RESEARCH ARTICLE

Potentially adaptive effects of maternal nutrition during gestation on offspring phenotype of a viviparous reptile

Chloé D. Cadby, Susan M. Jones and Erik Wapstra*

School of Zoology, Private Bag 05, University of Tasmania, Hobart, 7001, Australia

*Author for correspondence (Erik.Wapstra@utas.edu.au)

SUMMARY

Viviparous reptiles have been used as model species for many studies that seek to explain the evolution of viviparity. The vast majority of such studies have focused on the advantage viviparity provides with regards to maternal control of embryonic developmental temperature. However, viviparity may also allow increased control of nutrient transfer, such that mothers adaptively manipulate offspring phenotype through varying maternal nutritional support. Because maternal nutritional transfer is temperature dependent, maternal nutritional strategies may vary between climatically distinct populations. In this study we used an orthogonal experimental design in which mothers and offspring from climatically distinct populations of a viviparous skink (*Niveoscincus ocellatus*) were allocated randomly to either a protein-rich or a protein-poor diet. Our results suggest that *N. ocellatus* mothers are able to compensate for sub-optimal nutritional conditions and can adaptively manipulate offspring phenotype to best fit the postnatal nutritional environment. Furthermore, maternal nutritional strategies appear to vary between climatically distinct populations. These results suggest that in viviparous reptiles, matrotrophy provides a means of producing an adaptive offspring phenotype, in addition to maternal control of developmental temperature.

Key words: evolution of viviparity, maternal effects, matrotrophy, *Niveoscincus ocellatus.*

INTRODUCTION

In reptiles, viviparity has evolved from oviparity more than 100 times (Tinkle and Gibbons, 1977; Blackburn, 1985; Blackburn, 2006). These evolutionary transitions are commonly thought to have occurred because viviparity allows a prolonged and more refined control of embryonic developmental temperature (Shine, 1983; Shine, 1995), which is one of the most important sources of phenotypic plasticity in reptiles (reviewed in Deeming, 2004; Booth, 2006). However, in some species, viviparity may also allow extended and more refined control of nutrient transfer through the placenta, providing a further selective benefit of viviparity. In contrast to oviparous species, where the maternal nutritional input is typically limited to a narrow time period (i.e. egg formation) (Pike and Petrie, 2003), some viviparous species have placentae, which allow for transfer of nutrients, hormones and metabolites between the mother and the embryo throughout gestation (Stewart, 1989; Blackburn, 1992; Swain and Jones, 2000; Trexler and DeAngelis, 2003; Thompson and Speake, 2006; Cadby et al., 2010a; Itonaga et al., 2011a; Itonaga et al., 2011b). In these species, this may allow manipulation of offspring phenotype after egg formation, as allowed by maternal control of developmental temperature through thermoregulation in viviparous species (Shine, 1983; Shine, 1995).

In viviparous reptiles, placental provisioning has been described as increasing opportunities for maternal decision-making and maternal sampling of the environment (Olsson and Shine, 1997; Swain and Jones, 2000; Trexler and DeAngelis, 2003; Atkins et al., 2006; Uller et al., 2008). This suggests that maternal manipulation of offspring phenotype through nutritional transfer is possible. Importantly, differences in maternal strategies are often observed in widespread species of reptiles, especially with regards to maternal control of

developmental temperatures (e.g. Gvozdik, 2002; Doody et al., 2006; Doody, 2009; Hoffman and Sgro, 2011). For example, in the viviparous common lizard, *Zootica vivipara*, females that live in cold high-altitude regions spend 50% more time basking during gestation than females from warmer low-altitude regions (Gvozdik, 2002). Low temperatures select for females that bask more in the highlands as they maintain developmental temperature high enough for proper embryonic development (Gvozdik, 2002). The difference in selection pressure between climatically distinct regions has therefore selected for different maternal strategies (see also Uller et al., 2011). In reptiles, maternal nutrient transfer is influenced by the thermal environment (Tinkle and Gibbons, 1977; Shine, 1983). Thus, it is very likely that if maternal nutritional support allows for manipulation of offspring phenotype, then we should observe differences in maternal strategies between populations that have evolved in different environments.

