Male mating success in a North American pitviper: influence of body size, testosterone, and spatial metrics

CHARLES F. SMITH[1,2,3,4*], GORDON W. SCHUETT[3,4,5] and MELISSA AMARELLO[3,6]

[1]*Department of Ecology and Evolutionary Biology, The University of Connecticut, 75 N Eagle Road Unit 3043, Storrs, CT 06269-3043, USA*

[2]*Department of Biology, Wofford College, 429 North Church Street, Spartanburg, SC 29303, USA*

[3]*The Copperhead Institute, P.O. Box 6755, Spartanburg, SC 29304, USA*

[4]*Chiricahua Desert Museum, P.O. Box 376, Rodeo, NM 88056, USA*

[5]*Department of Biology and Center for Behavioural Neuroscience, Georgia State University, 33 Gilmer Street, S. E., Unit 8, Atlanta, GA 30303, USA*

[6]*Advocates for Snake Preservation (A.S.P.), P.O. Box 40493, Tucson, AZ 85717, USA*

Males with enhanced traits relative to conspecifics often show increased mating and reproductive success and thus have a fitness advantage. The opportunity or potential for sexual selection is predicted to occur under these conditions. Here, we investigated proximate determinants of mating success in male copperhead snakes (*Agkistrodon contortrix*), a medium-sized pitviper of North America. Specifically, we investigated the relationships of body size (snout-vent length, body mass), body condition index, spatial metrics (total distance moved, home range size), and plasma testosterone concentration on mating success in males. The single mating season lasts from August through September. We compared a set of candidate linear mixed models and selected the best-fitting one using the adjusted Akaike Information Criterion (AICc). The AICc-selected model (model 2), with testosterone, body condition index, and home range size as predictor variables, showed that male mating success was positively correlated with testosterone. To our knowledge, this is the first report to show the relationship of testosterone and individual mating success in any snake species. A parallel study conducted on male fitness in *A. contortrix* of the same population used microsatellite markers to assign parentage of fathers (known mothers). Unlike our study, they found that snout-vent length was positively correlated with reproductive success and that males were experiencing greater sexual selection. This relationship has been detected under natural conditions in other species of snakes. Although behavioural data are important in any mating system analysis, they should not stand alone to infer parentage, relationships or selection metrics (e.g. Bateman gradients). Long-term sperm storage by females, female cryptic choice, and other factors contribute to the complexity of mating success of males. Accordingly, we thus conclude that estimates of reproductive success and fitness in cryptic species, such as copperheads and other snakes, require robust molecular methods to draw accurate conclusions regarding proximate and evolutionary responses. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, **115**, 185–194.

ADDITIONAL KEYWORDS: *Agkistrodon contortrix* – copperhead – male reproductive success – mating systems – reptiles – sex steroid hormones – sexual selection – snakes.

INTRODUCTION

Theoretical and empirical studies of animal mating systems have repeatedly demonstrated that males are the primary mate searching sex (Darwin, 1871; Bateman, 1948; Trivers, 1972; Emlen & Oring, 1977; Thornhill & Alcock, 1983; Kokko & Wong, 2007). Furthermore, male mating success is often correlated with visible phenotypic traits (e.g. horns, ornaments, body size) that increase priority-of-access to females. These traits are used in competitive fights with

*Corresponding author. E-mail: smithcf@wofford.edu

conspecifics, mate-searching tactics, and attracting females, hence mate choice (Thornhill & Alcock, 1983; Andersson, 1994; Emlen, 2001; Shuster & Wade, 2003; Smith *et al.,* 2008; Klose, Welbergen & Kalko, 2009). However, mating success can also be influenced by non-visible traits such as performance ability (Arnold, 1983; Husak & Fox, 2008; Careau & Garland, 2012), personality (Dall *et al*., 2012; Wolf & Weissing, 2012), sexual and fighting experience (Riechert, Chastel & Becker, 2012), and concentrations of circulating sex hormones (Sinervo *et al*., 2000; Adkins-Regan, 2005; John-Alder *et al*., 2009; Neff & Knapp, 2009). In males, elevated levels of plasma testosterone (T) can increase aggression, vigilance at territories, quality of traits (e.g. colours of feathers and song type), courtship skills, and whole-body performance, including locomotor capacity during mate searching (Husak *et al*., 2006; Hau, 2007; Careau & Garland, 2012). Males with enhanced traits relative to conspecifics often show differential reproductive success (progeny) and thus a fitness advantage (Shuster & Wade, 2003). Under the aforementioned conditions, the opportunity is present for sexual selection to act on males (Darwin, 1871; Arnold & Duvall, 1994; Shuster & Wade, 2003; Jones, 2009).

Among terrestrial vertebrates, most research on mating systems and traits associated with mating success has concerned birds and mammals, with far fewer studies on amphibians and non-avian reptiles such as lizards and snakes. Taxon biases are even found within reptiles, with far less information available on snakes, for instance, than in other lineages (Bonnet, Shine & Lourdais, 2002; Shine, 2003). Secretiveness, cryptic habits, rarity, and small body size play a significant role in explaining this historic gap of information in snakes (Bonnet *et al*., 2002; Clark *et al*., 2014). Nonetheless, in recent years, radio-telemetry has circumvented many logistical problems in locating and observing wild individuals for extended periods (Reinert, 1992; Dorcas & Willson, 2009). Several lineages in particular, the viperids (Jellen *et al*., 2007; Glaudas & Rodríguez-Robles, 2011; Clark *et al*., 2014), several species of colubrids (Blouin-Demers, Gibbs & Weatherhead, 2005; Dubey *et al*., 2009), boids (Rivas & Burghardt, 2005) and pythonids (Slip & Shine, 1988) have proved particularly valuable in radiotelemetric studies of mating systems of snakes (Duvall, Arnold & Schuett, 1992; Aldridge & Duvall, 2002; Clark *et al*., 2014). This success is largely due to large body size, high visibility, spatial predictability, and high population densities (Duvall *et al*., 1992; Duvall, Schuett & Arnold, 1993), which have permitted the field study of their mating systems, reproductive success, and sexual selection (Duvall & Schuett, 1997; Coupe, 2002; Greene *et al*., 2002; Rivas & Burghardt, 2005; Jellen

et al., 2007; Dugan, Figueroa & Hayes, 2008; Glaudas & Rodríguez-Robles, 2011; Schuett, Repp & Hoss, 2011; Schuett *et al*., 2013; Clark *et al*., 2014).

