19

Evolution of Primary Sexual Characters in Reptiles

TOBIAS ULLER, DEVI STUART-FOX, AND MATS OLSSON

INTRODUCTION

Variation in sexual traits within and among species arises from past and current biotic and abiotic selective regimes, environmental conditions experienced during ontogeny, and developmental and phylogenetic constraints (Andersson 1994). Our aim with this review is to assess variation in primary sexual characters in relation to abiotic and biotic factors in reptiles. However, as a consequence of modern evolutionary biology, the main focus will be on processes directly targeting the size and function of gametes and their storage from a perspective of ongoing selection, that is mostly postcopulatory sexual selection. In order to do that without replicating previous work in this area, we review the literature with most emphasis on work published since 1997, that is, the year of submission of the last major review in this field (Olsson and Madsen 1998). Pre-1997 information is only considered if crucial for a more complete understanding of conceptual issues. We also deliberately minimize treatment of some areas that we think are still essentially up to date in Olsson and Madsen (1998), including hormone cycles and copulatory organs. A substantial part of the current review is also a phylogenetic analysis of variation in testis size lacking in Olsson and Madsen's (1998) descriptive presentation, now extended with additional data from the published literature since 1997.

To put our treatment into perspective, we start with a brief introduction into general reptilian reproductive biology.

A Brief Overview of Reptilian Biology

Reptilia (excluding birds) is a diverse group of ectotherm animals comprising Rhynchocephalians (tuataras), crocodilians, turtles, and squamates (lizards and snakes) (see Hedges & Poling 1999 and Townsend et al. 2004 for phylogenies). In most species, parental care of hatchlings is absent or rudimentary (Shine 1988) and males do not provide any direct resources to the female before, during, or after mating. Mating systems are generally categorized by intense male–male competition for females and, in many species, female or resource defense polygyny (e.g., Martins 1994; Shine 2003). Female mate choice on male quantitative traits has rarely been documented (Tokarz 1995; Olsson & Madsen 1995; but see, e.g., Lopez et al. 2003) and females frequently mate with more than one male within each ovarian cycle, both in captivity and in the wild (e.g., crocodilians: Davis et al. 2001; turtles: Pearse et al. 2002; Jensen et al. 2006; lizards: Zamudio & Sinervo 2000; Laloi et al. 2004; snakes: Schwartz et al. 1989; Prosser et al. 2002; reviewed in Uller & Olsson 2008). Such polyandrous mating is a prerequisite for the operation of postcopulatory sexual

selection (e.g., sperm competition and cryptic female choice).

Reproductive intervals range from days and weeks to years within lizards and turtles (e.g., Cogger 1978; Pearse & Avise 2001), whereas they are more consistently long (≥ 1 year) in snakes and crocodilians (e.g., Seigel & Ford 1987). Breeding normally occurs according to a seasonal pattern even in tropical species (e.g., James & Shine 1985; Seigel & Ford 1987). In most species of vertebrates, sperm production, insemination, and fertilization show close temporal association. In reptiles, however, and in particular in snakes, sperm production, insemination, and fertilization is frequently decoupled in time (reviewed in Duvall et al. 1982; Saint-Girons 1982; Crews 1984; Schuett 1992; Aldridge & Duvall 2002). This may have important implications for the evolution of male and female primary sexual traits for a number of reasons, for example, by generating selection on sperm storage and ejaculates. Clutch size ranges from one to over 50 in squamates (Fitch 1970), whereas turtles and crocodilians can lay more than 100 eggs in a single clutch (Greer 1975; Lutz & Musick 1996).

The weak evidence for wide-spread pre-copulatory mate choice (Tokarz 1995; Olsson & Madsen 1995), high incidence of multiple mating (Olsson & Madsen 1998; Uller & Olsson 2008), and female sperm storage (Sever & Hamlett 2002) suggests that post-copulatory sexual selection on male primary sexual traits, such as testis size and ejaculates should be strong in reptiles (Olsson & Madsen 1998). Similarly, in females, high multiple mating should strongly select for traits that facilitate post-copulatory paternity bias (cryptic female choice, Eberhard 1996) and may influence selective regimes on patterns of ovulation and sperm storage capacity. Many reptiles are thus highly suited for addressing the role of sexual selection for the evolution of primary sexual traits. Here, we provide an overview of our current understanding of primary sexual traits in reptiles and the evidence for sexual selection as a driving force in generating within- and among-species variation.

