z. vivipara* ayudaría a esclarecer la relación entre el grosor de la cáscara y la retención prolongada de la puesta. Sí se constató en nuestro trabajo una disminución de la concentración de calcio durante la fase de incubación externa del huevo, lo cual sugiere que la cáscara es una fuente importante de calcio para el embrión durante su desarrollo y concuerda con el estudio de Stewart *et al.* (2009) en el que se muestra que el 81% del calcio de las crías de *Z. vivipara* proviene de la cáscara. Ji y Braña (1999) demuestran en otro lacértido (*Podarcis muralis*) que la cáscara aporta el 53% del Calcio y el 20% del Magnesio que constituye los tejidos, principalmente el esqueleto, de los neonatos. La dependencia por parte del embrión del calcio de la cáscara constituiría, según Packard *et al.* (1977), una limitación en la evolución del viviparismo en esta especie, ya que este proceso traería consigo el adelgazamiento y descalcificación progresiva de la cáscara. Sin embargo, la disminución de la cantidad de calcio en las cáscaras a medida que aumenta la duración de la retención de la puesta no hace que disminuya la cantidad de calcio disponible para el embrión de *Z. vivipara*, puesto que la transferencia de calcio a través de secreciones uterinas, que evolucionaría simultáneamente a la evolución del viviparismo (Stewart *et al.*, 2011), compensaría el adelgazamiento de la capa calcárea (Stewart *et al.*, 2009) y además la capacidad del embrión para tomar calcio, ya sea de la cáscara o de las secreciones uterinas, no se ve modificada durante la transición entre los modos de reproducción (Stewart *et al.*, 2011).

Uno de los objetivos abordados en esta memoria ha sido evaluar la hipótesis de optimización térmica de la embriogénesis como motor de los cambios hacia mayor extensión de la retención de los huevos en los oviductos (Shine, 1995; Webb *et al.*, 2006; Braña y Ji, 2007), otra de las más ampliamente aceptadas sobre la evolución del viviparismo en reptiles. Esta hipótesis predice que la retención de la puesta es ventajosa porque permite que los embriones estén sometidos durante un mayor periodo de tiempo a la temperatura óptima para su desarrollo, basándose en que las hembras modifican sus preferencias térmicas durante la gestación (Shine, 2006) y en que la temperatura es un factor con importantes efectos sobre el desarrollo embrionario (Braña y Ji, 2007).

El estudio de la biología térmica de *Z. vivipara* nos permitió comprobar que, al igual que ocurre en poblaciones vivíparas de esta especie (Van Damme *et al.*, 1990; Gvoždík y Castilla, 2001), no hay adaptación de las preferencias térmicas a las condiciones locales, pues no existen diferencias entre las temperaturas seleccionadas por las lagartijas de los diferentes ambientes térmicos que representan las zonas altas y bajas. Tampoco las temperaturas corporales medidas en el campo difieren entre poblaciones de diferentes altitudes y tanto en zonas altas como bajas estas temperaturas están por debajo de las temperaturas seleccionadas en el gradiente térmico, lo cual pone de manifiesto la dificultad de las lagartijas para termorregular en el hábitat de baja calidad térmica, húmedo y con densa cobertura vegetal, en el que habitan, ya que las temperaturas disponibles en él están muy por debajo de las preferidas por las lagartijas, independientemente de la altitud (Hertz *et al.*, 1993; Blouin-Demers y Nadeau, 2005). Sin embargo, al igual que ocurre en otras poblaciones vivíparas (Van Damme *et al.*, 1986; Gvoždík y Castilla, 2001) y ovíparas (Carretero *et al.*, 2005) de *Z. vivipara*, las hembras de las poblaciones de nuestro estudio seleccionan una temperatura corporal más baja durante la gestación y este hecho podría ocurrir por dos motivos (Beuchat, 1986; Braña, 1993; Andrews *et al.*, 1997): 1) porque la temperatura óptima para el desarrollo de los embriones es más baja que para otros procesos fisiológicos de las hembras, de modo que éstas seleccionan activamente temperaturas más bajas; 2) porque la carga del peso o volumen de la puesta hace que las hembras tengan dificultades para termorregular y acepten pasivamente temperaturas corporales bajas. Debido a que a lo largo del gradiente térmico creado en el laboratorio no hay diferencias en cuanto a costes de depredación o a la disponibilidad de alimento o refugios se puede concluir que las bajas temperaturas seleccionadas por las hembras gestantes en esas condiciones no fueron una consecuencia de la movilidad limitada de las hembras o de una modificación de su comportamiento antidepredador (Braña, 1993). En cambio podría deberse, de acuerdo con la hipótesis de “optimización térmica de la embriogénesis”, a que las hembras gestantes evitan temperaturas altas que podrían tener efectos negativos sobre el desarrollo de los embriones. Esta hipótesis se vio reforzada al comprobar que una temperatura de incubación similar a la temperatura que seleccionan las hembras no gestantes en el gradiente térmico afecta negativamente al éxito de eclosión, principalmente, así como al fenotipo de las crías. También se comprobó que, al igual que en otras especies de lacértidos (Braña y Ji, 2007), la temperatura de incubación influye sobre la duración del desarrollo embrionario en *Z. vivipara*, según una función exponencial negativa, de manera que incubar los huevos a 21ºC reduce el tiempo a la mitad comparado con incubarlos a 17ºC, mientras que apenas hay diferencia en el tiempo de incubación a 32ºC y 34ºC (ver Fig. 6.1).

Las consecuencias de someter a las hembras gestantes a la temperatura seleccionada por las hembras no gestantes para evaluar su efecto sobre el desarrollo embrionario durante la fase de incubación intrauterina fueron similares a las que resultaron de someter a los huevos a altas temperaturas durante la incubación externa. Esto, junto con el cambio en las preferencias térmicas de las hembras gestantes, concuerda con la predicción de la hipótesis de “optimización térmica” de la embriogénesis, pues las hembras gestantes evitarían las temperaturas de incubación altas, que tienen importantes efectos negativos sobre aspectos del desarrollo que influyen en la supervivencia de la descendencia.

Se puede concluir, en consecuencia, que las dos principales hipótesis sobre la evolución del viviparismo no son incompatibles en *Z. vivipara*. Por un lado, las hembras de zonas altas retienen la puesta durante una mayor fracción del desarrollo embrionario que las de zonas bajas, coincidiendo con las predicciones de la hipótesis de clima frío. Y, aunque la selección de una temperatura corporal más baja durante la gestación podría parecer contraria a esta hipótesis, ese cambio en la temperatura seleccionada durante la gestación apenas retrasa la eclosión debido a que la duración del desarrollo embrionario varía en función de la temperatura siguiendo una función exponencial negativa, de manera que la diferencia entre incubar los huevos a 32ºC (temperatura seleccionada durante la gestación) o a 34ºC (temperatura seleccionada por las hembras no gestantes) apenas influye en el tiempo de incubación. Y por otro lado, también serían válidas las predicciones de la hipótesis de optimización térmica de la embriogénesis, pues la temperatura corporal seleccionada por las hembras durante la gestación se aproxima a las temperaturas que optimizan los fenotipos de las crías.

VII. Conclusiones

1. La totalidad de las poblaciones del norte de la Península Ibérica de la especie con bimodalidad reproductora *Zootoca vivipara* son ovíparas y presentan un nivel avanzado de retención oviductal de la puesta en relación con otros lacértidos.

2. El nivel de retención de la puesta en poblaciones de zonas altas es mayor que en poblaciones de zonas bajas. La relativa discontinuidad entre poblaciones de *Z. vivipara* a lo largo de la Cordillera Cantábrica y los Pirineos debido a la distribución discontinua de su hábitat sugiere que la retención prolongada en zonas altas podría haber tenido varios orígenes independientes.

3. Existe variación contra gradiente en la tasa de desarrollo embrionario, que, a igual estado inicial de desarrollo, es más rápida para los embriones de zonas altas, de modo que el efecto que las bajas temperaturas producen sobre el tiempo de incubación queda parcialmente contrarrestado.

4. El avance en la retención y una tasa de desarrollo mayor implican la reducción del periodo de incubación externa en las poblaciones de montaña y esto, junto con la distribución geográfica de los modos de reproducción de *Z. vivipara*, vivípara en la mayor parte de su distribución y ovípara solo en el límite meridional, concuerda con las predicciones derivadas de la hipótesis sobre la evolución del viviparismo en climas fríos.