Here, we tested hypotheses concerning the mating system of copperhead snakes (*Agkistrodon contortrix*), a medium-sized pitviper of North America (Fitch, 1960; Gloyd & Conant, 1990) using linear mixed models (Burnham & Anderson, 2004). Specifically, we asked the following questions: (1) Is body size (snout-vent length, body mass) and body condition index (BCI) in males correlated with their mating (copulation) success? This taxon exhibits male-biased sexual size dimorphism (Fitch, 1960). (2) Do males that move greater distances and have larger home ranges locate and copulate with more females? and (3) Are concentrations of plasma testosterone in males correlated with their mating success (which we define as the frequency of copulations with unique mates)?

MATERIAL AND METHODS

RESEARCH SITE

The study site was located in a 485 ha parcel of basalt trap rock ridge ecosystem situated 4.75 km NW of Meriden, Connecticut. Details of the topography and climate of this region are presented elsewhere (Smith, 2007; Smith *et al*., 2009).

SUBJECTS

After their initial capture in spring (April), subjects were transported to the laboratory for processing. Under Isoflurane anesthesia, body mass (± 0.5 g) was determined using a triple beam balance, and snout-vent length (SVL: cm ± 0.2) was measured using a non-stretchable cloth measuring tape. Ten adult males were surgically implanted with standard radio-transmitters (per Smith *et al*., 2009; Smith, Schuett & Schwenk, 2010). Using procedures of Waye & Mason (2008), the BCI of each male we selected to radio-track was calculated as the residual score from the general linear regression of ln-transformed mass against ln-transformed SVL.

Regardless of size, all copperheads encountered at the field site were permanently marked for future identification using passive integrated transponder (PIT) tags (125 kHz 12 mm, Biomark, Boise, Idaho, USA; Gibbons & Andrews, 2004). PIT tags were injected one-third of the body length anterior from the cloaca and the last three characters of the 10-character PIT code were used as an identification code for all records pertaining to an individual.

RADIO-TRACKING AND BEHAVIOURAL OBSERVATIONS

The ten male subjects implanted with radio-transmitters were located every 48–72 h in August

and September on foot using radio-tracking equipment described in Smith *et al.* (2009). Reproductive behaviours (i.e., bisexual pairings, courtship, and coitus) were determined by direct observation (Smith *et al.*, 2009, 2010). Only observations involving coitus were used in the present analysis.

SPATIAL MEASUREMENTS

Subjects implanted with radio-transmitters were located and observed in the field on 17 occasions during August and 19 times during September (Smith *et al.*, 2009). Movement distances and home range sizes for males were calculated using the Animal Movement Extension option in ArcView 3.2 (Hooge, Eichenlaub & Solomon, 1999). Home range size was estimated using 100% minimum convex polygon (MCP) and kernel 95% and 50% contour intervals as determined by ArcView. Because a single point lying outside the normal range of an animal can artificially inflate activity range size, range estimates were recalculated after removing 5% and 10% of outliers using the Animal Movement harmonic mean outlier removal procedure (Hooge *et al.*, 1999). Comparisons of values prior to and following outlier removal showed no significant outlier effect; therefore, only values prior to outlier removal were used in the statistical analyses. For kernel estimates of home range size, smoothing values were determined using least-squares cross-validation (Seaman *et al.*, 1999).

We were unable to monitor every movement made by each male; hence, we relied on analyzing their movements as straight-lines. Although straight-line estimates of movement can underestimate the actual distance moved by as much as 50% (Madsen, 1984; Tiebout & Carey, 1987; Secor, 1992), based on our tracking protocol we assumed underestimation of straight-line movement to be equivalent for all individuals (Secor, 1994; see Table 1 herein).

COLLECTION OF BLOOD FOR MEASURING HORMONES

Blood for subsequent hormone analysis was sampled from all radio-tracked males at four equally spaced times; twice during August and twice during September. From these samples, radio-immunoassays (RIAs) were conducted for testosterone (T). Measurement of T in this copperhead population is detailed in Smith *et al.* (2010). Briefly, following their capture, blood was collected immediately in the field. Collection of blood samples to obtain plasma for sex steroid analysis was accomplished by gently restraining subjects in a clear acrylic tube. A small volume of blood (0.5-1 mL; less than 4% of total blood volume) was collected via heart-puncture (cardiocentesis) using a sterile-disposable 1 mL tuberculin syringe (coated with sodium heparin), fitted with a sterile-disposable 26-gauge needle. Sampling typically required < 60 s from initial capture until the blood sample was obtained (Schuett *et al.*, 2004a) and the subject released (or held for further processing). Blood was transported in individual 1.5 mL centrifuge tubes at ambient (~20 °C) temperature. Steroid levels are unaffected when blood is maintained at ambient temperatures for short (< 24-h) periods (Taylor & Schuett, 2004).