We used the spotted skink, *Niveoscincus ocellatus* Gray 1845, to test whether prolonged maternal nutritional support during gestation provides further selective advantages to the evolution of viviparity by allowing maternal manipulation of offspring phenotype, and to test for evidence of different maternal strategies in climatically distinct populations. This species has two key advantages for the purpose of this study. First, it has significant placentotrophy, with approximately half of the nutrients provided to the embryo transferred through the placenta (Thompson et al., 2001; Stewart and Thompson, 2009), providing a potential vehicle for maternal manipulation of offspring phenotype. Second, it is geographically widespread, with a distribution extending from warm coastal regions to cold highland regions (Cadby et al., 2010b; Pen et al., 2010; Uller et al., 2011). The difference in thermal conditions between regions has selected for difference in maternal

thermal strategies (Atkins et al., 2007; Uller et al., 2011; Cadby et al., in review), suggesting there may also be differences in maternal nutritional strategies. Additionally, food availability and quality differs between regions (Wapstra and Swain, 1996), which could also affect maternal nutritional strategies (e.g. Ballinger, 1977; Bonnet et al., 1998; Stephens et al., 2009). Consequently, to determine whether viviparity allows for manipulation of offspring phenotype *via* maternal nutritional support and to examine whether differences in nutritional strategies occur between climatically distinct regions, we designed an orthogonal experiment using females and offspring originating from two populations located at the climatic extremes of the species' distribution (Fig. 1).

MATERIALS AND METHODS
Field and laboratory protocol

Niveoscincus ocellatus is a small skink [3–10 g, 60–80 mm snout–vent length (SVL)] that is widespread in Tasmania, Australia. Fifty-nine gravid females from a warm, coastal site (42°34S, 147°52E; elevation 30 m) and 68 gravid females from a cold, mountain site (41°59S, 146°44E; elevation 1200 m) were collected by noosing or mealworm 'fishing' early in gestation [timing determined from Wapstra et al. and Uller et al. (Wapstra et al. 1999; Wapstra et al., 2009; Wapstra et al., 2010; Uller et al., 2011)]. Both collection sites are adjacent to those used in previous studies that have documented genotypic (Melville and Swain, 2000) and phenotypic differences between these populations (Wapstra et al., 1999; Wapstra et al., 2001; Wapstra et

al., 2009; Cadby et al., 2010b; Pen et al., 2010; Uller et al., 2011). Females were brought to the laboratory where they were measured (SVL ± 1 mm) and weighed (±1 mg). Female body condition was calculated from the residuals of the regression of mass and SVL. Females were housed in plastic terraria (200×300×100 mm) that contained a rock for basking and a terracotta pot for shelter. The laboratory was air-conditioned and maintained at 15±1°C. Visible and UV fluorescent lighting reproduced daylight conditions (20,000 lx, 14 h:10 h light:dark). Basking was supplied by a 25 W spotlight positioned 120 mm above the basking rock (10 h:14 h light:dark). When the basking light was on, the temperature reached 35°C on the basking rock and diminished on a gradient with distance from the basking rock to a minimum of 15°C. Females could, therefore, thermoregulate freely. Terraria were repositioned weekly on the shelves to minimize position effects.

