

Between-population variation in body size and growth rate of hatchling Asian yellow pond turtles, *Mauremys mutica*

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Two morphologically and genetically distinct populations were recently recognized in the Asian yellow pond turtle (*Mauremys mutica*), but little information is available on between-population variation in life-history traits. We collected eggs from Zhejiang (northern) and Hainan (southern) populations, and incubated them at constant temperatures of 26 °C and 30 °C. Hatchling body mass and early growth were determined in a common garden experiment to identify the intrinsic between-population difference in these life-history traits. Relative to egg size, hatchling body mass was larger in the Zhejiang population than in the Hainan population. Hatchlings from the Hainan population grew faster than their counterparts from the Zhejiang population. Growth rates differed between the sexes in the Hainan population (females > males), but not in the Zhejiang population. These results suggest that 1) eggs from the Zhejiang population might contain more energy and thus produce larger hatchlings than eggs of the same size from the Hainan population, and 2) the intrinsic fast growth rate of hatchlings could be the reason for the larger adult size observed in the Hainan population.

Key words: geographic variation, post-hatching growth, temperature

INTRODUCTION

Understanding life-history variation and its causes has long been an important topic in evolutionary biology (Stearns, 1992; Roff, 2002). Life-history variation, both among and within species, stems from the combined effects of genetic and environmental factors (Ewert, 1985; Dunham et al., 1988). In wide-ranging species, life-history traits show substantial variation among geographically separated populations (e.g. Dunham & Gibbons, 1990; Du et al., 2005a). Therefore, comparative studies on geographically separated populations provide an opportunity to identify the underlying causes of life-history variation.

Body size is an important trait that can vary substantially among populations (Du et al., 2005a; Yom-Tov & Geffen, 2006). Body size at hatching may directly affect growth and survival at the juvenile stage (Janzen, 1993; Sinervo, 1993), as well as body size at maturity (Dunham & Gibbons, 1990; Lovich et al., 1990). At the adult stage, body size is closely related to reproductive success (Congdon & van Loben Sels, 1991; Shine, 1992; Du et al., 2005b). One of the proximate mechanisms causing such variation may involve differences in hatchling size and post-hatching growth, which are determined by genetic as well as environmental factors.

Identifying the genetic and environmental causes of inter-population variation in hatchling size and growth is critical to understanding the evolution of these life-history traits. In oviparous ectotherms, body size and

post-hatching growth can be affected by environmental factors (e.g. temperature and food availability) during embryonic development (see review in Deeming, 2004) and in post-hatching development (Smith & Ballinger, 1994; Angilletta et al., 2004a; Zhang et al., 2009). Given the combined effects of genetic and environmental factors on these life-history traits, it is difficult to tease them apart from comparisons among natural populations. Common garden experiments can largely control variations arising from environmental factors that may differ among populations, and thus provide a powerful tool to identify the intrinsic (mostly genetic) sources of inter-population variation (Aday et al., 2003; Du et al., 2005a).

The Asian yellow pond turtle (*Mauremys mutica*) is an aquatic geoemydid species distributed in China, Japan and Vietnam (Iverson, 1992; Yasukawa et al., 1996; Zhang et al., 1998; Chen et al., 2000; Iverson et al., 2000). In China, there are two geographically separated populations that differ both morphologically and genetically (Fong et al., 2007; Zhu et al., 2008). Adults from the southern population (mainly distributed in Guangdong, Guangxi and Hainan Provinces) are larger than those from the northern population (mainly in Jiangsu, Zhejiang and Anhui Provinces) (Zhu et al., 2008). Southern and northern populations differ in colour, with the carapace and head being brown to dark-brown for both adults and hatchlings from the southern population, and yellowish-brown or green for individuals from the northern population (Zhu et al., 2008). However, little information is available on between-population variations in body size

and the growth of hatchlings, which are important for elucidating the proximate causes of between-population differences in morphology. In *M. mutica*, eggs produce mainly male hatchlings if incubated at 26 °C, but mainly females at 30 °C (Zhu et al., 2006; Du et al., 2010b). We thus incubated eggs from southern (Hainan) and northern (Zhejiang) populations at these two temperatures to produce both male and female hatchlings, raising them in a common environment to monitor their growth. Given that adults are larger in the southern population than the northern population (Zhu et al., 2008), we predicted that hatchlings from the southern population would grow faster than their counterparts from the northern population.