In the laboratory, the blood samples were placed in disposable microcentrifuge tubes (1 mL) and centrifuged at 13 000 *g* at room temperature (21–23 °C) for 5 min to separate plasma. Plasma was collected using a micropipette fitted with a sterile-disposable tip and transferred to another microcentrifuge tube that was

Table 1. Mating success variables used in linear mixed models (Table 2) involving ten adult male copperhead subjects (*Agkistrodon contortrix*). Measurements were obtained in the mating season (August and September, 2003)

ID	SVL	BM	BCI	T (ng mL⁻¹)	TDM	HRS	MS
15a	76.2	322.8	0.4598	35.90	817.70	0.29	1
e36	68.5	194.1	−0.0003	35.73	962.03	1.04	1
d54	95.1	347.8	0.4340	27.27	1985.69	2.92	5
96c	60.4	160.2	−0.1351	32.31	2068.30	3.03	2
71c	78.0	321.9	0.4466	26.74	2761.87	7.54	5
4gh	71.6	274.5	0.3260	41.32	2741.11	6.96	3
06a	62.1	166.0	−0.1121	39.96	2460.16	8.03	5
825	78.2	361.0	0.5599	34.79	1481.10	1.96	4
263	75.4	283.1	0.3344	28.35	4658.69	14.91	5
104	83.2	468.1	0.7916	35.92	1438.27	1.97	2

ID = male subject; SVL = snout-vent length (cm); BM = body mass (g); BCI = body condition index; T = mean plasma testosterone (ng mL⁻¹) level for the mating season; TDM = total distance moved (m); HRS = home range size (minimum convex polygon, hectare); MS = frequency of male mating success (coitus) with unique females for the mating season.

permanently labeled with the specimen identification code and date. Plasma samples were placed in an ultra-low freezer (−80 °C) until RIAs could be performed.

RADIO-IMMUNOASSAY OF TESTOSTERONE

Procedures for conducting radio-immunoassays (RIAs) of testosterone (T) followed Schuett *et al.* (1997, 2004a, b, 2005, 2006). Briefly, in quantifying T, we used commercial RIA kits but with several appropriate modifications (e.g. use of snake plasma rather than rat plasma for validation procedures) to measure steroid concentrations from the collected plasma of subjects. Validation included both quantitative recovery and parallelism. All values for plasma T concentrations are in ng mL^{-1}. Two different RIAs were performed for T and all samples were run in duplicate (duplicate reactions from a single extraction). The intra-assay coefficients of variation (CVs) were 9.1% and 11.1 %, and the inter-assay CV was 11.9%. Testosterone data (Table 1) represent the arithmetic means of four samples for each of the ten males during the mating season (August and September).

VARIABLES OF MALE MATING SUCCESS

The influence of body length (SVL), BCI, testosterone (T), total distance moved (TDM), and home range size (HRS) on male mating success (number of unique copulated females) were compared with five linear mixed models using the R statistical program (R Core Team, 2014) and packages lme4 (Bates *et al.*, 2014), lmerTest (Kuznetsova, Brockhoff & Christensen, 2014), and MuMIn (Barton, 2014). We included month (to account for serial autocorrelation) and ID (to account for non-independence of multiple records of individuals) as random variables, male mating success as a response variable, and SVL, BCI, TDM, HRS, and T as predictor variables. To select the best-fitting, most parsimonious model from among the five candidate models, we used Akaike's Information Criterion adjusted for small sample sizes (AICc) (Burnham & Anderson, 2004; Bolker, 2008).

RESULTS

SUBJECTS

Variables (SVL, BCI, TDM, HRS and T) analyzed in this study are presented in Table 1. Snout-vent length (SVL) and body mass (BM) were highly correlated ($F = 15.47$, $P = 0.004$, $R^2 = 0.66$, $N = 10$); therefore, owing to the stability of SVL (e.g. mass can change instantaneously with food or water consumption), it was used as the metric of male body size in all subsequent analyses. The SVLs of the ten radio-tracked males ranged from 62.1–85.2 cm (SVL mean = 74.87 cm, ± 3.21 SE, $N = 10$). Mean SVL of the radio-tracked males was not significantly different from mean SVL of all other adult males we measured (SVL mean = 75.20 cm ± 1.58 SE, $N = 47$, $P = 0.46$).

Adult males were significantly larger (SVL, BM) than females (SVL: $F = 18.8677$, $P < 0.0001$; body mass: $F = 3.9757$, $P = 0.0256$), a phenomenon termed male-biased sexual size dimorphism (SSD). This relationship has been reported in copperheads (Fitch, 1960, 1981) and many other snake species (Shine, 1994; Amarello *et al.*, 2010).

RADIO-TRACKING AND BEHAVIOURAL OBSERVATIONS

During August and September, mating was recorded in the ten males with radio-transmitters on 33 occasions involving 18 females (14 females fitted with radio-transmitters for a related concurrent study and 4 'incidental' females that were not fitted with radio-transmitters) (Table 1). Highly significant correlations were detected among the three parameters used to estimate male HRS (MCP, 95% kernel, 50% kernel home range, $R^2 > 0.63$, $P < 0.006$, but for reasons discussed by others (Row & Blouin-Demers, 2006), MCP was selected for all statistical comparisons. Total distance moved (TDM) and HRS were also highly correlated ($R^2 = 0.96$, $P = 0.0001$).

MALE MATING SUCCESS VARIABLES

The AICc-selected model (model 2) included HRS, T, and BCI as predictor variables (Fig. 1, Table 2). The frequency of male mating success was significantly correlated with T ($\beta = 0.051 \pm 0.02$, t = 2.99, $P = 0.013$), but not BCI ($\beta = 0.588 \pm 0.94$, t = 0.63, $P = 0.552$) or HRS ($\beta = 0.125 \pm 0.06$, t = 2.03, $P = 0.079$). None of the other candidate models were equally parsimonious (ΔAIC < 4), but model 1, which included HRS, T, and SVL as predictor variables, was close based on ΔAIC (ΔAIC = 5). In model 1, male mating success was significantly correlated with HRS ($\beta = 0.137 \pm 0.06$, t = 2.45, $P = 0.026$) and T ($\beta = 0.054 \pm 0.02$, t = 3.35, $P = 0.0004$), but not SVL ($\beta = 0.045 \pm 0.03$, t = 1.75, $P = 0.10$). Unlike model 2, HRS was significant in model 1, and in both models T was significant.