TESTIS SIZE AND SPERM PRODUCTION—A PHYLOGENETIC ANALYSIS

Sperm is produced in the testis and testis size in reptiles is maximal at the time of spermiogenesis,

suggesting that large testes are indicative of a high sperm production at the individual level (Licht 1984). Testis size is also sensitive to food intake and general male health (Olsson & Madsen 1998), which could provide a link between male phenotype and the size or quality of his ejaculate (at least when spermiogenesis and breeding are temporally associated). The link between testis size and sperm production at the intraspecific level could also lead to patterns at the inter-specific (or inter-population) level. More specifically, relative testis size is predicted to be positively related to the strength of sperm competition resulting from female polyandry (Short 1979; Møller & Briskie 1995; Parker et al. 1997; Birkhead & Møller 1998), which has been confirmed in both comparative (reviewed in Parker et al. 1997) and experimental (Hosken & Ward 2001) studies of other taxa. In reptiles, however, there has been no phylogenetically controlled test of this hypothesis. Furthermore, if production of sperm is energetically costly (Olsson et al. 1997), we may predict that species with a long breeding season would show lower peak testis mass compared to more "explosive breeders" where the cost is paid only during a brief period of time (Olsson & Madsen 1998).

Therefore, we conducted an analysis where we assessed the phylogenetically independent effects on relative testis size (Gonado-Somatic Index, GSI) of variables that we have reason to believe are related to sperm competition intensity or costs of sperm production (see Olsson & Madsen 1998): male–female synchrony of reproductive cycles (present vs. absent), and territoriality (present vs. absent), both of which could be related to the strength of sperm competition, for example, by adjusting the operational sex ratio and opportunity for multiple mating (Olsson & Madsen 1998); latitude (tropical vs. subtropical or temperate) and altitude (lowland or generalist vs. montane specialist), both related to the length of breeding season. Finally, we tested the effect of the mode of reproduction (viviparity vs. oviparity) since this is a fundamental aspect of reptilian life history with many potential carry-over effects on the strength of selection on both reproductive and non-reproductive traits (Shine 1983, 2005). The methods and results are summarized in Box 19.1.

Patterns of Testis Size in Reptiles

Our analyses provided only limited support for a relationship between testis size and intensity of

BOX 19.1 Methods and Results for a Phylogenetic Comparative Analysis of Testis Size in Reptiles

Methods

Because of differences of reporting relative testis size in the literature, we used two different indices as given in Olsson and Madsen (1998; taken directly from the literature when appropriate, or calculated from provided information in text and illustrations): (i) testis mass (g) divided by body mass (g) times 100, or (ii) testis mass (mg) divided by snout-vent length (mm). When testis mass was given as a volume, we converted volume to mass using the volume–mass relationship of ellipsoid testes in Swain and Jones (1994). When testis mass was given for one testis, we multiplied this figure by two. Thus, we ignored the possibility that the left and right testis may differ in size but any error in these estimates should be random across predictors. To ensure that the two indices of relative testis size were comparable, we first confirmed that there was no significant difference between the two (GSI index 1 N = 76, GSI index 2 N=72, $F_1 = 0.39$, $p = 0.54$) then standardized each to range between 0 and 1. We also assessed the ratio between the peak and trough GSI measurements (GSI ratio) when both were available for an annual cycle, to assess the possibility that seasonal variation in intensity of sperm competition selects for changes in testis size. All data are available from the authors upon request.

We compiled a phylogeny (Figure 19.1) for the 148 species in our dataset from recent published molecular and morphological phylogenies (see references) and assumed branches to be equal length for the purposes of the analysis. Comparative methods follow those in Ord and Stuart-Fox (2006) and Stuart-Fox and Moussalli (2007). Briefly, we used a Phylogenetic General Least Squares (PGLS) multiple regression model (Martins & Hansen 1997) with GSI or GSI ratio as the dependent variable with the following independent variables: snout-vent length (SVL), latitude, altitude, male–female synchrony, territoriality and mode of reproduction. Due to different sample sizes for different combinations of variables, we used the model selection procedure described in Purvis et al. (2000) to identify the best models. PGLS estimates a parameter α, which measures the extent of phenotypic variation across taxa that can be explained by phylogeny and subsequently controls for this effect in the model. If α is set to 0, results are identical to Felsenstein's (1985) independent contrasts (FIC), and when α is large (>15.5), it is equivalent to ignoring phylogeny (Tips). The parameter α can be interpreted as a measure of phylogenetic conservatism in the trait data (Martins & Hansen 1997). All analyses were carried out in COMPARE v4.6 (Martins 2004).