MATERIALS AND METHODS

It is difficult to collect eggs from wild populations in mainland China because most turtle species have been depleted dramatically due to habitat loss and overexploitation. *Mauremys mutica* is currently listed as endangered on the IUCN Red List (Wang & Zhao, 1998). We therefore collected eggs from two private farms located in Hainan and Zhejiang Provinces, in which the breeding populations of *M. mutica* were exclusively caught from local wild populations. In June 2009, 15 clutches of freshly laid eggs from the southern population were collected from Wenchang, Hainan, and 20 clutches of eggs from the northern population from Haining, Zhejiang. The eggs were weighed to ± 0.001 g using an electronic balance (Mettler Toledo AB135-S) and individually incubated in 160 ml jars with -220 kPa moist vermiculite (vermiculite: water = 1:1). One egg from each clutch was assigned to one of two incubators (Ningbo Life Science and Technology Ltd, China) set at 26 °C or 30 °C. In order to minimize any effects of thermal gradients inside the incubators, we rotated the jars among shelves twice a week.

Towards the end of incubation (about 60 days), the jars were monitored every day and hatchlings were collected promptly upon emerging and weighed to the nearest 0.001g. We then marked the hatchlings by cutting notches in the marginal scutes for future identification and raised them in a temperature-controlled room at 28 ± 1 °C for the growth experiment. The room lights were set to a 12-h light/12-h dark cycle. Four to five hatchlings were kept in a 300×200×185 mm plastic cage. Hatchlings from different populations were raised separately, and hatchlings from different incubation treatments were assigned equally to each cage. During the 90 days of the growth experiment, the young turtles were provided with an excess amount of commercial food once a day, and the body mass of each turtle was weighed at the end of the experiment to calculate growth rate (quantified as the daily rate of increase of body mass). Ninety days was chosen as the length of our experiment because previous growth experiments indicated that growth rate in the first three months was positively correlated with long-term growth in *M. mutica* (up to 30 months, female: $r=0.49$, $F_{1,56}=0.0001$; male: $r=0.42$, $F_{1,32}=0.01$) in this species (Du et al., 2010b).

Normality of distributions and homogeneity of variances were tested using the Kolmogorov–Smirnov test and Bartlett’s test, respectively. We used ANOVA or

Table 1. Between-population variation in body mass and growth rate of hatchling *Mauremys mutica* at different incubation temperatures. Body mass measurements indicated are weight at emergence from egg. Growth rates are represented as daily increments of body mass. Data are expressed as adjusted means (relative to egg mass) \pm SE for body mass, and mean \pm SE for growth rate.

	Incubation temperature	Zhejiang population (n=12)	Hainan population (n=7)
Body mass (g)	26	6.282 \pm 0.136	5.335 \pm 0.180
	30	6.238 \pm 0.147	5.566 \pm 0.182
Growth rate of body mass (g/d)	26	0.043 \pm 0.007	0.056 \pm 0.004
	30	0.049 \pm 0.005	0.109 \pm 0.006

ANCOVA to analyse the effect of temperature on, and between-population differences in, body size and post-hatching growth.

RESULTS

By the end of the incubation, 12 out of 20 clutches of eggs in the Zhejiang population and 7 out of 15 clutches of eggs in the Hainan population hatched successfully at both temperatures. The carapace was dark brown for hatchlings from the Hainan population, and yellowish brown for those from the Zhejiang population, confirming the different geographic origins of the two populations. Hatchling mass relative to egg mass differed significantly among populations ($F_{1,33}=21.71$, $P<0.0001$), but was not affected by incubation temperature ($F_{1,33}=0.35$, $P=0.56$) or the interaction between population and temperature ($F_{1,33}=0.74$, $P=0.40$). Controlling for egg size, hatchlings from the Zhejiang population were larger than those from the Hainan population (Table 1).