DISCUSSION

The AICc-selected model (model 2), with T, BCI, and HRS as predictor variables, showed that the frequency of male mating success was positively correlated only with T. Of our three hypotheses, we did not anticipate rejecting large body size (SVL) and BCI.
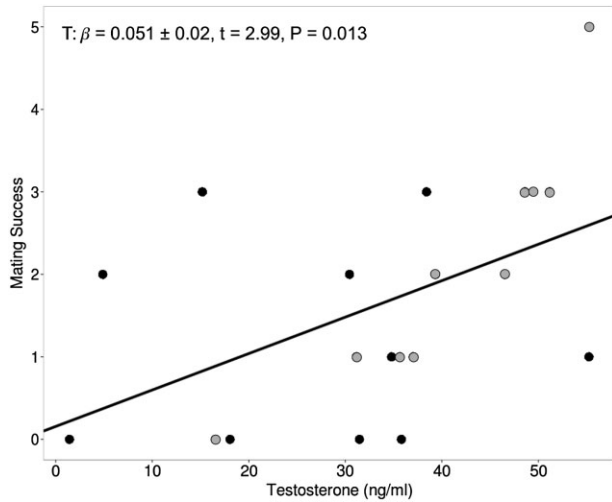
Figure 1. Relationship of plasma testosterone and mating success (frequency of copulation involving new females) in ten male copperheads (*Agkistrodon contortrix*). Gray circles denote August, and black circles denote September. See Table 1.

Table 2. Model selection criteria (Akaike's Information Criterion, AIC) and fitted parameters. Each model included month to account for serial autocorrelation and ID to account for non-independence of multiple records of individuals as random variables, and male mating success as a response variable. AICc = Akaike's Information Criterion adjusted for small sample sizes, ΔAICs = difference between AICc and most parsimonious model's AICc. HRS = home range size; T = testosterone; BCI = body condition index; TDM = total distance moved; and SVL = snout-vent length. See Table 1. For further details see *Methods*

Model	AICc	ΔAICc	Predictor variables
2	91	0	HRS, T, BCI
1	96	5	HRS, T, SVL
4	101	10	TDM, T, BCI
3	106	15	TDM, T, SVL
0	116	24	HRS, T, SVL, TDM

Schuett (1997), for example, reported that large body size in male copperheads was important in winning fights and obtaining priority-of-access to mates under laboratory conditions. Other studies concerning snakes support these results (Madsen *et al.*, 1993; Madsen & Shine, 1994; Shine, 2003; Jellen *et al.*, 2007; Dubey *et al.*, 2009). Presumably, large males also are capable of moving greater distances and having larger home ranges (Glaudas & Rodríguez-Robles, 2011). Nonetheless, several field studies of pit vipers and other snakes corroborate our

current findings with respect to SVL and male mating success (Duvall & Schuett, 1997; Blouin-Demers *et al.*, 2005; Jellen *et al.*, 2007; Dubey *et al.*, 2009; Ursenbacher, Erny & Fumagalli, 2009).

We also predicted that total distance moved (TDM) and HRS would be positively correlated with male mating success, but under model 2 TDM was not selected and HRS was rejected though approached significance ($P = 0.079$). In model 1, however, HRS was significant ($P = 0.026$). Other studies of snakes have shown a positive relationship with movements, HRS and mating (coitus) success in males (Duvall & Schuett, 1997; Prosser *et al.*, 2002; Weatherhead *et al.*, 2002; Jellen *et al.*, 2007; Glaudas & Rodríguez-Robles, 2011).

In model 2 we found support for our hypothesis that plasma testosterone levels of males would be significantly correlated with frequency of mating success (the number of copulations with unique mates). Studies investigating the relationship of T and mating success in wild snakes are essentially nonexistent, but studies of other vertebrates show that males with high levels of circulating androgens (e.g. T) often secure more mates and have greater mating success through displays and colour patterns influencing female choice, successfully holding territories, or by winning fights, challenges and guarding mates (Wingfield, 1984; Alatalo *et al.*, 1996; Ketterson & Nolan, 1999; Westneat & Stewart, 2003; Hau, 2007; Ouyang *et al.*, 2011). Furthermore, males with higher T levels show greater locomotor and whole-body performance (Irschick *et al.*, 2008; Higham & Irschick, 2013), which can lead to increased encounters with females (Garamszegi *et al.*, 2005) and greater reproductive success (Sinervo *et al.*, 2000; Gowan, McBrayer & Rostal, 2010). Importantly, Garland, Bennett & Daniels (1990) showed that locomotor performance in garter snakes (*Thamnophis s. fitchi*) is heritable and thus a target of selection (Husak *et al.*, 2006, 2009; Irschick *et al.*, 2008). In general, high levels of activity coupled with mate searching and interactions with females, a sort of endurance rivalry, can often predict male mating and reproductive success (Keogh *et al.*, 2012).

King and colleagues (King, 2002; King, Cline & Hubbard, 2004; King & Bowden, 2013) provide groundbreaking research on the inheritance of testosterone (T) levels in North American natricine snakes (genus *Thamnophis*). Importantly, significant effects were detected with respect to heritability estimates (h^2) and family differences. However, whether greater T levels in adult male *Thamnophis* translates to increased reproductive success remains for future studies (King & Bowden, 2013). Though not known in non-avian reptiles, selection can act on testosterone production in wild birds (McGlothin *et al.*, 2010).

Heritability, family differences, and selection on T levels and production have not been studied in copperheads or other snakes and is thus a rich area for future research.

Finally, with few exceptions (Schuett & Grober, 2000), most studies concerning steroid levels in snakes report only means and standard errors rather than individual values for individuals (Taylor & DeNardo, 2011). Our previous work on copperheads from the population we report here, for example, only provided means and standard errors (Smith *et al*., 2010). Accordingly, we support the view that a full understanding of variance in reproductive success and endocrinological metrics will need to incorporate the shifting paradigm of individual differences and animal personalities (Kempenaers, Peters & Foerster, 2007; Zera, Harshman & Williams, 2007; Dall *et al*., 2012; Wolf & Weissing, 2012). Instead of abandoning altogether the 'tyranny of the Golden Mean' (Bennett, 1987; Williams, 2008), we suggest also presenting data on individuals.