Results

The only significant predictor of GSI was latitude. Subtropical and temperate species had greater relative testis mass than tropical species (PGLS: $N = 125$, $r^2 = 0.05$, $p = 0.02$; Tips: $r^2 = 0.07$, $p < 0.001$). In non-phylogenetic analyses (Tips), territoriality was also a significant predictor of GSI in a multiple regression model with latitude ($N = 82$, model $r^2 = 0.15$, latitude $p < 0.001$ and territoriality $p = 0.02$), with territorial species tending to have greater GSI than non-territorial species. However territoriality was not a significant predictor in the equivalent PGLS model ($N = 82$, model $r^2 = 0.08$, latitude $p = 0.03$ and territoriality $p = 0.1$) because territoriality is highly conserved within genera and families. None of the other independent variables (altitude, SVL, male–female synchrony and mode of reproduction) predicted GSI. Similarly, none of the independent variables predicted the ratio of peak to trough relative testis size over an annual cycle. For most of the models we ran, α values were low to moderate, indicating that the variables are phylogenetically conservative.

(*Continued*)

BOX **19.1** (*Contd*)

FIGURE 19.1 Phylogeny used for comparative analysis of Gonado-Somatic Index (GSI). Sources used to construct the composite tree are given in Appendix 1. Polytomies were randomly resolved by inserting branch lengths of 0.00001. All other branch lengths were set to one.

sperm competition or length of breeding season. For example, we failed to identify any effect of territoriality on relative testis size when controlling for phylogeny. In fact, the only statistically significant pattern was larger testes in temperate regions, that is, where the mating season in general is more strictly defined. Because virtually all temperate zone reptiles in our data set have an associate reproductive pattern (i.e., maximum testis size and spermiogenesis co-occur with mating season; Crews 1984), we cannot with confidence separate the two effects at present. Thus, although the observed pattern may relate to a bias in the proportion of reproductively active males and females and therefore high scramble competition for fertilizations in temperate breeders, it could also arise because of a cost of maintaining large gonads through a prolonged mating season in tropical species (see Simmons & Emlen 2006). Cost of sperm production and testis size in reptiles include the direct energetic cost of ejaculate production (Olsson et al. 1997) but also costs associated with increased thermoregulation (Olsson et al. 1997; Herczeg et al. 2007). The latter should be relatively minor for tropical species, however.

Across a wide range of taxa, relative testis size has repeatedly been linked to mating system variation, with larger testis consistently being found in mating systems with more promiscuous females and hence stronger sperm competition (e.g., insects: Gage 1994; amphibians: Byrne et al. 2002; birds: Møller & Briskie 1995; mammals: Hosken 1998; early work summarized in Parker et al. 1997). Why do reptiles deviate from this pattern? One reason could be high phylogenetic conservatism of mating systems, which may be driven by coevolved neurosensory systems and reproductive traits. For instance, virtually all skinks are non-territorial mate guarders that rely on olfactory perception, whereas almost all iguanid and agamid species are visually orientated, territorial species. This reduces the power in a phylogenetically controlled analysis to dissect out the effect of mating system and associated effects. Furthermore, the classification scheme is necessarily coarse due to the limited data available from natural populations. Although both territoriality and breeding system (synchronous vs. asynchronous male and female reproductive cycles) have been suggested to co-vary with the strength of sperm competition, this may simply reflect our ignorance of factual patterns of female polyandry in the wild. For example, in contrast to the prediction that territorial species should have higher GSI

