Research	

# Allocation of offspring size and sex by female black ratsnakes

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How females allocate resources to each offspring and how they allocate the sex of their offspring are two powerful potential avenues by which mothers can affect offspring fitness. Previous research has focussed extensively on mean offspring size, with much less attention given to variance in offspring size. Here we focussed on variation in offspring size in black ratsnakes, Elaphe obsoleta. We collected and hatched 105 clutches (1283 eggs) over 9 years. We predicted that females should lay larger eggs, or more variable eggs, when the environment is less predictable. We also predicted that females laying early or laying larger eggs should produce mostly sons because adult males are larger than adult female ratsnakes. The largest hatchling was more than twice the length and almost four times the mass of the smallest hatchling. Variation in offspring size was itself highly variable, with CVs in offspring mass among clutches ranging from 1% to 25%. With one exception, the variables we expected should influence variation in offspring size had little effect. We found that clutch size increased with maternal size and that egg size decreased with clutch size, but we found no evidence that variance in egg size among clutches increased as the season progressed or that females increased the mean size of their offspring the later in the season they laid their eggs. Females in better condition after they finish laying their eggs did produce larger eggs. There was no relationship between within-clutch variation in egg size and laying date or mean egg size. Finally, sex ratio did not vary with mean egg size or hatching date. Given evidence that offspring size in snakes affects survival, selection should reduce variation in offspring size unless that variance enhances maternal fitness and yet we found little support for hypothesized advantages of varying offspring size.

How females allocate resources to each of their offspring (Smith and Fretwell 1974) and how they allocate the sex of their offspring (Trivers and Willard 1973, Charnov 1982) are two of the most powerful potential avenues by which mothers can affect offspring, and consequently their own, fitness. When size and sex affect offspring fitness, natural selection should have favoured female reproductive strategies whereby offspring size and sex are allocated to maximize maternal fitness. Among other features, large clutch sizes and the absence of parental care (in most species) make snakes well suited for investigating maternal allocation strategies, although efforts are only beginning to catch up to work on some other taxa (Shine 2003). Here we use data from a long-term study to test hypotheses about offspring size and sex allocation by female black ratsnakes, Elaphe obsoleta, an oviparous, New World colubrid.

#### **Offspring size**

A fundamental reproductive decision made by females is how to divide the resources available for egg formation. Investing more per egg should increase each offspring's probability of survival (Jayne and Bennett 1990, Azevedo et al. 1997, Giron and Casas 2003, Kissner and Weatherhead 2005), but at the expense of producing fewer offspring. For a given environment there should be a single solution to the tradeoff between offspring size and number that maximizes maternal fitness (Smith and Fretwell 1974). If the environment is the same for all females in a population, we should expect all females to produce offspring of the optimal size, and thus offspring size should vary little. Thus, females with different amounts of energy to invest should vary the number but not the size of their offspring. Note that if the optimal offspring size is not an integer, or if resources are not evenly divisible by the optimal offspring size, some variation in offspring size would result, but this problem becomes trivial at larger litter sizes (Ricklefs 1968, Smith and Fretwell 1974) typical of snakes. Contrary to the expectation of offspring size not varying, however, empirical evidence indicates that substantial offspring size variation is the norm both among and within litters (Roff 1992, Stearns 1992, Bernardo 1996), probably because the environment is not the same for all females or because the environment is unpredictable. In northern watersnakes, Nerodia sipedon, for example, neonate mass varied four-fold within a population and as much as two-fold within litters (Weatherhead et al. 1999). Thus, the concept of optimal offspring size is not useful for understanding how females trade off offspring size and number, a view expressed for some time (Capinera 1979, Kaplan and Cooper 1984) and which continues to be reinforced (Bernardo 1996, Weatherhead et al. 1999, Koops et al. 2003). Our focus in this study will therefore be on variation in offspring size. We consider variation both among and within clutches.