MEASURING SEXUAL SELECTION

In other species of snakes, including viperids, standard variances on mating frequencies ($Is = \sigma^2/\overline{\chi}^2$, Wade, 1979; Wade & Arnold, 1980) have been used as estimates of the potential for sexual selection (Madsen & Shine, 1994; Duvall & Schuett, 1997; Prosser *et al*., 2002). For example, based on our mating data in Table 1, we found that *Is* for copperheads showed no sex differences (Kolmogorov–Smirnov two-sample test, ♂ = 0.45, ♀ = 0.40, $P = 0.61$; Zar, 1999). However, based on Arnold & Duvall (1994) and Shuster & Wade (2003), mating success and the potential for sexual selection is best expressed as a function of the number of progeny produced by an individual in a given reproductive season. Furthermore, methods have been generated to overcome statistical biases associated with analyzing sexual selection gradients in open populations (Mobley & Jones, 2012).

Our research group (Levine *et al.,* 2014) conducted a parallel study on male fitness in *A. contortrix* from the population discussed herein, but analyzed mating success per Arnold & Duvall (1994) using 22 microsatellite markers to assign the parentage of fathers (all mothers were known) and the methods outlined in Mobley & Jones (2012) for open populations. Unlike the results of this study, Levine *et al*. (2014) found that SVL was positively correlated with male reproductive success (progeny) and males were experiencing greater sexual selection than females. With a range of alternative reproductive tactics operating in copperheads and other snakes, such as long-term sperm storage, cryptic female choice, sneaky males, and mate mimicry (Schuett, 1992; Zamudio & Sinervo, 2000; Booth & Schuett, 2011; Jellen & Aldridge, 2011; Clark *et al*., 2014; Levine *et al*., 2014), the relationship of male body size, copulation success and production of progeny to individual fitness is complex and requires further investigation.

REFERENCES

Adkins-Regan E. 2005. *Hormones and animal social behavior*. Princeton, NJ: Princeton University Press.

Alatalo RV, Hoglund J, Lundberg A, Rintamaki PT, Silverin B. 1996. Testosterone and male mating success on the black grouse leks. *Proceedings of the Royal Society of London Series B-Biological Sciences* **263:** 1697–1702.

Aldridge RD, Duvall D. 2002. Evolution of the mating season in the pitvipers of North America. *Herpetological Monographs* **16:** 1–25.

Amarello M, Nowak EM, Taylor EN, Schuett GW, Repp RA, Rosen PC, Hardy DL Sr. 2010. Potential environmental influences on variation in body size and sexual size dimorphism among Arizona populations of the western diamond-backed rattlesnake (*Crotalus atrox*). *Journal of Arid Environments* **74:** 1443–1449.

Andersson M. 1994. *Sexual selection*. Princeton, NJ: Princeton University Press.

Arnold SJ. 1983. Morphology, performance and fitness. *American Zoologist* **23:** 347–361.

Arnold SJ, Duvall D. 1994. Animal mating systems: a synthesis based on selection theory. *American Naturalist* **143:** 317–348.

Barton K. 2014. *MuMIn: multi-model inference. R package version 1.10.5*. Available at: http://CRAN.R-project.org/package=MuMIn

Bateman AJ. 1948. Intra-sexual selection in *Drosophila*. *Heredity* **2:** 349–368.

Bates D, Maechler M, Bolker B, Walker S. 2014. *lme4: linear mixed-effects models using Eigen and S4_. R package version 1.1-7.* Available at: http://CRAN.R-project.org/package=lme4

Bennett AF. 1987. Interindividual variability: an underutilized resource. In: Feder ME, Bennett AF, Burggren WW, Huey RB, eds. *New directions in ecological physiology*. Cambridge, UK: Cambridge University Press, 147–169.

Blouin-Demers G, Gibbs HL, Weatherhead PJ. 2005. Genetic evidence for sexual selection in black rat snakes, *Elaphe obsoleta*. *Animal Behaviour* **69:** 225–234.

Bolker BM. 2008. *Ecological models and data in R*. Princeton, NJ: Princeton University Press.

Bonnet X, Shine R, Lourdais O. 2002. Taxonomic chauvinism. *Trends in Ecology and Evolution* **17:** 1–3.

Booth W, Schuett GW. 2011. Molecular genetic evidence for alternative reproductive strategies in North American pitvipers (Serpentes: Viperidae): long-term sperm storage and facultative parthenogenesis. *Biological Journal of the Linnean Society* **104:** 934–942.

Burnham KP, Anderson DR. 2004. Multimodel inference: understanding AIC and BIC in model selection. *Sociological Methods and Inference* **33:** 261–304.

Careau V, Garland T Jr. 2012. Performance, personality, and energetics: correlation, causation and mechanisms. *Physiological and Biochemical Zoology* **85:** 543–571.

Clark RW, Schuett GW, Repp RA, Amarello M, Smith CF. 2014. Mating systems, reproductive success, and sexual selection in secretive species: a case study of the western diamond-backed rattlesnake, *Crotalus atrox*. *PLoS ONE* **9:** e90616.

Coupe B. 2002. Pheromones, search patterns, and old haunts: how do male timber rattlesnakes (*Crotalus horridus*) locate mates? In: Schuett GW, Höggren M, Douglas ME, Greene HW, eds. *Biology of the vipers*. Eagle Mountain, UT: Eagle Mountain Publishing, 139–148.

Dall SRX, Bell AM, Bolnick DI, Ratnieks FLW. 2012. An evolutionary ecology of individual differences. *Ecology Letters* **15:** 1189–1198.

Darwin C. 1871. *The descent of man, and selection in relation to sex*. London: Murray.

Dorcas ME, Willson JD. 2009. Innovative methods for studies of snake ecology and conservation. In: Mullin SJ, Seigel RA, eds. *Snakes: ecology and conservation*. Ithaca, NY: Cornell University Press, 5–37.