Gravid females were randomly assigned either to a diet poor in protein or a diet rich in protein. Gravid females offered a protein-rich diet were fed three times weekly with fruit puree 10% enriched with soy protein powder (Nature's Way® Instant Natural Protein Powder, Warriewood, NSW, Australia) and crickets (family *Gryllidae*) that had been fed on a mixture of soy protein powder and green vegetables for at least 2 weeks prior to being offered to the lizards. Gravid females given the protein-poor diet were fed three times weekly with fruit puree (without soy protein powder) and crickets that had been fed on green vegetables only. The overall quantity of food given to each lizard did not differ between treatment groups [see Warner et al. for
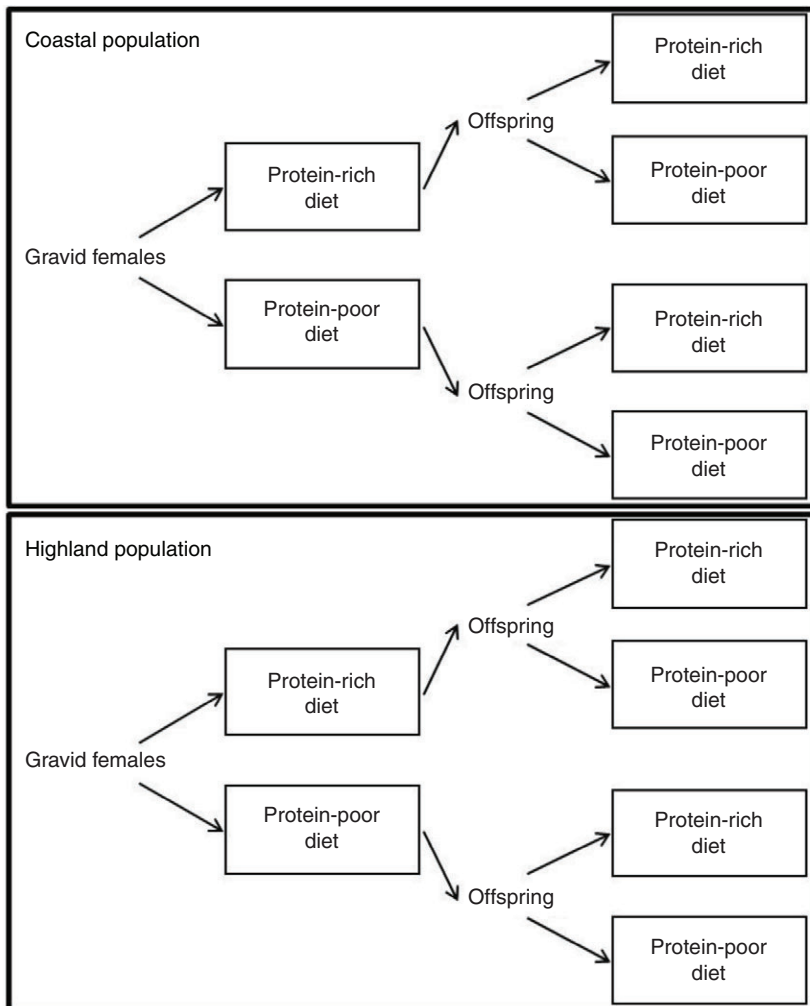

Fig. 1. Orthogonal experimental design in which gravid *Niveoscincus ocellatus* females from a coastal and a highland population were allocated randomly to either a protein-rich or protein-poor diet at the beginning of gestation. Once offspring were born, each litter was allocated randomly to either a protein-rich or protein-poor diet.

similar methods (Warner et al., 2007)]. Protein is essential for building embryonic tissue in reptiles (Thompson and Speake, 2003) and amino acids have been demonstrated to pass through the placenta in species with placentae of similar complexity to *N. ocellatus* (Thompson, 1977; Swain and Jones, 1997).