We first assess whether clutch size increases with maternal size and whether egg size decreases with clutch size. Both patterns are widely reported in other species (Seigel and Ford 1987) and confirmation that ratsnakes adhere to these norms would suggest that other results we report may also be broadly applicable. We consider three hypotheses proposed to explain variation in offspring size among clutches. All three hypotheses attribute variation in egg size among clutches to environmental predictability. Kaplan and Cooper (1984) proposed that variation in egg size among females could be favored by environmental unpredictability, such that among-clutch variation in egg size would increase as environmental predictability decreases. The ratsnake population we studied lives in a highly seasonal environment. The conditions confronted by young from eggs that hatch early in the season should be predictably good, whereas young from eggs hatching late could find conditions ranging from benign to harsh. Thus, we test the prediction that variance in egg size among clutches should increase as the season progresses.

The second hypothesis we consider follows from the common observation that the tradeoff between offspring size and number in reptiles varies seasonally (Ferguson and Snell 1986, Sinervo et al. 1992, King 1993, Sinervo and Doughty 1996, Weatherhead et al. 1999). Weatherhead et al. (1999) proposed that if neonates born early in the season have more time to acquire resources before hibernation, females giving birth early could invest less per offspring and thus increase the size of their litters. Thus, we test the prediction that females should increase the mean size of their offspring the later in the season they lay their eggs.

Several studies of fish have found that females that have more food or that have experienced faster growth produce smaller eggs (Reznick and Yang 1993, Jonsson et al. 1996, Johnston and Leggett 2002). This pattern suggests that females predict the environmental conditions their offspring will face based on their own recent experience - if conditions are good then offspring require less provisioning, allowing females to produce more, smaller eggs (Koops et al. 2003). If females differ in their perception of the future, that should also affect the tradeoff they make between resources allocated to their own survival vs reproduction. Thus, if females perceive that future conditions will be harsh, they should retain more reserves for self-preservation, particularly in long-lived species such as ratsnakes (Blouin-Demers et al. 2002). Those same females should also produce larger eggs to enhance offspring viability. Therefore, we test the prediction that females in better condition after they finish laying their eggs (i.e. those saving more reserves) will produce larger eggs. In shortlived species, this pattern could be harder to detect if age affects investment in reproduction such that senescent females are more willing to sacrifice survival in favour of reproduction, irrespective of their condition. This should be a minor issue in long-lived species such as ratsnakes, however.

Within-clutch variation in offspring size has received much less attention than among-clutch variation, although three hypotheses have been proposed to explain this variation. First, similar to the first hypothesis for among-clutch variation, decreased environmental predictability could favor females increasing size variation among their eggs (Koops et al. 2003). Thus, we test the prediction that within-clutch variation in egg size will increase the later in the season the clutch is laid.

An alternative, the imperfect information hypothesis, proposed by Koops et al. (2003) rests on the assumption that females lack the information to predict accurately the environment their offspring will face. Furthermore, the consequences of misjudging future environments are different for females producing small eggs compared to those producing large eggs. A female that incorrectly predicts a harsh environment and produces few large eggs suffers only the cost of missing the opportunity to have produced more eggs. Conversely, a female incorrectly predicting a favourable environment that produces many small eggs could suffer entire clutch failure. By producing variably sized eggs a female can improve her fitness when her prediction of the future environment is incorrect. An asymmetry in outcomes still persists, however. The advantage of producing variably sized eggs will be greater for a female predicting good conditions than for a female predicting harsh conditions. By varying egg size, the former will have some large eggs in a clutch of otherwise smaller eggs, where the large eggs will survive if conditions prove not to be good. By contrast, a female that predicts harsh conditions and produces some small eggs will suffer a small reduction in fecundity if conditions turn out to be good. Given that varying egg size should be more valuable to females predicting good conditions (i.e. those producing smaller eggs), we test the prediction that egg size variability will decrease as mean egg size increases. Koops et al. (2003) found strong support for this prediction in brook trout, Salvelinus fontinalis, but Einum and Fleming (2004) did not find such support for this prediction in their theoretical model. Seigel and Ford (1992) found that checkered garter snakes, Thamnophis marcianus, that received more food produced more variably sized offspring, although they did not produce smaller offspring, so these results are only partially consistent with the prediction.

Finally, within-clutch variation could be non-adaptive, such as a result of maternal inability to control precisely resource allocation to developing follicles (McGinley et al. 1987). If so, then we would not expect variance in egg size to be correlated with any of the variables considered in the other hypotheses.