Dubey S, Brown GP, Madsen T, Shine R. 2009. Sexual selection favours large body size in males of a tropical snake (*Stegonotus cucullatus*, Colubridae). *Animal Behaviour* **77:** 177–182.

Dugan EA, Figueroa A, Hayes WK. 2008. Home range size, movements, and mating phenology of sympatric red diamond (*Crotalus ruber*) and southern Pacific (*C. oreganus helleri*) rattlesnakes in southern California. In: Hayes WK, Beaman KR, Cardwell MD, Bush SP, eds. *The biology of rattlesnakes*. Loma Linda, CA: Loma Linda University Press, 353–364.

Duvall D, Arnold SJ, Schuett GW. 1992. Pitviper mating systems: ecological potential, sexual selection, and microevolution. In: Campbell JA, Brodie ED Jr, eds. *Biology of the pitvipers*. Tyler, TX: Selva, 321–336.

Duvall D, Schuett GW. 1997. Straight-line movement and competitive mate searching in prairie rattlesnakes, *Crotalus viridis viridis*. *Animal Behaviour* **54:** 329–334.

Duvall D, Schuett GW, Arnold SJ. 1993. Ecology and evolution of snake mating systems. In: Seigel RA, Collins JT, eds. *Snakes: ecology and behavior*. New York, NY: McGraw-Hill, 165–200.

Emlen DJ. 2001. Costs and the diversification of exaggerated animal structures. *Science* **291:** 1534–1536.

Emlen ST, Oring LW. 1977. Ecology, sexual selection and the evolution of mating systems. *Science* **197:** 215–223.

Fitch HS. 1960. Autecology of the copperhead. *University of Kansas, Museum of Natural History Miscellaneous Publication* **13:** 85–288.

Fitch HS. 1981. Sexual size differences in reptiles. *University of Kansas, Museum of Natural History Miscellaneous Publication* **70:** 1–72.

Garamszegi LZ, Eens M, Boussès S, Møller AP. 2005. Testosterone, testes size, and mating success in birds: a comparative study. *Hormones and Behaviour* **47:** 389–409.

Garland T Jr, Bennett AF, Daniels CB. 1990. Heritability of locomotor performance and its correlates in a natural population of vertebrates. *Experientia* **46:** 530–533.

Gibbons JW, Andrews KM. 2004. PIT tagging: simple technology at its best. *BioScience* **54:** 447–454.

Glaudas X, Rodríguez-Robles JA. 2011. Vagabond males and sedentary females: spatial ecology and mating system of the speckled rattlesnake (*Crotalus mitchellii*). *Biological Journal of the Linnean Society* **103:** 681–695.

Gloyd HK, Conant R. 1990. *Snakes of the Agkistrodon complex: a monographic review*. Contributions in Herpetology 6. Oxford, OH: Society for the Study of Amphibians and Reptiles.

Gowan TA, McBrayer LD, Rostal DC. 2010. Seasonal variation in testosterone and performance in males of a non-territorial lizard species. *Physiology & Behavior* **100:** 357–363.

Greene HW, May PG, Hardy DL Sr, Sciturro JM, Farrell TM. 2002. Parental behavior by vipers. In: Campbell JA, Brodie ED Jr, eds. *Biology of the pitvipers*. Tyler, TX: Selva, 179–206.

Hau M. 2007. Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. *BioEssays* **29:** 133–144.

Higham TE, Irschick DJ. 2013. Springs, steroids, and slingshots: the roles of enhancers and constraints in animal movements. *Journal of Comparative Physiology B* **183:** 583–595.

Hooge PN, Eichenlaub W, Solomon E. 1999. *The animal movement program*. Anchorage, Alaska: USGS, Alaska Biological Science Center.

Husak JF, Fox SF. 2008. Sexual selection on locomotor performance. *Evolution Ecology Research* **10:** 213–228.

Husak JF, Fox SF, Lovern MB, Van Den Bussche RA. 2006. Faster lizards sire more offspring: sexual selection on whole-animal performance. *Evolution* **60:** 2122–2130.

Husak JF, Irschick DJ, McCormick SD, Moore IT. 2009. Hormonal regulation of whole-animal performance: implications for selection. *Integrative and Comparative Biology* **49:** 349–353.

Irschick DJ, Meyers JJ, Husak JF, Le Galliard JF. 2008. How does selection operate on whole-organism function performance capabilities? A review and synthesis. *Evolutionary Ecology Resources* **10:** 177–196.

Jellen BC, Aldridge RD. 2011. Paternity patterns. In: Aldridge RD, Sever DM, eds. *Reproductive biology and phylogeny of snakes, vol. 9*. Boca Raton, FL: CRC Press, 619–644.

Jellen BC, Shepard DB, Dreslik MJ, Phillips CA. 2007. Male movement and body size affect mating acquisition in the eastern massasauga (*Sistrurus catenatus*). *Journal of Herpetology* **41:** 451–457.

John-Alder HB, Cox RM, Haenel GJ, Smith LC. 2009. Hormones, performance and fitness: natural history and endocrine experiments on a lizard (*Sceloporus undulatus*). *Integrative and Comparative Biology* **49:** 393–407.

Jones AG. 2009. On the opportunity for sexual selection, the Bateman gradient and the maximum intensity of sexual selection. *Evolution* **63:** 1673–1684.

Kempenaers B, Peters A, Foerster K. 2007. Sources of individual variation in plasma testosterone levels. *Philosophical Transactions of the Royal Society B* **363:** 1711–1723.

Keogh JS, Noble DWA, Wilson EE, Whiting MJ. 2012. Activity predicts male reproductive success in a polygynous lizard. *PLoS ONE* **7:** e38856. doi:10.1371/journal.pone.0038856.

Ketterson ED, Nolan VJ. 1999. Adaptation, exaptation, and constraint: a hormonal perspective. *American Naturalist* **154:** S4–S25.

King RB. 2002. Family, sex and testosterone effects on garter snake behaviour. *Animal Behaviour* **64:** 345–359.