From the beginning of December, terraria were checked three times daily for neonates. Females that had given birth were weighed (±1 mg) and their body condition was calculated from the residuals of the regression of mass and SVL. Gestation length was calculated from the date of ovulation [1 October for coastal females and 15 October for mountain females, timing determined from Wapstra et al. (Wapstra et al., 1999)] to the date of parturition (see Wapstra et al., 2009; Cadby et al., 2010a; Pen et al., 2010; Uller et al., 2011). Unsuccessful pregnancies (i.e. females did not give birth) were also recorded. All females were released at their site of capture within 7 days of giving birth. Offspring were measured using digital callipers (SVL ± 0.01 mm), weighed (±1 mg) and given a unique toe clip for permanent identification. Offspring body condition at birth was calculated as the residual from mass and SVL at birth. We allocated each litter randomly into either a protein-rich or protein-poor treatment group (following the same protocol as for gravid females). To determine growth rate and mass gain rate, each offspring was measured (SVL ± 0.01 mm; mass ±1 mg) every 20 days for 9 weeks. Early growth is linear in this species (Wapstra, 2000); consequently, growth rate was calculated using the slope of the least-squares regression line of length for mass and time.

The sprint speed of offspring was assessed as a measure of whole-body performance (Wapstra, 2000; Cadby et al., 2010a) within 24 h of birth and 1 day prior to being released. To measure sprint speed, offspring were placed in a sealed container held in warm (27°C) water (the optimum temperature to maximize sprint speed) for 20 min to allow equilibration to the ambient temperature (Wapstra and O'Reilly, 2001). They were then sprinted individually along a 1 m 'racetrack' and encouraged to run by tapping their tail lightly with a paintbrush. Sprint time along the track was recorded by five equally spaced (0.20 m) infrared light beams. Offspring were sprinted only once and the best of the five times recorded during the test was retained as a measure of maximal sprint speed. Offspring that refused to run, stopped or turned and ran in the opposite direction were excluded from the analysis.

All protocols in the present study were approved by the Animal Ethics Committee at the University of Tasmania.

Statistical analyses

Analyses were conducted using SAS 9.1 (SAS Institute, Cary, NC, USA). In all cases, variables were either normally distributed, or were so following log transformation. We used one-way ANOVA to compare female traits between treatments. The effects of maternal diet and population on the success of pregnancy were evaluated using a two-way logistic regression. A non-parametric test (Kruskal–Wallis) was used to compare litter size between maternal treatments and populations. We used a multi-factorial mixed model to assess the effects of maternal diet, population and their interaction on offspring SVL, mass and sprint speed at birth. The 'litter effect' was taken into account by considering maternal identity as a random factor. Similarly, we used a multi-factorial mixed model to assess the effects of maternal diet, offspring diet and population on offspring growth rate, mass gain rate and sprint speed after 9 weeks in captivity. For all mixed models, the degrees of freedom were calculated using the Kenward–Roger approximation (Littell et al., 1996). All values are reported as means ± s.e.m.

RESULTS
Maternal traits

The SVL, mass and body condition of females were not significantly different between treatment groups at collection (Table 1). However, maternal diet had a significant effect on post-partum maternal mass and a marginal (though not significant) effect on post-partum maternal body condition (Table 1). Maternal mass and body condition post-partum were higher for females in the protein-rich treatment group (mass: 6.1±0.18 g; body condition: student residual=0.22±0.156) than for females in the protein-poor treatment group (mass: 5.5±0.14 g; body condition: student residual=–0.17±0.127). Maternal diet had a significant effect on the success of pregnancy, with more unsuccessful pregnancies occurring in mothers on the protein-rich diet (N=18) than on the protein-poor diet (N=7; Table 1). We also observed significantly more unsuccessful pregnancies in coastal population females (N=18) than in mountain population females (N=7; Table 1). Mean gestation length did not vary between treatments but was significantly shorter (99±0.6 days) for females from the mountain population than for females from the coastal population (103±1.9 days; Table 1). Litter

Table 1. Effects of maternal diet, population and their interaction on maternal and offspring traits of the spotted skink, *Niveoscincus ocellatus*