5. Si bien la retención forzada de los huevos en los oviductos maternos permite alcanzar un estadio de desarrollo más avanzado en el momento de la puesta, el retraso que la retención produce en la embriogénesis intrauterina, el escaso éxito de eclosión o las deficiencias de los fenotipos de las crías obtenidos tras la retención forzada de la puesta señalan que existen dificultades para el avance del desarrollo embrionario intrauterino más allá del momento normal de la oviposición y que la retención prolongada de la puesta no representa claras ventajas en la población estudiada.

6. Parece haber un límite superior alrededor del estadio 35 de la escala de desarrollo de Dufaure y Hubert (1961) en la extensión de desarrollo embrionario alcanzable en los oviductos maternos para las poblaciones ovíparas de *Z. vivipara*, pues al someter a las hembras gestantes a una temperatura alta o al forzarlas a retener la puesta más allá del tiempo normal no se sobrepasa ese estadio de desarrollo y además aumenta considerablemente la mortalidad de los embriones.

7. La cantidad de calcio en la cáscara del huevo es mayor en el momento de la puesta que en el de la eclosión, lo cual apoya el hecho de que la cáscara del huevo de *Z. vivipara* es una fuente de minerales para el embrión durante su desarrollo. No se ha constatado una reducción del grosor de la cáscara paralela al avance del desarrollo embrionario intrauterino favorecido por la retención forzada de la puesta o por las altas temperaturas a las que fueron sometidas las hembras durante la gestación, tal vez porque ese avance, de solo una unidad en la escala de desarrollo embrionario, no fue lo suficientemente importante.

8. Los diferentes ambientes térmicos que representan las zonas altas y bajas no han ocasionado la adaptación de las preferencias térmicas de *Z. vivipara* a las condiciones locales, ya que no hay diferencias en la temperatura seleccionada en un gradiente térmico por lagartijas de diferentes altitudes. Tampoco difieren entre poblaciones las temperaturas corporales medidas en el campo, que son considerablemente más bajas que las preferidas en el gradiente térmico, señalando la dificultad de las lagartijas para termorregular en el hábitat de baja calidad térmica, húmedo y con densa cobertura vegetal, en el que viven.

9. Las hembras gestantes presentan temperaturas corporales más bajas que los machos y las hembras no gestantes de la misma población tanto en el campo como en un gradiente térmico creado en el laboratorio. Este hecho indica que las bajas temperaturas de las hembras gestantes no se deben a que éstas tengan pocas oportunidades para termorregular a causa de su limitada movilidad. La diferencia podría deberse, de acuerdo con la hipótesis de "optimización térmica de la embriogénesis", a que las hembras gestantes evitan temperaturas altas que podrían tener efectos negativos sobre el desarrollo de los embriones.

10. La temperatura durante la fase de incubación externa de los embriones de *Z. vivipara* afecta al tiempo de desarrollo, al éxito de eclosión y al fenotipo de las crías. Al incubar los huevos a temperaturas en el intervalo de 17 a 34ºC se comprobó que las temperaturas más altas, y especialmente la de 34ºC, tienen importantes efectos negativos sobre aspectos del desarrollo que influyen en la supervivencia de la descendencia. En este contexto cabe interpretar que las hembras gestantes evitan las temperaturas de incubación altas, de acuerdo con las predicciones de la hipótesis de "optimización térmica de la embriogénesis".

11. Una temperatura de incubación intrauterina elevada, similar a la seleccionada por las hembras no gestantes, hace que los embriones alcancen mayor desarrollo en el momento de la puesta, pero influye negativamente en el éxito de eclosión y en varios rasgos del fenotipo de las crías. Por lo tanto, el cambio en las preferencias térmicas de las hembras durante la gestación evita los efectos negativos que tienen sobre la descendencia las altas temperaturas seleccionadas por las hembras no gestantes, tal y como predice la hipótesis de "optimización térmica de la embriogénesis".

12. Las dos principales hipótesis sobre la evolución del viviparismo no parecen ser excluyentes en *Z. vivipara*. Por un lado, las temperaturas corporales seleccionadas por las hembras durante la gestación se aproximan a las temperaturas que optimizan los fenotipos de las crías, según las predicciones de la hipótesis de "optimización térmica de la embriogénesis". Por otra parte, la selección de temperaturas corporales más bajas por parte de las hembras gestantes solo produce un corto retraso en el desarrollo debido a que el tiempo de incubación varía en función de la temperatura siguiendo una función exponencial negativa que se vuelve prácticamente asintótica a partir de 30ºC. Sin embargo, puesto que la temperatura seleccionada por las hembras gestantes es más alta que la temperatura media en los nidos, la retención acorta considerablemente el tiempo de incubación, tal y como asume la hipótesis de "clima frío".

VIII. Bibliografía

Adolph S.C., Porter W.P. 1993. Temperature, activity, and lizard life histories. *The American Naturalist,* 142: 273-295.

Adolph S.C., Porter W.P. 1996. Growth, seasonality, and lizard life histories: age and size at maturity. *Oikos,* 77: 267-278.

Andrews R.M. 2000. Evolution of viviparity in squamate reptiles (*Sceloporus* spp.): a variant of the cold-climate model. *Journal of Zoology,* 250: 243-253.

Andrews R.M. 2002. Low oxygen: a constraint on the evolution of viviparity in reptiles. *Physiological and Biochemical Zoology,* 75: 145-154.

Andrews R.M. 2004. Patterns of embryonic development. In Deeming D.C. (ed.). *Reptilian incubation: environment, evolution, and behaviour*. Nottingham University Press, Nottingham, pp. 75-102.

Andrews R.M., Mathies T. 2000. Natural history of reptilian development: constraints on the evolution of viviparity. *BioScience,* 50: 229-238.

Andrews R.M., Rose B.R. 1994. Evolution of viviparity: constraints on egg retention. *Physiological Zoology,* 67: 1006-1024.

Andrews R.M., Mathies T., Warner D.A. 2000. Effect of incubation temperature on morphology, growth, and survival of juvenile *Sceloporus undulatus*. *Herpetological Monographs,* 14: 420-431.

Andrews R.M., Méndez de la Cruz F.R., Villagrán-Santa Cruz M. 1997. Body temperatures of female *Sceloporus grammicus*: thermal stress or impaired mobility? *Copeia,* 1997: 108-115.

Andrews R.M., Díaz-Paniagua C., Marco A., Portheault A. 2008. Developmental arrest during embryonic development of the common chameleon (*Chamaeleo chamaeleon*) in Spain. *Physiological and Biochemical Zoology,* 81: 336-344.

Andrews R.M., Mathies T., Qualls C.P., Qualls F.J. 1999. Rates of embryonic development of Sceloporus lizards: do cold climates favor the evolution of rapid development? *Copeia,* 1999: 692-700.

Angilletta M.J. 2001. Thermal and physiological constraints on energy assimilation in a widespread lizard (*Sceloporus undulatus*). *Ecology,* 82: 3044-3056.

Angilletta M.J. 2009. Thermal adaptation: A theoretical and empirical synthesis. *Oxford University Press,* Oxford.

Angilletta M.J., Sears M.W., Pringle R.M. 2009. Spatial dynamics of nesting behavior: lizards shift microhabitats to construct nests with beneficial thermal properties. *Ecology,* 90: 2933-2939.

Angilletta M.J., Winters R.S., Dunham A.E. 2000. Thermal effects on the energetics of lizard embryos: implications for hatchling phenotypes. *Ecology,* 81: 2957-2968.

Angilletta M.J., Niewiarowski P.H., Dunham A.E., Leaché A.D., Porter W.P. 2004a. Bergmann's clines in ectotherms: illustrating a life-history perspective with sceloporine lizards. *The American Naturalist,* 164: 168-183.

Angilletta M.J., Oufiero C.E., Sears M.W. 2004b. Thermal adaptation of maternal and embryonic phenotypes in a geographycally widespread ectotherm. *International Congress Series,* 1275: 258-266.

Arrayago M.J., Bea A., Heulin B. 1996. Hybridization experiment between oviparous and viviparous strains of *Lacerta vivipara*: a new insight into the evolution of viviparity in reptiles. *Herpetologica,* 52: 333-342.