King RB, Bowden RM. 2013. Seasonal, condition-dependent, and individual variation in testosterone in a natricine snake. *Journal of Herpetology* **47:** 179–183.

King RB, Cline JH, Hubbard CJ. 2004. Heritable variation in testosterone levels in male garter snakes (*Thamnophis sirtalis*). *Journal of Zoology* **264:** 143–147.

Klose SM, Welbergen JA, Kalko EKV. 2009. Testosterone is associated with harem maintenance ability in free-ranging grey-headed flying foxes, *Pteropus poliocephalus*. *Biology Letters* **5:** 758–761.

Kokko H, Wong BBM. 2007. What determines sex roles in mate searching? *Evolution* **61:** 1162–1175.

Kuznetsova A, Brockhoff PB, Christensen RHB. 2014. *lmerTest: tests for random and fixed effects for linear mixed effect models (lmer objects of lme4 package). R package version 2.0-11*. Available at: http://CRAN.R-project.org/package=lmerTest

Levine BA, Smith CF, Schuett GW, Douglas MR, Davis MA, Douglas ME. 2014. Bateman-Trivers in the 21st century: sexual selection in a North American pitviper. *Biological Journal of the Linnean Society* **114:** 436–445.

Madsen T. 1984. Movements, activity range size and habitat use of radio-tracked grass snakes (*Natrix natrix*) in southern Sweden. *Copeia* **1984:** 707–713.

Madsen T, Shine R. 1994. Components of lifetime reproductive success in adders, *Vipera berus*. *Journal of Animal Ecology* **63:** 561–568.

Madsen T, Shine R, Loman J, Håkansson T. 1993. Determinants of mating success in male adders, *Vipera berus*. *Animal Behaviour* **45:** 491–499.

McGlothin JW, Whittaker DJ, Schrock SE, Gerlach NM, Jawor JM, Snajdr EA, Ketterson ED. 2010. Natural selection on testosterone production in a wild songbird population. *American Naturalist* **175:** 687–701.

Mobley KB, Jones AG. 2012. Overcoming statistical bias to estimate genetic mating systems in open populations. in open populations: a comparison of Bateman's principles between the sexes in a sex-role reversed pipefish. *Evolution* **67:** 646–660.

Neff BD, Knapp R. 2009. Paternity, parental behavior and circulating steroid hormone concentrations in nest-tending male bluegill. *Hormones and Behavior* **56:** 239–245.

Ouyang JQ, Sharp PJ, Dawson A, Quetting M, Hau M. 2011. Hormone levels predict individual differences in reproductive success in a passerine bird. *Proceedings of the Royal Society B* **278:** 2537–2545.

Prosser MR, Weatherhead PJ, Gibbs HL, Brown GP. 2002. Genetic analysis of the mating system and opportunity for sexual selection in northern water snakes (*Nerodia sipedon*). *Behavioral Ecology* **13:** 800–807.

R Core Team. 2014. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Available at: http://www.R-project.org/

Reinert HK. 1992. Radiotelemetric field studies of pitvipers: data acquisition and analysis. In: Campbell JA, Brodie ED Jr, eds. *Biology of the pitvipers*. Tyler, TX: Selva, 185–197.

Riechert J, Chastel O, Becker PH. 2012. Why do experienced birds reproduce better? Possible endocrine mechanisms in a long-lived seabird, the common tern. *General and Comparative Endocrinology*. **178:** 391–399.

Rivas JA, Burghardt GM. 2005. Snake mating systems, behavior, and evolution: the revisionary implications of recent findings. *Journal of Comparative Psychology* **119:** 447–454.

Row JR, Blouin-Demers G. 2006. Kernels are not accurate estimators of home range size for herpetofauna. *Copeia* **2006:** 797–802.

Schuett GW. 1992. Is long-term sperm storage an important component of the reproductive biology of temperate pitvipers? In: Campbell JA, Brodie ED Jr, eds. *Biology of the pitvipers*. Tyler, TX: Selva, 169–184.

Schuett GW. 1997. Body size and agonistic experience affect dominance and mating success in male copperheads. *Animal Behaviour* **54:** 213–224.

Schuett GW, Grober MS. 2000. Post-fight levels of plasma lactate and plasma corticosterone in male copperheads, *Agkistrodon contortrix* (Serpentes, Viperidae): differences between winners and losers. *Physiology and Behavior* **71:** 335–341.

Schuett GW, Grober MS, Van Kirk EA, Murdoch WJ. 2004b. Long-term sperm storage and plasma steroid profile of pregnancy in a western diamond-backed rattlesnake (*Crotalus atrox*). *Herpetological Review* **35:** 328–333.

Schuett GW, Hardy DL Sr, Greene HW, Earley RL, Grober MS, Van Kirk EA, Murdoch WJ. 2005. Sympatric rattlesnakes with contrasting mating systems show differences in seasonal patterns of plasma sex steroids. *Animal Behaviour* **70:** 257–266.

Schuett GW, Harlow HJ, Rose JD, Van Kirk EA, Murdoch WJ. 1997. Annual cycle of plasma testosterone in male copperheads, *Agkistrodon contortrix* (Serpentes, Viperidae): relationship to timing of spermatogenesis, mating, and agonistic behavior. *General and Comparative Endocrinology* **105:** 417–424.

Schuett GW, Repp RA, Amarello M, Smith CF. 2013. Unlike most vipers, female rattlesnakes (*Crotalus atrox*) continue to hunt and feed throughout pregnancy. *Journal of Zoology* **289:** 101–110.

Schuett GW, Repp RA, Hoss SK. 2011. Frequency of reproduction in female western diamond-backed rattlesnakes (*Crotalus atrox*) from the Sonoran Desert of Arizona is variable in individuals: potential role of rainfall and prey densities. *Journal of Zoology* **284:** 105–113.