	Maternal diet	Population	Maternal diet × Population
Maternal trait at collection			
SVL	$F_{1,125}$=0.33, P>0.1	$F_{1,125}$=84.54, **P<0.0001**	$F_{1,125}$=0.02, P>0.1
Mass	$F_{1,125}$=0.99, P>0.1	$F_{1,125}$=31.00, **P<0.0001**	$F_{1,125}$=0.03, P>0.1
Body condition	$F_{1,125}$=1.47, P>0.1	$F_{1,125}$=12.59, **P=0.0006**	$F_{1,125}$=0.03, P>0.1
Maternal trait post partum			
SVL	$F_{1,100}$=2.03, P>0.1	$F_{1,100}$=35.64, **P<0.0001**	$F_{1,100}$=0.36, P>0.1
Mass	$F_{1,101}$=5.11, **P=0.0259**	$F_{1,101}$=22.44, **P<0.0001**	$F_{1,101}$=0.06, P>0.1
Body condition	$F_{1,100}$=3.46, P=0.0657	$F_{1,100}$=0.07, P>0.1	$F_{1,100}$=0.10, P>0.1
Success of pregnancy	$\chi^2_{1,125}$=5.36, **P=0.0205**	$\chi^2_{1,125}$=4.61, **P=0.0317**	$\chi^2_{1,125}$=0.00, P>0.1
Gestation length	$F_{1,99}$=0.27, P>0.1	$F_{1,99}$=0.7.16, **P=0.0087**	$F_{1,99}$=0.02, P>0.1
Litter size	$\chi^2_{1,103}$=1.18, P>0.1	$\chi^2_{1,103}$=7.57, **P=0.0059**	–
Offspring trait at birth			
SVL	$F_{1,96.4}$=0.07, P>0.1	$F_{1,96.4}$=2.76, P>0.05	$F_{1,96.4}$=0.77, P>0.1
Mass	$F_{1,96.2}$=1.27, P>0.1	$F_{1,96.2}$=1.90, P>0.1	$F_{1,96.2}$=0.03, P>0.1
Body condition	$F_{1,92.7}$=2.50, P>0.1	$F_{1,92.7}$=0.04, P>0.1	$F_{1,92.7}$=1.30, P>0.1
Sprint speed	$F_{1,92.3}$=2.53, P>0.1	$F_{1,92.3}$=3.03, P>0.05	$F_{1,92.3}$=0.28, P>0.1

SVL, snout–vent length.
Values in bold indicate significance at P<0.05.

size differed between populations but not between maternal diets (Table 1): females from the mountain population had larger litters (2.5±0.12 offspring) than females from the coastal population (2.0±0.16 offspring).

Offspring phenotypic traits

Offspring SVL (29.75±0.068 mm), mass (0.51±0.003 g), condition (student residual=−0.05±0.064) and sprint speed (0.400±0.010 m s^{-1}) at birth were similar between maternal treatment groups and between populations (Table 1). Offspring growth rate and sprint speed were affected by an interaction between maternal diet and offspring diet (Table 2). Offspring offered the same diet as their mothers grew faster (Fig. 2A) and ran faster (Fig. 2B) compared with those offspring offered a different diet from their mothers. Offspring sprint speed was marginally affected by an interaction between population and offspring diet, with offspring from the coastal population running faster when offered the high-protein diet and offspring from the mountain population running just as fast between treatment groups, but these results were not significant ($F_{1,111}$=3.52, P=0.0631). Offspring growth rate was affected by the independent effect of population (Table 2), with offspring from the mountain population growing faster (0.135±0.0032 mm day^{-1}) than offspring from the coastal population (0.119±0.0083 mm day^{-1}). Population and offspring diet affected rate of mass gain. Offspring from the mountain population gained mass faster (0.012±0.0002 mg day^{-1}) than offspring from the coastal population (0.010±0.0004 mg day^{-1}). Offspring fed the protein-rich diet gained mass faster (0.012±0.0003 mg day^{-1}) than offspring fed the protein-poor diet (0.009±0.0003 mg day^{-1}).