Arribas O. 2009. Morphological variability of the Cantabro-Pyrenean populations of *Zootoca vivipara* (Jacquin, 1787) with description of a new subspecies (Squamata: Sauria: Lacertidae). *Herpetozoa,* 21: 123-146.

Ashton K.G. 2001. Body size variation among mainland populations of the western rattlesnake (*Crotalus viridis*). *Evolution,* 55: 2523-2533.

Ashton K.G. 2002. Patterns of within-species body size variation of birds: strong evidence for Bergmann's rule. *Global Ecology and Biogeography,* 11: 505-523.

Ashton K.G., Feldman C.R. 2003. Bergmann's rule in non-avian reptiles: turtles follow it, lizards and snakes reverse it. *Evolution,* 57: 1151-1163.

Atkinson D. 1994. Temperature and organism size: a biological law for ectotherms? *Advances in Ecological Research,* 25: 1-58.

Bauwens D., Castilla A.M., Van Damme R., Verheyen R.F. 1990. Field body temperatures and thermoregulatory behavior of the high altitude lizard, *Lacerta bedriagae*. *Journal of Herpetology*, 24: 88-91.

Beuchat C.A. 1986. Reproductive influences on the thermoregulatory behavior of a live-bearing lizard. *Copeia,* 4: 971-979.

Beuchat C.A. 1988. Temperature effects during gestation in a viviparous lizard. *Journal of Thermal Biology,* 13: 135-142.

Beuchat C.A., Ellner S. 1987. A quantitative test of life history theory: thermoregulation by a viviparous lizard. *Ecological Monographs,* 57: 45-60.

Birchard G.F. 2004. Effects of incubation temperature. In Deeming D.C. (ed.). *Reptilian incubation: environment, evolution, and behaviour.* Nottingham University Press, Nottingham, pp. 103-123.

Blackburn D.G. 1982. Evolutionary origins of viviparity in the Reptilia. I. Sauria. *Amphibia-Reptilia,* 3: 185-205.

Blackburn D.G. 1985. Evolutionary origins of viviparity in the Reptilia. II. Serpentes, Amphisbaena, and Ichtyosauria. *Amphibia-Reptilia,* 6:259-291.

Blackburn D.G. 1995. Saltationist and punctuated equilibrium models for the evolution of viviparity and placentation. *Journal of Theoretical Biology,* 174: 199-216.

Blackburn D.G. 1998. Reconstructing the evolution of viviparity and placentation. *Journal of Theoretical Biology,* 192: 183-190.

Blackburn D.G. 1999a. Viviparity and oviparity: evolution and reproductive strategies. En Knobil E., Neill J.D. (eds.). *Encyclopedia of reproduction,* Vol. 4. Academic Press, San Diego, pp. 994-1003.

Blackburn D.G. 1999b. Are viviparity and egg-guarding evolutionarily labile in Squamates? *Herpetologica,* 55: 556-573.

Blackburn D.G. 2006. Squamate reptiles as model organisms for the evolution of viviparity. *Herpetological Monographs,* 20: 131-146.

Blackburn D.G., Evans H.E. 1986. Why are there no viviparous birds? *The American Naturalist,* 128: 165-190.

Blackburn T.M., Gaston K.J., Loder N. 1999. Geographic gradients in body size: a clarification of Bergmann's rule. *Diversity and Distributions,* 5.

Blouin-Demers G., Nadeau P. 2005. The cost-benefit model of thermoregulation does not predict lizard thermoregulatory behavior. *Ecology,* 86: 560-566.

Booth D.T. 2006. Influence of incubation temperature on hatchling phenotype in reptiles. *Physiological and Biochemical Zoology,* 79: 274-281.

Braña F. 1986. Ciclo reproductor y oviparismo de *Lacerta vivipara* en la Cordillera Cantábrica. *Revista Española de Herpetología,* 1: 273-291.

Braña F. 1993. Shifts in body temperature and escape behaviour of female *Podarcis muralis* during pregnancy. *Oikos,* 66**:** 216-222.

Braña F. 1996. Sexual dimorphism in lacertid lizards: male head increase vs female abdomen increase? *Oikos,* 75: 511-523.

Braña F. 2003. Morphological correlates of burst speed and field movement patterns: the behavioural adjustment of locomotion in wall lizards (*Podarcis muralis*). *Biological Journal of the Linnean Society,* 80: 135-146.

Braña F. 2008. Sex of incubation neighbours influences hatchling sexual phenotypes in an oviparous lizard. *Oecologia,* 156: 275-280.

Braña F., Bea A. 2002. *Lacerta vivipara*. In *Atlas y libro rojo de los anfibios y reptiles de España.* Pleguezuelos J.M., Márquez R., Lizana M. (eds). Dirección General de Conservación de la Naturaleza - Asociación Herpetológica Española, Madrid, pp. 236-237.

Braña F., Ji X. 2000. Influence of incubation temperature on morphology, locomotor performance, and early growth of hatchling wall lizards (*Podarcis muralis*). *Journal of Experimental Zoology,* 286: 422-433.

Braña F., Ji X. 2007. The selective basis for increased egg retention: early incubation temperature determines hatchling phenotype in wall lizards (*Podarcis muralis*). *Biological Journal of the Linnean Society,* 92: 441-447.

Braña F., Bea A., Arrayago M.J. 1991. Egg retention in lacertid lizards: relationships with reproductive ecology and the evolution of viviparity. *Herpetologica,* 47: 218-226.

Braña F., Arrayago M.J., Bea A., Barahona A. 1990. Ciclo reproductor y de cuerpos grasos en los machos de *Lacerta monticola cantabrica*. Comparación entre dos poblaciones situadas a diferente altitud. *Amphibia-Reptilia*, 11: 41-52.

Buckley D., Alcobendas M., García-París M., Wake M.H. 2007. Heterochrony, cannibalism, and the evolution of viviparity in *Salamandra salamandra*. *Evolution and Development,* 9: 105-115.

Calderón-Espinosa M.L., Andrews R.M., Méndez de la Cruz F.R. 2006. Evolution of egg retention in the *Sceloporus spinosus* group: exploring the role of physiological, environmental, and phylogenetic factors. *Herpetological Monographs,* 20: 147-158.

Carretero M.A., Roig J.M., Llorente G.A. 2005. Variation in preferred body temperature in an oviparous population of *Lacerta* (*Zootoca*) *vivipara*. *Herpetological Journal,* 15: 51-55.

Chang J.L., Doughty S., Wade J., Lovern M.B. 2006. Sexual dimorphism in the second-to-fourth digit length ratio in green anoles, *Anolis carolinensis* (Squamata: Polychrotidae) from the southeastern United States. *Canadian Journal of Zoology,* 84: 1489-1494.

Civantos E., Salvador A., Veiga J.P. 1999. Body size and microhabitat affect winter survival of hatchling *Psammodromus algirus* lizards. *Copeia,* 1999: 1112-1117.

Clobert J., Oppliger A., Sorci G., Ernande B., Swallow J.G., Garland T. 2000. Trade-offs in phenotypic traits: endurance at birth, growth, survival, predation and susceptibility to parasitism in a lizard, *Lacerta vivipara. Functional Ecology,* 14: 675–684.

Conover D.O., Schultz E.T. 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends in Ecology and Evolution,* 10: 248-252.

Conover D.O., Duffy T.A., Hice L.A. 2009. The covariance between genetic and environmental influences across ecological gradients. Reassessing the evolutionary significance of countergradient and cogradient variation. *Annals of the New York Academy of Sciences,* 1168: 100-129.

Daut E.F., Andrews R.M. 1993. The effect of pregnancy on selected body temperatures of the viviparous lizard *Chalcides ocellatus*. *Journal of Herpetology,* 27: 6-13.

De Fraipont M., Clobert J., Barbault R. 1996. The evolution of oviparity with egg-guarding and viviparity in lizards and snakes: a phylogenetic analysis. *Evolution,* 50: 391-400.

Deeming D.C. 2004. Post-hatching phenotypic effects of incubation in reptiles. In Deeming D.C. (ed.). *Reptilian incubation: Environment, Evolution, and Behaviour.* Nottingham University Press, Nottingham, pp. 229-251.

Dhouailly D., Saxod R. 1974. Les stades du développement de *Lacerta muralis* Laurent entre la ponte et l'éclosion. *Bulletin de la Société Zoologique de France,* 99: 489-494.