Schuett GW, Repp RA, Taylor EN, DeNardo DF, Earley RL, Van Kirk EA, Murdoch WJ. 2006. Winter profile of plasma sex steroid levels in free-living male western diamond-backed rattlesnakes, *Crotalus atrox* (Serpentes: Viperidae). *General and Comparative Endocrinology* **149:** 72–80.

Schuett GW, Taylor EN, Van Kirk EA, Murdoch WJ. 2004a. Handling stress and plasma corticosterone levels in captive male western diamond-backed rattlesnakes (*Crotalus atrox*). *Herpetological Review* **35:** 229–233.

Seaman DE, Millspaugh JJ, Kerohan BJ, Brundige GC, Raedeke Gitzen RA. 1999. Effects of sample size on kernel activity range estimates. *Journal of Wildlife Management* **63:** 739–747.

Secor SM. 1992. A preliminary analysis of the movement and home range size of the sidewinder, *Crotalus cerastes*. In: Campbell JA, Brodie ED Jr, eds. *Biology of the pitvipers*. Tyler, TX: Selva, 389–393.

Secor SM. 1994. Ecological significance of movements and activity range for the sidewinder, *Crotalus cerastes*. *Copeia* **1994:** 631–645.

Shine R. 1994. Sexual size dimorphism in snakes revisited. *Copeia* **1994:** 326–346.

Shine R. 2003. Reproductive strategies in snakes. *Proceedings of the Royal Society of London Series B – Biological Sciences* **270:** 995–1004.

Shuster SM, Wade MJ. 2003. *Mating systems and strategies*. Princeton, NJ: Princeton University Press.

Sinervo B, Miles DB, Frankino A, Klukowski M, DeNardo DF. 2000. Testosterone, endurance, and Darwinian fitness: natural and sexual selection on the physiological bases of alternative male behaviors in side-blotched lizards. *Hormones and Behavior* **38:** 222–233.

Slip DJ, Shine R. 1988. The reproductive biology and mating system of diamond pythons, *Morelia spilota* (Serpentes: Boidae). *Herpetologica* **44:** 396–404.

Smith CF. 2007. Sexual dimorphism, and the spatial and reproductive ecology of the copperhead snake (*Agkistrodon contortrix*). Unpublished D. Phil. Thesis, University of Connecticut, USA.

Smith CF, Schuett GW, Earley RL, Schwenk K. 2009. The spatial and reproductive ecology of the copperhead (*Agkistrodon contortrix*) at the northeastern extreme of its range. *Herpetological Monographs* **23:** 45–73.

Smith CF, Schuett GW, Schwenk K. 2010. Relationship of plasma sex steroids to the mating season of copperheads (*Agkistrodon contortrix*) at the north-eastern extreme of their range. *Journal of Zoology* **280:** 362–370.

Smith CF, Schwenk K, Earley RL, Schuett GW. 2008. Sexual size dimorphism of the tongue in a North American pitviper. *Journal of Zoology* **274:** 367–374.

Taylor EN, DeNardo DF. 2011. Hormone and reproductive cycles in snakes. In: Norris DO, Lopez KH, eds. *Hormones and reproduction in vertebrates, vol. 2, reptiles*. San Diego, CA: Academic Press, 355–372.

Taylor EN, Schuett GW. 2004. Effect of temperature and storage duration on the stability of steroid hormones in blood samples from western diamond-backed rattlesnakes (*Crotalus atrox*). *Herpetological Review* **35:** 14–17.

Thornhill R, Alcock J. 1983. *The evolution of insect mating systems*. Cambridge, MA: Harvard University Press.

Tiebout HM, Carey JR. 1987. Dynamic spatial ecology of the water snake, *Nerodia sipedon*. *Copeia* **1987:** 1–18.

Trivers RL. 1972. Parental investment and sexual selection. In: Campbell B, ed. *Sexual selection and the descent of man, 1871-1971*. Chicago, IL: Aldine, 136–179.

Ursenbacher S, Erny C, Fumagalli L. 2009. Male reproductive success and multiple paternity in wild, low-density populations of the Adder (*Vipera berus*). *Journal of Heredity* **100:** 365–370.

Wade MJ. 1979. Sexual selection and variance in reproductive success. *American Naturalist* **114:** 742–747.

Wade MJ, Arnold SJ. 1980. The intensity of sexual selection in relation to male sexual behavior, female choice and sperm precedence. *Animal Behaviour* **28:** 446–461.

Waye HL, Mason RT. 2008. A combination of body condition measurements is more informative than conventional condition indices: temporal variation in body condition and corticosterone in brown tree snakes (*Boiga irregularis*). *General and Comparative Endocrinology* **155:** 607–612.

Weatherhead PJ, Prosser MR, Gibbs HL, Brown GP. 2002. Male reproductive success and sexual selection in northern water snakes determined by microsatellite DNA analysis. *Behavioral Ecology* **13:** 808–815.

Westneat DF, Stewart IRK. 2003. Extra-pair paternity in birds: causes, correlates, and conflicts. *Annual Review of Ecology Evolution and Systematics* **34:** 365–396.

Williams TD. 2008. Individual variation in endocrine systems: moving beyond the 'tyranny of the Golden Mean'. *Philosophical Transactions of the Royal Society B* **363:** 1687–1698.

Wingfield JC. 1984. Androgens and mating systems: testosterone-induced polygyny in normally monogamous birds. *Auk* **101:** 665–671.

Wolf M, Weissing FJ. 2012. Animal personalities: consequences for ecology and evolution. *Trends in Ecology and Evolution* **27:** 452–461.

Zamudio KR, Sinervo B. 2000. Polygyny, mate-guarding, and posthumous fertilization as alternative male mating strategies. *Proceedings of the National Academy of Sciences of the United States of America* **97:** 14427–14432.

Zar JH. 1999. *Biostatistical Analysis*, 4th edn. Upper Saddle River, NJ: Prentice Hall.

Zera AJ, Harshman LG, Williams TD. 2007. Evolutionary endocrinology: the developing synthesis between endocrinology and evolutionary genetics. *Annual Review of Ecology Evolution and Systematics* **38:** 793–781.