DISCUSSION

In reptiles, offspring phenotypic traits at birth are typically affected by the environment experienced by the embryo during development (reviewed in Deeming, 2004; Booth, 2006) and often reflect later
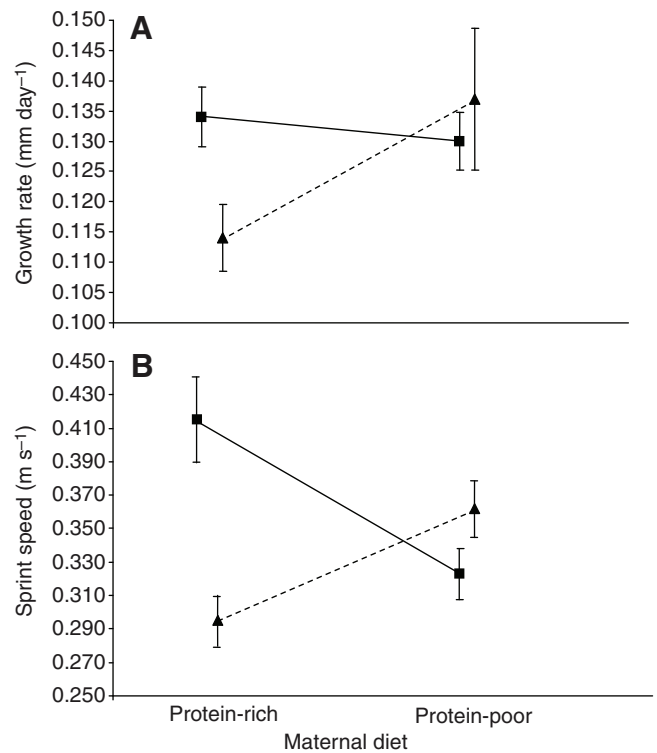

Fig. 2. The effects of maternal diet, population and offspring diet (dashed line, protein-poor diet; solid line, protein-rich diet) on the (A) growth rate and (B) sprint speed of offspring of *Niveoscincus ocellatus*.

fitness (e.g. Chamaillé-Jammes et al., 2006; Warner and Shine, 2007; Uller and Olsson, 2010; Wapstra et al., 2010). Viviparity is considered to be advantageous over oviparity as it provides prolonged maternal control of developmental temperatures (Shine, 1983; Shine, 1995). Our results suggest that placental provisioning may allow for prolonged control of maternal nutritional input to the embryo, which may prove to be a further benefit of viviparity.

Diet quality during gestation affected maternal body condition. Mothers placed in the protein-poor diet group weighed less after parturition than females offered the protein-rich diet. Interestingly, the effects of diet on mothers did not result in differences in offspring morphology at birth between offspring born to mothers from the two nutritional treatments. There are several explanations for this. Maternal diet protein content during gestation may have no effect on the amount of nutrients transferred across the placenta, and, thus, on embryonic development. Alternatively, the observed results could be the consequence of maternal buffering of sub-optimal nutritional conditions rather than a lack of treatment effect. In both oviparous and viviparous reptiles, offspring phenotypic characteristics are not entirely determined at the time of ovulation; significant phenotypic variation occurs as a result of the environment experienced by the embryo during development (reviewed in Deeming, 2004; Booth, 2006). Specifically, in some viviparous species, such as *N. ocellatus*, that exhibit significant placentotrophy (Thompson et al., 2001; Stewart and Thompson, 2009), potentially including maternal transfer of proteins *via* the placenta (Swain and Jones, 1997), any difference in maternal diet during gestation could, in principle, influence offspring phenotype. Importantly, offspring phenotype at birth typically has long-lasting consequences on fitness and survival of reptiles (Chamaillé-Jammes et al., 2006; Warner and Shine, 2007; Uller and Olsson, 2010), including *N. ocellatus* (Pen et al., 2010;

Table 2. Effects of maternal diet, offspring diet, population and their interactions on offspring traits of the spotted skink, *Niveoscincus ocellatus*