Du W., Ji X. 2006. Effects of constant and fluctuating temperatures on egg survival and hatchling traits in the northern grass lizard (*Takydromus septentrionalis*, Lacertidae). *Journal of Experimental Zoology,* 305: 47-54.

Du W., Warner D.A., Langkilde T., Robbins T., Shine R. 2010. The physiological basis of geographic variation in rates of embryonic development within a widespread lizard species. *The American Naturalist,* 176: 522-528.

Dufaure J.P., Hubert J. 1961. Table de développement du lézard vivipare: *Lacerta* (*Zootoca*) *vivipara* Jacquin. *Archives d'Anatomie Microscopique et de Morphologie Expérimentale,* 50: 309-328.

Elphick M.J., Shine R. 1998. Longterm effects of incubation temperatures on the morphology and locomotor performance of hatchling lizards

(*Bassiana duperreyi*, Scincidae). *Biological Journal of the Linnean Society,* 63: 429-447.

Ferguson G.W., Bohlen C.H. 1978. Demographic analysis: A tool for the study of natural selection of behavioural traits. In Greenberg N., Maclean P.D. (eds.). *Behavior and Neurology of lizards*. US Department of Health, Education and Welfare, National Institute of Mental Health, Rockville, pp. 227-243.

Flatt T., Shine R., Borges-Landaez P.A., Downes S.J. 2001. Phenotypic variation in an oviparous montane lizard (*Bassiana duperreyi*): the effects of thermal and hydric incubation environments. *Biological Journal of the Linnean Society,* 74: 339-350.

Forsman A., Shine R. 1995. Parallel geographic variation in body shape and reproductive life history within the Australian scincid lizard *Lampropholis delicata*. *Functional Ecology,* 9: 818-828.

Garland T., Losos J.B. 1994. Ecological morphology of locomotor performance in Squamate reptiles. In Wainwright P.C., Reilly S.M. (eds.). *Ecological morphology. Integrative organismal biology.* University of Chicago Press, Chicago, pp. 240-302.

Garland T., Hankins E., Huey R.B. 1990. Locomotor capacity and social dominance in male lizards. *Functional Ecology,* 4: 243-250.

Gasc J.P., Cabela A., Crnobrnja-Isailovic J., Dolmen D., Grossenbacher K., Haffner P., Lescure J., Martens H., Martínez Rica J.P., Maurin H., Oliveira M.E., Sofianidou T.S., Veith M., Zuiderwijk A. (eds.). 1997. Atlas of amphibians and reptiles in Europe. Collection Patrimoines Naturels, 29. Societas Europaea Herpetologica, Muséum National d'Histoire Naturelle and Service du Patrimoine Naturel, Paris.

Grenot C.J., Garcin L., Dao J., Hérold J., Fahys B., Tséré-Pagès H. 2000. How does the European common lizard, *Lacerta vivipara*, survive the cold of winter? *Comparative Biochemistry and Physiology,* 127: 71-80.

Grigg G.C., Seebacher F. 1999. Field test of a paradigm: hysteresis of heart rate in thermoregulation by a free-ranging lizard (*Pogona barbata*). *Proceedings of the Royal Society B,* 266: 1291-1297.

Guillaume C.P., Heulin B., Arrayago M.J., Bea A., Braña F. 2000. Refuge areas and suture zones in the Pyrenean and Cantabrian regions: geographic variation of the female MPI sex-linked alleles among oviparous populations of the lizard *Lacerta (Zootoca) vivipara*. *Ecography,* 23: 3-10.

Guillette L.J. 1991. The evolution of viviparity in amniote vertebrates: new insights, new questions. *Journal of Zoology,* 223: 521-526.

Gvoždík L. 2002. To heat or to save time? Thermoregulation in the lizard *Zootoca vivipara* (Squamata: Lacertidae) in different thermal environments along an altitudinal gradient. *Canadian Journal of Zoology,* 80: 479-492.

Gvoždík L., Castilla A.M. 2001. A comparative study of preferred body temperatures and critical thermal tolerance limits among populations of *Zootoca vivipara* (Squamata: Lacertidae) along an altitudinal gradient. *Journal of Herpetology,* 35: 486-492.

Gvoždík L., Van Damme R. 2003. Evolutionary maintenance of sexual dimorphism in head size in the lizard *Zootoca vivipara*: a test of two hypotheses. *Journal of Zoology,* 259: 7-13.

Harlow P.S. 1996. A harmless technique for sexing hatchling lizards. *Herpetological Review,* 27: 71-72.

Herczeg G., Kovács T., Hettyey A., Merilä J. 2003. To thermoconform or thermoregulate? An assessment of thermoregulation opportunities for the lizard *Zootoca vivipara* in the subartic. *Polar Biology,* 26: 486-490.

Herrel A., Van Damme R., Vanhooydonck B., De Vree F. 2001.The implications of bite performance for diet in two species of lacertid lizards. *Canadian Journal of Zoology,* 79: 662-670.

Hertz P.E., Huey R., Stevenson R.D. 2003. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *The American Naturalist,* 142: 796-818.

Heulin B. 1987. Température diurne d'activité des males et des femelles de *Lacerta vivipara*. *Amphibia and Reptilia,* 8: 393-400.

Heulin B. 1990. Étude comparative de la membrane coquillère chez les souches ovipare et vivipare de *Lacerta vivipara*. *Canadian Journal of Zoology*, 68: 1015-1019.

Heulin B., Osenegg K., Lebouvier M. 1991. Timing of embryonic development and birth dates in oviparous and viviparous strains of *Lacerta vivipara*: testing the predictions of an evolutionary hypothesis. *Acta Oecologica,* 12: 517-528.

Heulin B, Arrayago M.J., Bea A., Braña F. 1992. Caractéristiques de la coquille des oeufs chez la souche hybride (ovipare x vivipare) du lézard *Lacerta vivipara*. *Canadian Journal of Zoology*, 70: 2242-2246.

Heulin B., Ghielmi S., Vogrin N., Surget-Groba Y., Guillaume C.P. 2002. Variation in eggshell characteristics and in intrauterine egg retention between two oviparous clades of the lizard *Lacerta vivipara*: insight into the oviparity-viviparity continuum in squamates. *Journal of Morphology,* 252: 255-262.

Heulin B., Guillaume C.P., Vogrin N., Surget-Groba Y., Tadic Z. 2000. Further evidence of the existence of oviparous populations of *Lacerta* (*Zootoca*) *vivipara* in the NW of the Balcan Peninsula. *Comptes Rendus de l'Académie des Sciences, Series III,* 323: 462-468.

Heulin B., Surget-Groba Y., Sinervo B., Miles D.B., Guiller A. 2011. Dynamics of haplogroup frequencies and survival rates in a contact zone of two mtDNA lineages of the lizard *Lacerta vivipara*. *Ecography,* 34: 436-447.

Hodges W.L. 2004. Evolution of viviparity in horned lizards (*Phrynosoma*): testing the cold-climate hypothesis. *Journal of Evolutionary Biology,* 17: 1230-1237.

Hofmann S., Henle K. 2006. Male reproductive success and intrasexual selection in the common lizard determined by DNA-microsatellites. *Journal of Herpetology,* 40: 1-6.

Hubert J. 1985. Embryology of the Squamata. In Gans C., Billet F. (eds.). *Biology of the Reptilia*, Vol 15. John Wiley and Sons, New York, pp. 1-34.

Huey R., Gilchrist G.W., Carlson M.L., Berrigan D., Serra L. 2000. Rapid evolution of a geographic cline in size in an introduced fly. *Science,* 287: 308-309.

Husak J.F. 2006. Does speed help you survive? A test with collared lizards of different ages. *Functional Ecology,* 20: 174-179.

Iraeta P., Monasterio C., Salvador A., Díaz J. 2006. Mediterranean hatchling lizards grow faster at higher altitude: a reciprocal transplant experiment. *Functional Ecology,* 20: 865-872.

Ji X., Braña F. 1999. The influence of thermal and hydric environments on embryonic use of energy and nutrients, and hatchling traits, in the wall lizards (*Podarcis muralis*). *Comparative Biochemistry and Physiology*, 124: 205-213.