# Offspring sex

Most research on reptilian sex ratios has focussed on temperature-dependent sex determination, a phenomenon unknown in snakes (Shine 2003). There is evidence from snakes, however, that sex allocation is non-random. Among viviparous snakes, offspring sex ratios vary with female size and/or age (Lemen and Voris 1981, Dunlap and Lang 1990, Madsen and Shine 1992, Weatherhead et al. 1998). Trivers and Willard (1973) proposed that females should produce more of the sex that performs best in the current environment. For example, females with the resources to produce larger offspring should produce more of the sex that benefits most from being large. Weatherhead et al. (1998) tested this hypothesis with northern watersnakes and obtained equivocal results. They assumed that because female watersnakes grow much larger than males, large size is more beneficial to females and, thus, that females should favour female-biased litters when offspring were larger or born earlier. Earlier birth should provide neonates a head start for growth, which could affect growth through their lifetime (Madsen and Shine 2000). We test the same hypotheses with ratsnakes. Because male black ratsnakes grow larger than females (Blouin-Demers et al. 2002) and larger males sire more offspring than smaller males (Blouin-Demers et al. 2005), however, our predictions were reversed. Clutches of larger eggs and clutches laid earlier should be male biased.

# **Methods**

We conducted this study from 1996 to 2004 at the Queen's Univ. Biol. Stn in eastern Ontario, Canada (45°37'N, 76°13'W). As part of a larger study, all ratsnakes were individually marked with PIT tags when first captured. Most eggs were obtained from nests that we had located by radio tracking gravid females until they oviposited (Blouin-Demers et al. 2004). Females had radio-transmitters surgically implanted in their body cavity using sterile techniques and under isoflurane anaesthesia (Blouin-Demers et al. 2000, Weatherhead and Blouin-Demers 2004). Over the nine years of the study, we followed 72 females, 31 of which were gravid at least once. Collectively these females allowed us to locate 19 nests. Eggs were always laid in decaying organic matter in both natural (e.g. hollow trees) and man-made (e.g. leaf piles) locations and included sites used by single females and by multiple females (Blouin-Demers et al. 2004).

We obtained eggs from nests in several ways. Once a female we tracked to a nest had laid her eggs (determined by capturing and palpating the female after she moved away from the nest), we excavated the nest substrate. If we found a single clutch we assigned it to the female we had tracked. If we found additional clutches, maternity was unknown for all of them. Because females lay all their eggs in a tight cluster, however, we were often able to assign eggs to clutches. When the number of eggs in a cluster exceeded the range of sizes of known single clutches, we assumed more than one female had contributed to the clutch. Once we had found a nest we revisited it every summer after nesting was well underway and excavated it for eggs. In addition to finding eggs at these nests, we regularly encountered gravid females. We brought these gravid females back to the lab, housed them individually, and provided them with a nesting box filled with a mixture of moist sphagnum and peat moss in which they laid within a few days. After laying, females were measured for snout-vent length (SVL) to the nearest 1 cm with a flexible measuring tape run along their body (Blouin-Demers 2003) and weighed to the nearest 1 g with an electronic scale. Females were subsequently released where they had been captured.

We weighed each clutch to the nearest 0.1 g with an electronic scale. We incubated the eggs at 30°C in two incubators ( $120 \times 90 \times 30$  cm) built with fiberboard laminated with melamine. Temperature was maintained in each incubator with a 100-W light bulb activated by an electronic thermostat. Eggs were grouped by clutch in  $15 \times 15 \times 6$  cm plastic containers with perforated lids and were half-buried in 3 cm of moist vermiculite (2:1 ratio by mass of water and vermiculite). The containers with the eggs and incubation medium were weighed at the onset of incubation and, to compensate for evaporation, water was added weekly to maintain total mass. The weekly watering also permitted us to change the position of containers within the incubators to avoid possible location effects. Toward the end of incubation in late August, we checked egg containers regularly to remove hatchlings.