Within the 90 days of the growth experiment, growth rate was not related to the initial body mass of hatchlings in either population ($F_{1,33}=0.001$, $P=0.98$). The growth rate of hatchlings differed vastly between the populations ($F_{1,34}=36.23$, $P<0.0001$), with Hainan hatchlings growing much larger than their Zhejiang counterparts (Table 1). Growth rate was also significantly affected by incubation temperature ($F_{1,34}=24.24$, $P<0.0001$) and the interaction between population origin and incubation temperature ($F_{1,34}=15.29$, $P<0.001$). Further analyses using separate ANCOVAs on each population indicated that temperature significantly affected growth rates in the Hainan population ($F_{1,12}=54.39$, $P<0.0001$), but not in the Zhejiang population ($F_{1,22}=0.57$, $P=0.46$). The Hainan hatchlings at 30 °C grew faster than their siblings at 26 °C (Table 1).

DISCUSSION

Egg size did not differ between the two populations, but hatchling size relative to egg size was larger in the Zhe-

jiang population than the Hainan population (Table 1). One plausible explanation for this phenomenon is that females from the Zhejiang population invest more energy (more yolk or a higher percentage of lipids) in eggs than those from the southern population. Such geographic patterns of energy investment in eggs have been found in some lizards, and are regarded as adaptations to cold environments (Nagle et al., 1998; Oufiero et al., 2007; Du et al., 2010a). Geographic variation in energy investment in eggs also has been found in some turtle species (e.g. Finkler et al., 2004). Alternatively, the observed between-population variation in hatchling size may be related to energy expenditure during development, which could be lower in eggs from the northern population than the southern population.

Post-hatching growth is one of the proximate causes that may determine adult body size. How can individuals from the southern population grow to a larger size than those from the northern population? Hatchlings from the southern population may reach a large size at maturity by rapid growth. As predicted, hatchlings from the southern population grew faster than those from the northern population (Table 1). The common garden experiment used in the present study may have largely controlled the effects of environmental factors on hatchling growth, and thus the between-population difference in growth is likely to be attributable to intrinsic (genetic) differences rather than environmentally induced variations. However, the tendency of larger adults to grow faster at the juvenile stage does not apply to other reptiles. For example, due to delayed maturation, eastern fence lizards (*Sceloporus undulatus*) from northern populations are larger than those from southern populations despite a lower juvenile growth rate (Angilletta et al., 2004b; Sears & Angilletta, 2004). Except for the intrinsic difference in growth rates, the growth of turtles from the southern population may benefit from more suitable environments for growth in their natural habitat. Such a relationship between hatchling growth and the local environment has been found in a number of turtle species (Gibbons et al., 1981; Gibbons & Lovich, 1990; Lindeman, 1996).

Incubation temperatures can significantly affect post-hatching growth of reptilian hatchlings (Deeming, 2004). The early growth of *M. mutica* hatchlings was affected by the incubation temperature in the Hainan population, but not in the Zhejiang population (Table 1). The between-temperature variation in the Hainan population also translates into a between-sex difference in the early growth of hatchlings, because hatchlings at 26 °C were mainly males, and those at 30 °C mainly females (Zhu et al., 2006; Du et al., 2010b). Unfortunately, our experiment could not identify whether the difference in hatchling growth was attributable to incubation temperature or sex differences, for which hormone manipulation, for example, would be required (Rhen & Lang, 1995; Du et al., 2010c). In addition, between-sex differences in early growth cannot explain the sexual size dimorphism of this species, because adult males are larger than adult females (Zhu et al., 2008). An earlier study on the Zhejiang population indicated that male hatchlings are larger than female hatchlings in captivity at six months (Du et al., 2010b). These findings

suggest that sexual size dimorphism may occur at later stages of post-hatching development.

ACKNOWLEDGEMENTS

We thank B. Sun for his assistance in the laboratory. We are grateful to J.J. Fong, R.M. Bowden and an anonymous reviewer for their valuable comments on an earlier version of this manuscript. This work was supported by grants from the Zhejiang Provincial Foundation of Natural Science (No. G5080004), the “One Hundred Person Project” of the Chinese Academy of Sciences, and the Program for New Century Excellent Talents in University.

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Accepted: 10 January 2011