Ji X., Lin C., Lin L., Qiu Q., Du Y. 2007. Evolution of viviparity in warm-climate lizards: an experimental test of the maternal manipulation hypothesis. *Journal of Evolutionary Biology,* 20: 1037-1045.

Ji X., Lin L., Luo L., Lu H., Gao J., Han J. 2006. Gestation temperature affects sexual phenotype, morphology, locomotor performance, and growth of neonatal brown forest skinks, *Sphenomorphus indicus. Biological Journal of the Linnean Society,* 88: 453-463.

Johnston I.A., Bennett A.F. 1996. Animals and temperature: phenotypic and evolutionary adaptation. Society for Experimental Biology, Seminar Series, 59. Cambridge University Press, Cambridge.

Köhler F., Von Rintelen T., Meyer A., Glaubrecht M. 2004. Multiple origin of viviparity in Southeast Asian gastropods (Cerithioidea: Pachychilidae) and its evolutionary implications. *Evolution,* 58: 2215-2226.

Laloi D., Richard M., Lecomte J., Massot M., Clobert J. 2004. Multiple paternity in clutches of common lizard *Lacerta vivipara*: data from microsatellite markers. *Molecular Ecology,* 13: 719-723.

Lantz L.A. 1927. Quelques observations nouvelles sur l'herpétologie des Pyrénées Centrales. *Revue d'Histoire Naturelle Appliquée,* 8: 54-61.

Le Galliard J.F., Clobert J., Ferrière R. 2004. Physical performance and darwinian fitness in lizards. *Nature,* 432: 502-505.

Le Galliard J., Le Bris M., Clobert J. 2003. Timing of locomotor impairment and shift in thermal preferences during gravidity in a viviparous lizard. *Functional Ecology,* 17: 877-885.

Lee M.S.Y., Shine R. 1998. Reptilian viviparity and Dollo's law. *Evolution,* 52: 1441-1450.

Li H., Qu Y., Hu R., Ji X. 2009. Evolution of viviparity in cold-climate lizards: testing the maternal manipulation hypothesis. *Evolutionary Ecology,* 23: 777-790.

Liao W., Lu X. 2012. Adults body size = *f* (initial size + growth rate x age): explaining the proximate cause of Bergman's cline in a toad along altitudinal gradients. *Evolutionary Ecology,* 26: 579-590.

Lin C., Zhang L., Ji X. 2008. Influence of pregnancy on locomotor and feeding performances of the skink, *Mabuya multifasciata*: Why do females shift thermal preferences when pregnant? *Zoology,* 111: 188-195.

Lindtke D., Mayer W., Böhme W. 2010. Identification of a contact zone between oviparous and viviparous common lizards (*Zootoca vivipara*) in central Europe: reproductive strategies and natural hybridization. *Salamandra,* 46: 73-82.

Lourdais O., Bonnet X., Doughty P. 2002. Costs of anorexia during pregnancy in a viviparous snake (*Vipera aspis*). *Journal of Experimental Zoology,* 292: 487-493.

Lourdais O., Shine R., Bonnet X., Guillon M., Naulleau G. 2004. Climate affects embryonic development in a viviparous snake, *Vipera aspis. Oikos,* 104: 551-560.

Mathies T. 1998. Constraints on the evolution of viviparity in the lizard genus *Sceloporus.* D. Phil. Thesis, Virginia Polytechnic Institute and State University.

Mathies T., Andrews R.M. 1995. Thermal and reproductive biology of high and low elevation populations of the lizard *Sceloporus scalaris*: implications for the evolution of viviparity. *Oecologia,* 104: 101-111.

Mathies T., Andrews R. 1996. Extended egg retention and its influence on embryonic development and egg water balance: implications for the evolution of viviparity. *Physiological Zoology,* 69: 1021-1035.

Mathies T., Andrews R.M. 1997. Influence of pregnancy on the thermal biology of the lizard, *Sceloporus jarrovi*: why do pregnant females exhibit low body temperatures? *Functional Ecology,* 11: 498-507.

Mathies T., Andrews R. 1999. Determinants of embryonic stage at oviposition in the lizard *Urosaurus ornatus*. *Physiological and Biochemical Zoology,* 72: 645-655.

Mathies T., Andrews R. 2000. Does reduction of the eggshell occur concurrently with or subsequent to the evolution of viviparity in phrynosomatid lizards? *Biological Journal of the Linnean Society,* 71: 719-736.

Monasterio C., Shoo L.P., Salvador A., Siliceo I., Díaz J. 2011. Thermal constraints on embryonic development as a proximate cause for elevational range limits in two Mediterranean lacertid lizards. *Ecography,* 34: 1030-1039.

Niewiarowski P.H., Roosenburg W. 1993. Reciprocal transplant reveals sources of variation in growth rates of the lizard *Sceloporus undulatus*. *Ecology,* 74: 1992-2002.

Olsson M., Gullberg A., Shine R., Madsen T., Tegelstrom H. 1996. Paternal genotype influences incubation period, offspring size, and offspring shape in an oviparous reptile. *Evolution,* 50: 1328-1333.

Oufiero C.E., Angilletta M.J. 2006. Convergent evolution of embryonic growth and development in the eastern fence lizard (*Sceloporus undulatus*). *Evolution,* 60: 1066-1075.

Packard G.C. 1991. Physiological and ecological importance of water to embryos of oviparous reptiles. In Deeming D.C., Ferguson M.W.J. (eds.). *Egg incubation: its effects on embryonic development in birds and reptiles*. Cambridge University Press, Cambridge, pp. 213-228.

Packard G.C., Packard M.J. 1988. The physiological ecology of reptilian eggs and embryos. In Gans C., Huey R.B. (eds.). *Biology of the reptilia,* Vol 16. Alan R. Liss, New York, pp. 523-606.

Packard G.C., Tracy C.R., Roth J.J. 1977. The physiological ecology of reptilian eggs and embryos, and the evolution of viviparity within the class reptilia. *Biological Reviews,* 52: 71-105.

Packard M.J., Packard G.C., Gutzke, W.H.N. 1984. Calcium metabolism in embryos of the oviparous snake *Coluber constrictor. Journal of Experimental Biology,* 110: 99-112.

Panigel M. 1956. Contribution a l'étude de l'ovoviviparité chez les reptiles: gestation et parturition chez le lézard vivipare *Zootoca vivipara. Annales des Sciences Naturelles - Zoologie,* 18: 569-668.

Parker S.L., Andrews R. 2006. Evolution of viviparity in sceloporine lizards: in utero Po2 as a developmental constraint during egg retention. *Physiological and Biochemical Zoology,* 79: 581-592.

Parker S.L., Andrews R. 2007. Incubation temperature and phenotypic traits of *Sceloporus undulatus*: implications for the northern limits of distribution. *Oecologia,* 151: 218-231.

Parker S.L., Andrews R., Mathies T. 2004. Embryonic responses to variation in oviductal oxygen in the lizard *Sceloporus undulatus* from Ney Jersey and South Carolina, USA. *Biological Journal of the Linnean Society,* 83: 289-299.

Pincheira-Donoso D., Hodgson D.J., Tregenza T. 2008. The evolution of body size under environmental gradients in ectotherms: why should Bergmann's rule apply to lizards? *BMC Evolutionary Biology,* 8: 68.

Qualls C.P. 1996. Influence of the evolution of viviparity on eggshell morphology in the lizard, *Lerista bougainvillii. Journal of Morphology*, 228: 119-125.

Qualls C.P., Andrews R. 1999a. Cold climates and the evolution of viviparity in reptiles: cold incubation temperatures produce poor-quality offspring in the lizard, *Sceloporus virgatus. Biological Journal of the Linnean Society,* 67: 353-376.

Qualls C.P., Andrews R. 1999b. Maternal body volume constrains water uptake by lizard eggs *in utero. Functional Ecology,* 13: 845-851.

Qualls C.P., Shine R. 1998a. *Lerista bougainvillii*, a case study for the evolution of viviparity in reptiles. *Journal of Evolutionary Biology,* 11: 63-78.

Qualls C.P., Andrews R., Mathies T. 1997. The evolution of viviparity and placentation revisted. *Journal of Theoretical Biology,* 185: 129-135.

Qualls C.P., Shine R., Donnellan S., Hutchinson M. 1995. The evolution of viviparity within the Australian scincid lizard *Lerista bouganvillii*. *Journal of Zoology,* 237: 13-26.