Factor	F	d.f.	P
Offspring growth			
Maternal diet	2.69	1, 229	>0.1
Offspring diet	0.71	1, 229	>0.1
Population	5.24	1, 229	**0.0230**
Maternal diet × Offspring diet	4.06	1, 229	**0.0450**
Maternal diet × Population	2.62	1, 229	>0.1
Offspring diet × Population	0.13	1, 229	>0.1
Maternal diet × Offspring diet × Population	0.42	1, 229	>0.1
Offspring weight gain			
Maternal diet	0.02	1, 86.6	>0.1
Offspring diet	29.64	1, 105	**<0.0001**
Population	14.23	1, 86.6	**0.0003**
Maternal diet × Offspring diet	2.09	1, 86.6	>0.1
Maternal diet × Population	0.65	1, 86.6	>0.1
Offspring diet × Population	0.48	1, 105	>0.1
Maternal diet × Offspring diet × Population	2.80	1, 105	>0.1
Offspring sprint speed			
Maternal diet	0.49	1, 93.1	>0.1
Offspring diet	4.80	1, 111	**0.0305**
Population	0.01	1, 93.1	>0.1
Maternal diet × Offspring diet	15.24	1, 111	**0.0002**
Maternal diet × Population	0.05	1, 93.1	>0.1
Offspring diet × Population	3.52	1, 111	>0.1
Maternal diet × Offspring diet × Population	0.96	1, 111	>0.1

Values in bold are significant at P<0.05.

Wapstra et al., 2010; Uller et al., 2011), and thus there should be strong selection for maternal optimization of offspring phenotype, potentially through buffering of sub-optimal conditions during embryonic development. Such buffering has been demonstrated in response to sub-optimal thermal conditions in other reptiles (Gvozdik, 2002; Doody et al., 2006; Doody, 2009; Telemeco et al., 2009) as well as in *N. ocellatus* (Uller et al., 2011; Cadby et al., in review). For example, in two parallel studies on *N. ocellatus*, we showed that gravid females behaviourally thermoregulate during gestation to limit the effects of temperature on embryonic development and offspring phenotype at birth, resulting in good quality offspring even when thermal conditions are sub-optimal (Uller et al., 2011; Cadby et al., in review). Similarly, mothers may be expected to trade off their own energy stores under sub-optimal nutritional conditions if offspring fitness is enhanced sufficiently to overcome losses to female condition and future reproductive output (Marshall and Uller, 2007). Therefore, we suggest that the lack of effect on offspring phenotype at birth results from maternal buffering of nutritional variation so as to prevent environmental fluctuations from incurring large fitness costs for the offspring [as described in Uller et al. (Uller et al., 2011)].

Although maternal diet did not affect offspring phenotype at birth, it did influence two fitness-related offspring traits later in life. We observed a significant interaction between the effects of maternal diet and offspring diet on offspring growth rate and offspring sprint speed measured 9 weeks after birth. Offspring fed a protein-rich diet and born to females fed a protein-rich diet had the fastest growth rate and sprint speed, which is not surprising considering the role of protein in growth and muscle development (Avery et al., 1993; Oftedal and Allen, 1996). Offspring born to females offered a protein-poor diet appeared to be less affected by offspring diet than offspring born to females offered a protein-rich diet, suggesting that the former group of offspring has a broader metabolic capacity, allowing them to better tolerate variation in protein availability.