Within two days post-hatching, we removed hatchlings from the incubators and determined their sex by everting male hemipenes. Hatchlings were weighed individually to the nearest 0.1 g on an electronic scale. SVL and tail length (TL) were measured to the nearest 1 mm by laving the hatchlings against a metal ruler taped to a table (Blouin-Demers 2003). We marked all snakes individually by PITtagging and released them at their nest or at the capture location of their mother. Because all eggs were incubated at the same temperature and development time is temperature-dependent, we assumed that hatching dates were a reliable index of laying dates. Eggs found in nests would have undergone some incubation prior to being brought into the lab, but mean nest temperatures were within a few degrees of our incubators (Blouin-Demers et al. 2004), so the association between hatching and laying date should not have been affected substantially.

## Analyses

We computed an index of body condition for adult females using equations developed for the same population by Blouin-Demers et al. (2005). Briefly, we obtained equations relating mass to size of females by regressing the log of body mass on the log of SVL using measurements from the first capture for 329 females  $\geq 800$  mm snout-vent length between 1996 and 2001. We then obtained an index of relative body condition for all mothers by calculating the residuals of the regression equations using their SVL and mass (logtransformed) measured just after laying. We standardized our condition index by dividing it by the predicted mass (again log transformed) multiplied by 100. Our condition index thus represents a percentage above or below the predicted mass.

We calculated relative clutch mass (RCM) for females by expressing the mass of the clutch as a percentage of the post-oviposition mass of the female. Radio-implanted females were captured within one week after laying to measure their post-oviposition mass. We considered all eggs laid by a female to be part of her clutch, including occasional infertile or unviable eggs.

Because morphological data of siblings are not independent, we conducted our analyses on data averaged for each clutch. For analyses of within-clutch variance in offspring size we used the coefficient of variation (CV: the standard deviation expressed as a percentage of the mean). Because mean values calculated from few individuals are imprecise, we excluded clutches for which we measured fewer than five hatchlings. We inspected box plots and residual plots to verify the assumptions of normality and homogeneity of variance. All statistical analyses were two-tailed and were conducted using JMP version 6 (SAS Inst.). We considered statistical results significant at  $\alpha = 0.05$ .

# Results

# General

We collected 1944 eggs of which 661 could not be assigned to clutches with certainty. Eggs that could not be assigned to clutches are necessarily excluded from analyses based on clutch attributes. A total of 57 clutches (699 eggs) were from 48 known females (i.e. females that nested in the lab or alone in the field; mean clutch size =  $12.26 \pm 0.38$  eggs) and 47 clutches (584 eggs) were assumed to come from single females (i.e. separate clusters of eggs found in communal nests; mean clutch size =  $12.43 \pm 0.42$  eggs). There was no significant difference between the mean size of clutches from known females and those assumed to come from single females  $(t_{(102)} = 0.286, p = 0.776)$ , validating our assumption that these clusters were in fact single clutches. Overall, clutch size ranged from 6 to 18 eggs.

The 1944 eggs yielded 1631 offspring (83.9% hatching success): 821 females, 785 males. The sex of 25 offspring was undetermined because they were used in another experiment. The overall proportion of males (0.489) was not significantly different from 0.500 ( $\chi^2_{(1)} = 0.934$ , p = 0.334). For offspring that lived for at least 24 hours past hatching (n = 1599), SVL averaged  $277.0 \pm 0.42$  mm, but varied from 166 to 340 mm and mass averaged  $9.47 \pm 0.12$  g, but varied from 3.7 to 13.9 g. We did not measure the 32 offspring that died within 24 hours, but they were not notably different from the survivors. Because hatchling SVL and mass are highly correlated (r =0.574) and because mass is likely to have a smaller measurement error than SVL, hereafter we use only mass as our measure of offspring size. Within clutches, the CV of mass averaged  $7.64 \pm 0.57\%$ and ranged from 1.27 to 25.75%. Thus, there was ample variation in hatchling mass that could be under the influence of selection.