Qualls F.J., Shine R. 1998b. Geographic variation in lizard phenotypes: importance of the incubation environment. *Biological Journal of the Linnean Society,* 64: 477-491.

Robson M.A., Miles D.B. 2000. Locomotor performance and dominance in male tree lizards, *Urosaurus ornatus*. *Functional Ecology,* 14: 338-344.

Rock J., Cree A. 2003. Intraspecific variation in the effect of temperature on pregnancy in the viviparous gecko *Hoplodactylus maculatus*. *Herpetologica,* 59: 8-22.

Rock J., Andrews R., Cree A. 2000. Effects of reproductive condition, season, and site on selected temperatures of a viviparous gecko. *Physiological and Biochemical Zoology,* 73: 344-355.

Rodríguez-Díaz T., Braña F. 2011a. Shift in thermal preferences of female oviparous common lizards during egg retention: insights into the evolution of reptilian viviparity. *Evolutionary Biology,* 38: 352-359.

Rodríguez-Díaz T., Braña F. 2011b. Plasticity and limitations of extended egg retention in oviparous *Zootoca vivipara* (Reptilia: Lacertidae). *Biological Journal of the Linnean Society,* 102: 75-82.

Rodríguez-Díaz T., González F., Ji X., Braña F. 2010. Effects of incubation temperature on hatchling phenotypes in an oviparous lizard with prolonged egg retention: are the two main hypotheses on the evolution of viviparity compatible? *Zoology,* 113: 33-38.

Roff D.A. 1992. The evolution of life histories. Theory and analysis. Chapman and Hall, London.

Rubolini D., Pupin F., Sacchi R., Gentilli A., Zuffi M.A.L., Galeotti P., Saino N. 2006. Sexual dimorphism in digit length ratios in two lizard species. *Anatomical Record,* 288: 491-497.

Sears M.W., Angilletta M.J. 2004. Body size clines in *Sceloporus* lizards: proximate mechanisms and demographic constraints. *Integrative and Comparative Biology,* 44: 433-442.

Shadrix C.A., Crotzer D.R., McKinney S.L., Stewart J.R. 1994. Embryonic growth and calcium mobilization in oviposited eggs of the scincid lizard, *Eumeces fasciatus*. *Copeia,* 2: 493-498.

Shanbhag B.A., Saidapur S.K., Radder R.S. 2003. Lowering body temperature induces embryonic diapause during prolonged egg retention in the lizard, *Calotes versicolor*. *Naturwissenschaften,* 90: 33-35.

Shine R. 1980. "Costs" of reproduction in reptiles. *Oecologia,* 46: 92-100.

Shine R. 1983. Reptilian reproductive modes: the oviparity-viviparity continuum. *Herpetologica,* 39: 1-8.

Shine R. 1985. The evolution of viviparity in reptiles: an ecological analysis. In Gans C., Billett F. (eds.). *Biology of the Reptilia*, Vol. 15. John Wiley and Sons, New York, pp. 605-694.

Shine R. 1995. A new hypothesis for the evolution of viviparity in reptiles. *The American Naturalist*, 145: 809-823.

Shine R. 1999. Egg-laying reptiles in cold climates: determinants and consequences of nest temperatures in montane lizards. *Journal of Evolutionary Biology,* 12: 918-926.

Shine R. 2002a. Reconstructing an adaptationist scenario: what selective forces favor the evolution of viviparity in montane reptiles? *The American Naturalist,* 160: 582-593.

Shine R. 2002b. Eggs in autumn: responses to declining incubation temperatures by the eggs of montane lizards. *Biological Journal of the Linnean Society*, 76: 71-77.

Shine R. 2003. Effects of pregnancy on locomotor performance: an experimental study on lizards. *Oecologia*, 136: 450-456.

Shine R. 2004. Does viviparity evolve in cold climate reptiles because pregnant females maintain stable (not high) body temperatures? *Evolution,* 58: 1809–1818.

Shine R. 2006. Is increased maternal basking an adaptation or a pre-adaptation to viviparity in lizards? *Journal of Experimental Zoology Part A,* 305**:** 524-535.

Shine R., Bull J.J. 1979. The evolution of live-bearing in lizards and snakes. *The American Naturalist,* 113: 905-923.

Shine R., Downes S.J. 1999. Can pregnant lizards adjust their offspring phenotypes to environmental conditions? *Oecologia,* 119: 1-8.

Shine R., Harlow P. 1993. Maternal thermoregulation influences offspring viability in a viviparous lizard. *Oecologia,* 96: 122-127.

Shine R., Harlow P.S. 1996. Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology,* 77: 1808-1817.

Shine R., Lee M.S.Y. 1999. A reanalysis of the evolution of viviparity and egg-guarding in squamate reptiles. *Herpetologica,* 55: 538-549.

Shine R., Thompson M.B. 2006. Did embryonic responses to incubation conditions drive the evolution of reproductive modes in squamate reptiles? *Herpetological Monographs,* 20: 159-171.

Sinervo B., Hedges R., Adolph S.C. 1991. Decreased sprint speed as a cost of reproduction in the lizard *Sceloporus occidentalis*: variation among populations. *Journal of Experimental Biology,* 155: 323-336.

Smith S.A., Shine R. 1997. Intraespecific variation in reproductive mode within the scincid lizard *Saiphos equalis*. *Australian Journal of Zoology,* 45: 435-445.

Smith S.A., Austin C.C., Shine R. 2001. A phylogenetic analysis of variation in reproductive mode within an Australian lizard (*Saiphos equalis*, Scincidae). *Biological Journal of the Linnean Society,* 74: 131-139.

Sorci G., Clobert J. 1997. Environmental effects on locomotor performance in the common lizard (*Lacerta vivipara*). *Evolutionary Ecology,* 11: 531-541.

Sorci G., Swallow J.G., Garland T., Clobert J. 1995. Quantitative genetics of locomotor speed and endurance in the lizard *Lacerta vivipara. Physiological Zoology,* 68: 698-720.

Stamps J.A. 1976. Egg retention, rainfall and egg laying in a tropical lizard *Anolis aeneus*. *Copeia,* 1976: 759-764.

Stewart J.R., Ecay T.W. 2010. Patterns of maternal provision and embryonic mobilization of calcium in oviparous and viviparous squamate reptiles. *Herpetological Conservation and Biology,* 5: 341-359.

Stewart J.R., Ecay T.W., Heulin B. 2009. Calcium provision to oviparous and viviparous embryos of the reproductively bimodal lizard *Lacerta (Zootoca) vivipara. The Journal of Experimental Biology*, 212: 2520-2524.

Stewart J.R., Ecay T.W., Heulin B., Fregoso S.P., Linville B.J. 2011. Developmental expression of calcium transport proteins in extraembryonic membranes of oviparous and viviparous *Zootoca vivipara* (Lacertilia, Lacertidae). *Journal of Experimental Biology,* 214: 2999-3004.

Surget-Groba Y., Heulin B., Guillaume C.P., Thorpe R.S., Kupriyanova L., Vogrin N., Maslak R., Mazzotti S., Venczel M., Ghira I., Odierna G., Leontyeva O., Monney J. C., Smith N. 2001. Intraspecific phylogeography of *Lacerta vivipara* and the evolution of viviparity. *Molecular Phylogenetics and Evolution,* 18: 449–459.

Surget-Groba Y., Heulin B., Guillaume C.P., Puky M., Semenov D., Orlova V., Kupriyanova L., Ghira I., Smajda B. 2006. Multiple origins of viviparity, or reversal from viviparity to oviparity? The European common lizard (*Zootoca vivipara*, Lacertidae) and the evolution of parity. *Biological Journal of the Linnean Society*, 87: 1-11.

Swain R., Jones S.M. 2000. Maternal effects associated with gestation conditions in a viviparous lizard, *Niveoscincus metallicus*. *Herpetological Monographs,* 14: 432-440.

Telemeco R.S., Radder R.S., Baird T.A., Shine R. 2010. Thermal effects on reptile reproduction: adaptation and phenotypic plasticity in a montane lizard. *Biological Journal of the Linnean Society,* 100: 642-655.

Thompson M.B., Speake B.K. 2004. Egg morphology and composition. En Deeming D.C. (ed.). *Reptilian incubation: Environment, Evolution and Behaviou*r, Nottingham University Press, Nottingham, pp. 43-72.