Intriguingly, our results point to maternal 'pre-programming' or anticipatory maternal effects, in which mothers manipulate their offspring so as to best fit local conditions and, thus, maximize offspring fitness (Marshall and Uller, 2007; Uller, 2008; Badyaev and Uller, 2009). In our study, offspring born to mothers in the protein-rich diet and subsequently offered a protein-poor diet exhibited the lowest growth rate of all. Furthermore, offspring fed a similar diet to their mothers sprinted faster than offspring fed a different diet than their mothers. In reptiles, sprint speed and growth are typically linked to fitness and survival. Faster sprint speeds typically give offspring an advantage in terms of predator escape (Sorci and Clobert, 1997; Miles, 2004; Husak, 2006) as well as a feeding advantage (Wapstra and Swain, 1996), whereas growing faster increases survival (Madsen and Shine, 2000) and reproductive output, as well as leading to a larger size at maturity (Chamaillé-Jammes et al., 2006). Therefore, the benefits of increasing offspring fitness may have selected for maternal manipulation of offspring to fit the environmental conditions experienced during gestation (Marshall and Uller, 2007; Uller, 2008; Badyaev and Uller, 2009), especially for traits such as growth and performance that are themselves affected by nutritional conditions (Avery et al., 1993; Oftedal and Allen, 1996).

We suggest that maternal pre-programming to the nutritional environment is more likely to occur in viviparous species than in oviparous species. In contrast to oviparous species, in which maternal nutritional input is limited to the time prior to ovulation, in viviparous species maternal nutritional transfer can continue after ovulation (i.e. during gestation). This increases the potential for mothers to better match the offspring to the environment, as mothers have continuous feedback on the nutritional environment that surrounds them and thus can either reinforce a message (i.e. there is good quality food available) or change it if nutritional conditions change. Furthermore, previous studies have demonstrated that placental nutritional support to the embryo provides the advantage of increasing the flexibility of maternal input and allowing for better control of nutritional allocation (Swain and Jones, 2000; Atkins et al., 2006; Uller et al., 2008). To our knowledge, maternal pre-programming to nutritional conditions has only been described in humans, in which maternal caloric intake during gestation affects the glucose-insulin metabolism of embryos (Hales and Barker, 2001; Eriksson et al., 2007; Gluckman et al., 2008). Malnourishment during fetal development pre-programs children to living in an environment that provides limited caloric intake by reducing the capacity for insulin secretion and tolerance. If the children have access to sufficient or excessive calories later in life, they have an increased risk of developing Type 2 diabetes, cardiac diseases, hypertension or obesity (Barker, 1998; Hales and Barker, 2001; Eriksson et al., 2007; Gluckman et al., 2008). If results similar to ours are observed in other viviparous reptiles with significant placental transfer, then this may suggest further benefits of viviparity through adaptive manipulation of offspring phenotype.

If females are able to buffer unfavourable conditions and/or adaptively manipulate offspring to fit the environment, then the difference in selection pressure between climatically distinct regions may have selected for different maternal nutritional strategies in populations of *N. ocellatus* located at the climatic extremes of the species distribution. We know from a parallel study (Uller et al., 2011) that cold weather and short activity seasons in the highland populations increase the selection pressure to produce early and large offspring, as this maximizes chances of survival overwinter by allowing maximization of mass and body condition gain prior to hibernation (Olsson and Shine, 1998; Atkins et al., 2007; Uller et al., 2011). This increased pressure has selected for opportunistic basking behaviour: highland females tend to maintain high body temperature regardless of the thermal conditions (Uller et al., 2011; Cadby et al., in review). The results of the present study suggest that cold conditions in the highlands have selected for stronger canalization of nutrient transfer and more efficient use of nutrients. We observed more unsuccessful pregnancies in females from the warm coastal population than those from the mountain population, while offspring from the mountain population gained mass more rapidly than offspring from the coastal population regardless of offspring diet. Canalization of nutrient transfer and effective use of nutrients may have played an important role in the distribution of this species into colder regions through increasing the chances of mothers of carrying out successful pregnancies and offspring rapidly reaching good body conditions regardless of food availability (Uller et al., 2011).

In conclusion, our results introduce new evidence for the advantages of viviparity in reptiles. Our results emphasise the role of the female nutritional environment and offspring provisioning in optimising offspring fitness, in addition to the well-accepted thermal-based maternal manipulation hypothesis (Shine, 1995). However, further work in this species and other viviparous reptiles is clearly required to confirm the occurrence of maternal buffering and pre-programming to the nutritional environment.

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