RCM tended to decline with increasing female SVL ( $R^2 = 0.062$ ,  $F_{1,51} = 3.383$ , p = 0.072), but longer females produced more eggs ( $R^2 = 0.106$ ,  $F_{1,53} = 6.299$ , p = 0.015, Fig. 1). Mean egg mass, however,



Fig. 1. Clutch size increases as a function of maternal snoutvent length in an Ontario population of ratsnakes.

did not vary with female SVL ( $R^2 = 0.003$ ,  $F_{1,52} = 0.132$ , p = 0.718, Fig. 2). Mean offspring mass was strongly related to mean egg mass ( $R^2 = 0.577$ ,  $F_{1,89} = 121.205$ , p < 0.001).

Mean offspring mass declined with increasing clutch size ( $R^2 = 0.102$ ,  $F_{1,89} = 10.106$ , p = 0.002, Fig. 3). When we restricted the analysis to clutches with known maternal size, so that maternal SVL could be included as a control variable, mean offspring mass still declined



Fig. 2. Mean egg mass does not vary as a function of maternal snout-vent length in an Ontario population of ratsnakes.



Fig. 3. Tradeoff between mean offspring mass and clutch size in an Ontario population of ratsnakes.

with increasing clutch size, albeit weakly ( $R^2 = 0.075$ ,  $F_{1,44} = 3.586$ , p = 0.064).

#### Among-clutch variation in offspring size

To test the prediction that variation in offspring size among clutches should increase with laying date, we divided each year into early and late season based on the median hatching date for that year. We then computed a CV for early and late season of each year from the mean mass of hatchlings of each clutch. We found no support for the prediction of increased variance in offspring mass later in the season ( $R^2 =$ 0.148,  $F_{1,13} = 2.257$ , p = 0.157), and in fact the trend was in the opposite direction (mean CV for early clutches = 11.9 ± 1.9%; mean CV for late clutches = 7.9 ± 1.8%).

We used ANCOVA (with year and maternal SVL included as control variables) to test the prediction that mean offspring size should increase with hatch date. Offspring from clutches that hatched later were not heavier ( $R^2 = 0.011$ ,  $F_{1,38} = 0.815$ , p = 0.372).

We used ANCOVA (with year and maternal SVL included as control variables) to test the prediction that females in better condition after laying should have laid larger eggs. Females that were in better condition after laying had laid heavier eggs ( $R^2 = 0.137$ ,  $F_{1,51} = 8.122$ , p = 0.006, Fig. 4). Furthermore, linear regression indicated that RCM declined with female post-laying condition ( $R^2 = 0.073$ ,  $F_{1,50} = 3.969$ , p = 0.050, Fig. 4), consistent with those females having reduced their



Fig. 4. Females in better condition after laying laid heavier eggs (A), but invested less in reproduction (B) in an Ontario population of ratsnakes.

investment in reproduction in favour of greater self preservation.

#### Within-clutch variation in offspring size

We used ANCOVA (with year included as a control variable) to test the prediction that variation in offspring size should increase as the season progressed. Clutches hatched later did not yield more variable offspring (CV of mass:  $R^2 = 0.028$ ,  $F_{1,81} = 2.643$ , p = 0.108). In fact, the trend was in the opposite direction. We used linear regression to test the prediction that variance in offspring size should decrease with mean offspring size. The prediction was not supported (CV of offspring mass:  $R^2 = 0.002$ ,  $F_{1,90} = 0.20$ , p = 0.654).

#### **Offspring sex**

We found evidence for sexual size dimorphism (SSD) at birth. ANOVAs with clutch identity as a control variable revealed that sex had an effect on TL ( $R^2 =$ 0.237,  $F_{1,1475} = 853.093$ , p <0.0001), but not on SVL ( $R^2 < 0.001$ ,  $F_{1,1475} = 1.124$ , p =0.289) or on mass ( $R^2 < 0.001$ ,  $F_{1,1475} = 1.476$ , p = 0.225). Male hatchlings had longer tails ( $56.26 \pm 0.16$  mm) than female hatchlings ( $51.33 \pm 0.14$  mm), but were not longer or heavier. Thus, there appears to be no difference in the cost of producing sons and daughters that otherwise might have affected sex allocation decisions.