Thompson M.B., Speake B.K., Russell K.J., McCartney R.J. 2001. Utilisation of lipids, protein, ions and energy during embryonic development of Australian oviparous skinks in the genus *Lampropholis*. *Comparative Biochemistry and Physiology Part A*, 129: 313-326.

Tinkle D.W., Gibbons J.W. 1977. The distribution and evolution of viviparity in reptiles. *Miscellaneous Publications Museum of Zoology, University of Michigan,* 154: 1-55.

Tompa A.S. 1975. Embryonic use of egg shell calcium in a gastropod. *Nature*, 255: 232-233.

Touchon, J.C., Warkentin, K.M. 2008. Reproductive mode plasticity: aquatic and terrestrial oviposition in a treefrog. *Proceedings of the National Academy of Sciences of the United States of America,* 105: 7495-7499.

Van Damme R., Bauwens D., Verheyen R.F. 1986. Selected body temperatures in the lizard *Lacerta vivipara*: variation within and between populations. *Journal of Thermal Biology,* 11: 219-222.

Van Damme R., Bauwens D., Verheyen R.F. 1990. Evolutionary rigidity of thermal physiology: the case of the cool temperate lizard *Lacerta vivipara*. *Oikos,* 57: 61-67.

Van Damme R., Bauwens D., Verheyen R.F. 1991. The thermal dependence of feeding behaviour, food consumption and gut-passage time in the lizard *Lacerta vivipara* Jacquin. *Functional Ecology,* 5: 507-517.

Van Damme R., Bauwens D., Braña F., Verheyen R.F. 1992. Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. *Herpetologica,* 48: 220-228.

Verwaijen R., Van Damme R., Herrel A. 2002. Relationships between head size, bite force, prey handling efficiency and diet in two sympatric lacertid lizards. *Functional Ecology,* 16: 842-850.

Vleck D. 1991. Water economy and solute regulation of reptilian and avian embryos. En Deeming D.C., Ferguson M.W.J. (eds.). *Egg incubation: its effects on embryonic development in birds and reptiles*. Cambridge University Press, Cambridge, pp. 245-259.

Wake M.H. 1993. Evolution of oviductal gestation in amphibians. *Journal of Experimental Zoology,* 266: 394-413.

Wapstra E. 2000. Maternal basking opportunity affects juvenile phenotype in a viviparous lizard. *Functional Ecology,* 14: 345-352.

Wapstra E., Swain R. 2001. Geographic and annual variation in life-history traits in a temperate zone Australian skink. *Journal of Herpetology,* 35: 194-203.

Wapstra E., Swain R., O'Reilly J.M. 2001. Geographic variation in age and size at maturity in a small Australian viviparous skink. *Copeia,* 2001: 646-655.

Wapstra E., Uller T., While G.M., Olsson M., Shine R. 2010. Giving offspring a head start in life: field and experimental evidence for selection on maternal basking behaviour in lizards. *Journal of Evolutionary Biology,* 23: 651-657.

Warner D.A., Andrews R.M. 2003. Consequences of extended egg retention in the eastern fence lizard *(Sceloporus undulatus)*. *Journal of Herpetology,* 37: 309-314.

Warner D., Shine R. 2007. Fitness of juvenile lizards depends on seasonal timing of hatching, not offspring body size. *Oecologia,* 154: 65-73.

Webb J.K., Shine R., Christian K.A. 2006**.** The adaptive significance of reptilian viviparity in the tropics: testing the maternal manipulation hypothesis. *Evolution,* 60: 115-122.

Wourms J.P., Callard I.P. 1992. A retrospect to the symposium on evolution of viviparity in vertebrates. *American Zoologist,* 32: 251-255.

Wourms J.P., Grove B.D., Lombardi J. 1988. The maternal-embryonic relationship in viviparous fishes. En Hoar W.S., Randall D.J. (eds.). *Fish Physiology*, Vol. 11B. Academic Press, San Diego, pp. 1-134.

Yom-Tov Y., Geffen E. 2006. Geographic variation in body size: the effects of ambient temperature and precipitation. *Oecologia,* 148: 213-218.

Anexos

Informe de factores de impacto

A continuación se enumeran las publicaciones presentadas y los factores de impacto (2010) de las revistas correspondientes:

1. **Rodríguez-Díaz T.**, González F., Ji X., Braña F. 2010. Effects of incubation temperature on hatchling phenotypes in an oviparous lizard with prolonged egg retention: are the two main hypotheses on the evolution of viviparity compatible? **Zoology** 113: 33-38.

   Factor de impacto: 1.651

2. **Rodríguez-Díaz T.**, Braña F. 2011b. Plasticity and limitations of extended egg retention in oviparous *Zootoca vivipara* (Reptilia: Lacertidae). **Biological Journal of the Linnean Society** 102: 75-82.

   Factor de impacto: 2.166

3. **Rodríguez-Díaz T.**, Braña F. 2011a. Shift in thermal preferences of female oviparous common lizards during egg retention: insights into the evolution of reptilian viviparity. **Evolutionary Biology** 38: 352-359.

   Factor de impacto: 2.736

4. **Rodríguez-Díaz T.**, Braña F. Altitudinal variation in egg retention and rates of embryonic development in oviparous *Zootoca vivipara* fits predictions from the cold climate model on the evolution of viviparity. Actualmente en revisión, pendiente de cambios menores, en **Journal of Evolutionary Biology**.

   Factor de impacto: 3.656

Zoology 113 (2010) 33–38

Contents lists available at ScienceDirect

Zoology

journal homepage: www.elsevier.de/zool

Effects of incubation temperature on hatchling phenotypes in an oviparous lizard with prolonged egg retention: are the two main hypotheses on the evolution of viviparity compatible?

Tania Rodríguez-Díaz[a,*], Félix González[a], Xiang Ji[b], Florentino Braña[a]

[a] Departamento de Biología de Organismos y Sistemas, Universidad de Oviedo, 33071 Oviedo, Spain
[b] Jiangsu Key Laboratory for Biodiversity and Biotechnology, College of Life Sciences, Nanjing Normal University, Nanjing 210046, Jiangsu, PR China

ARTICLE INFO

Article history:
Received 22 January 2009
Received in revised form
11 May 2009
Accepted 11 May 2009

Keywords:
Incubation temperature
Egg retention
Maternal manipulation hypothesis
Cold-climate hypothesis
Zootoca vivipara

ABSTRACT

Females of several lizard species modify their body temperature during pregnancy, probably in connection with the optimisation of hatchling phenotypes. We studied variations in the temperature selected by gravid females compared with those selected by males and non-gravid females in an oviparous population of *Zootoca vivipara* (Jacquin, 1797) (Squamata: Lacertidae) of Northern Spain and examined the effects of incubation temperature on the phenotypic variation of hatchlings. Cloacal temperatures of gravid females active in the field were lower than those of males and non-gravid females, as well as the temperatures selected in a thermal gradient created in the laboratory (mean ± s.d.: 32.33 ± 1.27 °C for gravid females; 34.05 ± 1.07 °C for males and non-gravid females). Effects of temperature were assessed by incubating eggs at five constant temperatures (21, 25, 29, 32 and 34 °C). Incubation time decreased as temperature increased, following a negative exponential function. Incubation temperatures also affected the hatchlings' morphology: hatchlings incubated at 34 °C had shorter heads than those from other temperatures. Survival at 34 °C (58%) was significantly lower than at the other temperatures (mean 93%). Pregnant females select lower body temperature, approaching the temperatures that optimise hatchling phenotypes, according to predictions of the maternal manipulation hypothesis on the evolution of viviparity. The shift in preferred temperature by pregnant females would result in only a very short delay, if any, of hatching time and, because the temperature selected by pregnant females is much higher than average temperatures recorded in natural nests of *Z. vivipara*, egg retention considerably shortens incubation time, according to predictions of the cold-climate hypothesis. Our experimental results indicate that the two main hypotheses on the evolution of viviparity are compatible in our study model.

Introduction

Maternal effects and environmental conditions during embryonic development have a great influence on an organism's phenotype and can affect fitness in reptiles (Shine and Harlow, 1996; Deeming, 2004). Effects induced by developmental environment have special importance in oviparous species because eggs are exposed to a wide variation of environmental factors, which may have detrimental effects on embryo phenotypes if certain limits are reached (Flatt et al., 2001). Temperature is one of the most relevant environmental factors inducing phenotypic variation in ectothermic vertebrates (Johnston and Bennett, 1996). In squamate reptiles (lizards and snakes), incubation temperature influences incubation time, hatching success and several hatchling traits (e.g. Birchard, 2004; Deeming, 2004; Booth, 2006). There are, however, interspecific differences in the way that hatchlings are affected by incubation temperature. High incubation temperatures, within the range that produces viable offspring, are detrimental to hatchlings of some lizard species (e.g. *Podarcis muralis*: Van Damme et al., 1992; Braña and Ji, 2000), whereas low incubation temperatures produce "poor-quality" hatchlings in other species (e.g. *Sceloporus virgatus*: Qualls and Andrews, 1999). Furthermore, pregnant females of several lizard species modify their body temperature by increasing or decreasing it in comparison to non-pregnant females (Beuchat, 1986; Braña, 1993; Shine, 2006). The change in preferred temperature during pregnancy could be selected to provide suitable temperatures either for embryonic development or for physiological processes of the female (Beuchat and Ellner, 1987; Beuchat, 1988; Mathies and Andrews, 1997).

In addition to the choice of nest site or the season in which incubation takes place (Shine, 2002; Birchard, 2004), maternal

---

* Corresponding author.
E-mail address: rodrigueztania@uniovi.es (T. Rodríguez-Díaz).

0944-2006/$ - see front matter © 2009 Elsevier GmbH. All rights reserved.
doi:10.1016/j.zool.2009.05.001

The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2011, **102**, 75–82. With 2 figures

Plasticity and limitations of extended egg retention in oviparous *Zootoca vivipara* (Reptilia: Lacertidae)

TANIA RODRÍGUEZ-DÍAZ* and FLORENTINO BRAÑA

Departamento de Biología de Organismos y Sistemas, Universidad de Oviedo, 33071 Oviedo, Spain

Received 31 May 2010; revised 19 July 2010; accepted for publication 19 July 2010

The transition between oviparity and viviparity in reptiles is generally accepted to be a gradual process, the result of selection for increasingly prolonged egg retention within the oviduct. We examined egg retention plasticity in an oviparous strain of the lacertid lizard *Zootoca vivipara*, a species having both oviparous and viviparous populations. We forced a group of female *Z. vivipara* to retain their clutch *in utero* by keeping them in dry substrata, and assessed the effect on embryonic development and hatching success, along with offspring phenotype and locomotor performance. Forced egg retention for one additional week affected the developmental stage of embryos at oviposition, as well as hatchling robustness and locomotor performance. However, embryos from forced clutch retention treatment reached one stage unit more than control embryos at oviposition time. Embryos from control eggs were more developed than embryos from experimental eggs after approximately the same period of external incubation, showing that embryonic development is retarded during the period of extended egg retention, despite the high temperature inside the mother's body. Significant differences in external incubation time were only found in one of the two years of study. Hatching success was much lower in the experimental group with forced egg retention (21.1%) than in the control group (95.4%). Therefore, we conclude that there are limitations that hinder the advance of intrauterine embryonic development beyond the normal time of oviposition, and that extended egg retention does not represent clear advantages in this population of *Z. vivipara*. Nevertheless, the fact that some eggs are successful after forced egg retention could be advantageous for the females that are able to retain their clutch under unfavourable climatic conditions. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2011, **102**, 75–82.

ADDITIONAL KEYWORDS: developmental constraints – embryonic development – evolution of viviparity – hatching success – hatchling phenotype.

INTRODUCTION

It is generally accepted that viviparity in reptiles has evolved from oviparity, and that such an evolutionary transition has come about gradually through selection for increasingly prolonged egg retention within the oviducts, with its implied progressive increase in the degree of intrauterine embryonic development (Tinkle & Gibbons, 1977; Shine, 1983; Andrews, 2002). For this reason, egg retention is considered to be a key component of the evolutionary progression to viviparity in reptiles, and several studies have focused on the constraints and selective pressures that operate on the time that eggs are retained *in utero* before oviposition (Andrews & Rose, 1994; Mathies & Andrews, 1996; Warner & Andrews, 2003; Braña & Ji, 2007). Consequently, extended egg retention has achieved great importance in the current models of the evolution of viviparity, particularly in the 'cold-climate' model (Shine, 1985; Rodríguez-Díaz *et al.*, 2010). In this model, retaining eggs in cold environments is thought to be selectively advantageous, as retained eggs are generally subjected to warmer temperatures than those in nests, and therefore embryonic development proceeds faster *in utero*.

Considerable variation in the stage of development of the embryo at oviposition has been documented among Squamate reptiles, from chamaleonid lizards in which embryos are diapausing gastrulae at the

---

*Corresponding author. E-mail: rodriguezdiaz.tania@gmail.com

Evol Biol (2011) 38:352–359
DOI 10.1007/s11692-011-9122-y

RESEARCH ARTICLE

Shift in Thermal Preferences of Female Oviparous Common Lizards During Egg Retention: Insights into the Evolution of Reptilian Viviparity

Tania Rodríguez-Díaz · Florentino Braña

Received: 21 October 2010 / Accepted: 15 April 2011 / Published online: 3 May 2011

Abstract Pregnant female *Zootoca vivipara* select lower body temperatures than males or nonpregnant females, and this shift in the thermal preferendum is believed to be related to optimising the conditions for embryogenesis. Thus, subjecting embryos to the higher temperature selected by males and non-gravid females might have detrimental effects on embryonic development and on hatchling fitness, according to predictions of the "maternal manipulation" hypothesis on the evolution of viviparity. To test the role of gestation environment on embryonic development in oviparous *Z. vivipara*, we kept a number of gravid females at the temperature selected by non-gravid females in a laboratory thermal gradient, whereas control females were allowed to regulate their body temperature without restrictions. Developmental stage at oviposition was more advanced for embryos of the experimental clutches, which were heavier than those of the control group. Forced gestation temperature also affected hatching success (58.62% in the experimental treatment vs. 97.37% in the control group). In addition, hatchlings from females subjected to high temperatures during pregnancy were smaller, had shorter head length and performed worse in running trials. Our results fulfil the prediction of the "maternal manipulation" hypothesis, and suggest that the shift in female body temperature during pregnancy optimizes embryogenesis and hatchling phenotype by avoiding the negative effects of the high incubation temperatures preferred by non-gravid females.

T. Rodríguez-Díaz (✉) · F. Braña
Department of Biology of Organisms and Systems, Zoology Unit, University of Oviedo, and Research Unit of Biodiversity (CSIC-UO-PA), 33071 Oviedo, Spain
e-mail: rodriguezdiaz.tania@gmail.com

Keywords Gestation environment · Hatching success · Incubation time · Maternal manipulation hypothesis

Introduction

Environmental maternal effects, such as mother's feeding rate or exposition to predators, and several other aspects of the maternal gestation environment have been proved to influence the phenotype and behaviour of neonatal lizards (e.g. Sorci and Clobert 1997; Shine and Downes 1999). For example, maternal thermoregulation can affect egg temperature throughout embryonic development, and maternal temperature has been demonstrated to have a great influence on offspring fitness (Rock and Cree 2003; Birchard 2004; Ji et al. 2006). Research on this topic has mainly focused on viviparous species, in which the contact between mother and embryo exists over a long period from conception to hatching (Beuchat 1988; Swain and Jones 2000), but most oviparous squamates retain eggs in utero for a considerable proportion of the embryogenesis (Shine 1983; Braña et al. 1991; Blackburn 1995), so offspring phenotypes can also be influenced by variation in the preoviposition maternal environment.

Females of some squamate species prefer lower body temperatures during pregnancy (e.g. *Podarcis muralis*: Braña 1993; *Sceloporus virgatus*: Andrews and Rose 1994; *Zootoca vivipara*: Van Damme et al. 1986). In other species, gravid females prefer higher body temperatures than other individuals in the same population (e.g. *Chalcides ocellatus*: Daut and Andrews 1993; *Hoplodactylus maculatus*: Rock et al. 2000), and this increase in body temperature would accelerate embryonic development inside the mother's body (Mathies and Andrews 1997; Shine 2006). Shift in thermal preferendum during pregnancy, especially