We predicted that clutches with large offspring should be disproportionately male because of malebiased SSD in adult black ratsnakes. Contrary to this expectation, however, multiple regression indicated that the proportion of male offspring did not vary with the mean mass of offspring in the clutch  $(R^2 = 0.018)$ ,  $F_{1,88} = 1.623$ , p = 0.206). The proportion of male offspring also did not vary with maternal size  $(R^2 =$ 0.004,  $F_{1,47} = 0.317$ , p = 0.679) or with maternal condition after laying ( $R^2 = 0.003$ ,  $F_{1,45} = 0.131$ , p = 0.719), even though we showed above that females in better condition laid heavier eggs. ANCOVA (with year included as a control variable) provided some evidence that clutches that hatched late were more male-biased  $(R^2 = 0.043, F_{1.81} = 3.480, p = 0.066, Fig. 5)$ , contrary to our prediction.

### Discussion

Two general features characterized offspring size variation in black ratsnakes. First, enormous variation in offspring size occurred both within and among litters. Across all the eggs we sampled, the largest hatchling was more than twice the length and almost four times the mass of the smallest hatchling. Even variation in offspring size was itself highly variable, with CVs in offspring mass among clutches ranging from as little as 1% to over 25%. The second general feature of this variation was that, with one exception, the variables we expected should influence variation in offspring size had little effect. We found that clutch size increased with maternal size and that egg size decreased with clutch size, as documented in other snakes (Seigel and Ford 1987), but we found no evidence that variance in egg size among clutches increased as the season progressed or that females increased the mean size of their offspring



Fig. 5. Clutches laid late are more male-biased in an Ontario population of ratsnakes.

the later in the season they laid their eggs. Females in better condition after they finished laying their eggs did produce larger eggs. Finally, there was no relationship between within-clutch variation in egg size and laying date or mean egg size.

Pronounced variation in offspring size both within clutches and within populations, such as we found for black ratsnakes, appears to be the norm in snakes. For example, in corn snakes, *Elaphe guttata*, the largest eggs were approximately twice the mass of the smallest eggs and CVs of egg mass among clutches were between 18 and 23% (Seigel and Ford 1991). Even larger CVs of egg mass were found among clutches of brown house snakes, Lamphrophis fuliginosus, another oviparous species (Ford and Seigel 2006). In keelbacks, Tropidonophis mairii, a tropical oviparous colubrid, mean egg mass for clutches with the heaviest eggs was more than twice that of clutches with the lightest eggs (Brown and Shine 2002). Similar ranges of variation in neonate mass have also been documented for viviparous snakes (Ford and Seigel 1989, Seigel and Ford 1992, Weatherhead et al. 1999). It should be noted that in most analyses of offspring size, mean values for litters are used to control for family effects. If the ranges and variance in offspring size that are reported are only those from the analysis of mean values (e.g. the range in mean offspring size among litters), then the extent of variation in offspring size within litters and in the population will not be apparent, and will be greater than the values reported.

Although various studies have found weak associations between egg or neonate size and some other variable such as maternal size (Stewart 1989, Weatherhead et al. 1999, Ji and Wang 2005), much of

the variation remains unexplained. One reason this may not have attracted research attention is that the focus has been on mean offspring size within litters, which varies much less than other litter traits such as the number of young produced. Ford and Seigel (1989) and Seigel and Ford (1991) conducted feeding experiments with both a viviparous and an oviparous snake species. In both experiments, females on high energy diets produced more offspring per litter, whereas mean offspring mass was unaffected, leading to the conclusion that offspring/egg size was canalized, at least with respect to energy input. This conclusion did not mean that offspring size did not vary, however, but only that mean offspring size was unaffected by maternal feeding regimes. As cited above, CVs in offspring size in both studies were substantial.

How do we account for the variation in offspring size within and among litters? If size had no effect on offspring survival, selection should favour females that increase litter size at the expense of offspring size until size does affect survival, producing larger litters of uniformly small offspring. The inverse relationship between offspring size and litter size reported in many studies (Weatherhead et al. 1999, Ford and Seigel 2006) indicates that female snakes have the ability to make such tradeoffs. Evidence that size does affect offspring survival (Jayne and Bennett 1990, Azevedo et al. 1997, Giron and Casas 2003, Kissner and Weatherhead 2005), however, leads to the expectation that selection should optimize offspring size at a value where the combination of litter size and offspring survival maximizes maternal fitness (Smith and Fretwell 1974). The failure to observe pronounced shifts in mean offspring sizes in snakes in response to environmental factors (e.g. food variation, maternal condition, season) suggests that the interesting biology lies in understanding the variation around mean sizes.

Most of our predictions related to environmental variability rested on the assumption that, in our temperate study area, environmental variability should increase from late summer to early fall. Thus, we predicted that clutches laid late should consist of bigger and more variable eggs, both within and among clutches. One potential reason for the lack of support we found for the environmental variability hypothesis is that hatch date may not be a good proxy for environmental variability. Alternatively, it is possible that within-clutch variation in offspring size is non-adaptive in ratsnakes and that variation results from maternal inability to control precisely resource allocation to developing follicles (McGinley et al. 1987). If hatch date is a good proxy for environmental variability in temperate climates, this explanation would be consistent with our observation that none of the predicted variables correlated with variance in egg size. This non-adaptive explanation is problematic, however, given the extent of variation in offspring size on the one hand, and evidence that offspring size affects survival in snakes on the other. It seems clear that female ratsnakes could do a better job allocating the resources they invest in offspring. Either the variance we have documented is resistant to selection, or that variance provides some advantage we have yet to consider. Studies with captive snakes are required to assess heritability of egg size and variation in egg size, and field studies are required that determine how variance in offspring size affects variation in survival among litters.

One aspect of environmental variability that we did not consider here involves annual variation in the type of food available to hatchlings. Conceivably prey abundance could shift in ways that favour larger offspring in some years and smaller offspring in other years. If females cannot predict which prey will be available for their offspring, then variation in offspring size could be a form of bet hedging that allows all females to have some offspring matched to whatever conditions prevail. Logically it seems that such a scenario should lead to optimal variation in offspring size, such that the level of within-clutch variation that produces the highest mean maternal fitness through time would be favoured. Thus, one should find little variance among clutches in the degree of within-clutch variation. The 20-fold range we observed in within-clutch CV of offspring size is clearly at odds with this expectation.

The one prediction our data did support was that females that invested relatively less in reproduction, and thus were in better condition after laying, produced larger eggs. This prediction was based on our hypothesis that females that retain more reserves for selfpreservation are anticipating challenging environmental conditions. Because their offspring will face those same conditions, those females should increase the survival prospects of their offspring by making them bigger. To our knowledge this hypothesis has not been previously proposed or tested, so we do not know whether other species exhibit the same pattern. We can point to a potential logical difficulty with our results that present a challenge to this hypothesis, however. Female black ratsnakes do not associate with their offspring, so if a female anticipates environmental conditions that will be challenging for both her and her offspring, those conditions should be regional rather than local. In turn, that should lead to some consensus among females, and yet we did not find significant year effects that would be indicative of consensus. If future studies also find an association between female condition after laying and mean egg size but no evidence of consensus, a different explanation for that association will be required.

We did not find evidence of adaptive sex-ratio manipulation. We assumed that bigger offspring and offspring born early would benefit most from being male because of the male-biased SSD characteristic of the species (Blouin-Demers et al. 2002). SSD in ratsnakes is not extreme, however, suggesting that selection on body size is not markedly different for males and females. In indeterminate growers such as snakes, fertility selection favours large body size in females (Andersson 1994). Thus, it is possible that male and female ratsnakes benefit almost equally from being born large and that there is no strong selective advantage to sex-ratio manipulation. The fact that clutches laid late tended to be more male-biased is intriguing and requires further study.

In summary, ratsnakes (and other snake species) exhibit enormous variation in the size of eggs they produce, both among and within clutches in the same population. Given evidence that offspring size in snakes affects survival, selection should reduce the variation in offspring size unless that variance enhances maternal fitness and yet we find little support for hypothesized advantages of varying offspring size. Progress in resolving this issue requires that researchers interested in offspring size focus on the variance as much as on the mean. Adaptive sex allocation by snakes is also poorly resolved, but appears less problematic than offspring size, at least in part because we know little about whether factors such as size or timing of birth have sexspecific effects on fitness.

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