indices (Olsson & Madsen 1998), a negative relationship between territoriality and GSI is also plausible. Both pair-bonding skinks (Australian genera *Egernia* and *Tiliqua*) and highly territorial Australian agamids have lower multiple paternity than non-territorial lizards (Uller & Olsson 2008), suggesting that successful guarding of partners, and hence reduced sperm competition, could arise via multiple routes. More direct estimates may be obtained via studies of multiple mating and multiple paternity. Both are common in turtles and squamates (Olsson & Madsen 1998; Uller & Olsson 2008) but the overlap between species for which we have data on multiple mating, paternity and testis size is too low at present to allow a phylogenetically robust analysis. Furthermore, variation among populations in multiple mating and paternity, possibly arising from variation in operational sex ratios, is almost as high as the total interspecific variation for some species [e.g., multiple paternity ranges from 30 to 92% in Olive ridley sea turtles, *Lepidochelys olivacea* (Jensen et al. 2006) and 17 to 80% in adders, *Vipera berus* (Höggren 1995); Uller & Olsson 2008]. This suggests that species averages may not be informative, in particular for small sample sizes, and that robust tests will be difficult to generate. Similarly, despite our attempts to minimize errors in several ways, large intra-annual variation in testis size and problems with combining data obtained in different ways may introduce errors in the data set to significantly reduce the reliability of the results (Calhim & Birkhead 2007).

Finally, from a selection perspective, it may simply be that testis size and its correlate sperm production are relatively unimportant traits in sperm competition in reptiles. For example, under prolonged sperm storage (see below), scramble competition models of sperm competition do not capture the complexities of sperm survival in the female reproductive tract. Indeed, there is a growing awareness that other ejaculate characteristics than sperm number or sperm concentration may be just as, or even more, important in predicting fertilization success in polyandrous species (e.g., sperm longevity, sperm size, ejaculate composition; Snook 2005).

EJACULATES

Fertilization is internal in all reptiles. Thus, selection acting on sperm and ejaculate composition

arises from natural selection on sperm morphology and physiology due to (i) the environment of the female reproductive tract, (ii) temporal differences between ejaculation and fertilization, (iii) spatial differences between the site of insemination and the site of fertilization, and sexual selection arising via (iv) the presence of ejaculates from multiple males competing for fertilization, and (v) sexual conflict over fertilization. The first three categories are undisputed and could explain certain characteristics of both semen (such as the presence of antioxidants; Breque et al. 2003) and sperm (such as swimming ability; Snook 2005). These are undoubtedly of importance also in reptiles. However, given that multiple mating in reptiles is widespread with few exceptions (Olsson & Madsen 1998; Uller & Olsson 2008), post-copulatory sexual selection is also likely to mold ejaculate traits. We provide an overview of ejaculate composition and sperm morphology and discuss to what extent variation among taxa could be explained by natural selection (in particular temporal separation of sperm production, copulation, and fertilization) or post-copulatory sexual selection.

Seminal Fluids and Copulatory Plugs

Ejaculates of vertebrates and invertebrates contain a variety of fluids and substances that can increase sperm longevity, facilitate sperm storage, increase fertilization success under sperm competition, and manipulate female re-mating or reproductive investment (see Gillott 2003; Poiani 2006; Ram & Wolfner 2007 for reviews). In squamates, seminal fluid is produced by the epididymis and the renal sexual segment (RSS), a hypertrophied region of the distal urinary ducts (Fox 1977; Sever & Hopkins 2005). Both hypertrophy of the RSS and number and densities of sexual granules in the cytoplasm show seasonal variation that closely correspond to circulating levels of plasma testosterone and testicular action (Fox 1977; Krohmer 2004; Sever & Hopkins 2005). This implies a role for the RSS during spermiation and mating (at least in species with associated breeding cycles), suggesting that the primary role of the RSS is to provide seminal fluids during copulation. However, the exact functions of the RSS are not well understood. It has been suggested to produce courtship pheromones (Volsøe 1944; Devine 1975), sustain and activate sperm (e.g., Cuellar 1966), and form material for copulatory plugs (Volsøe 1944; Devine 1975) (see Fox 1977; Sever & Hamlett 2002; Sever & Hopkins 2005 for overviews). Although there is currently little evidence for sperm activation and nutrition, studies of garter snakes (*Thamnophis* spp.) have shown that seminal fluids (although not necessarily of RSS origin) have both pheromonal action and form copulatory plugs (Devine 1975; Ross & Crews 1977, 1978; Shine et al. 2000).

Copulatory plugs are more or less thick gelatinous structures that form in the female cloaca at the end of copulation subsequent to sperm transfer (Volsøe 1944; Devine 1975; Fox 1977; Shine et al. 2000). They are quite common in mammals and insects, but have also been described in several species of snakes and lacertid lizards (Devine 1984; in den Bosch 1994). Copulatory plugs were initially believed to prevent sperm from leaking out of the cloaca (Volsøe 1944; Fox 1977), a role that would benefit both males and females. However, with the advent of studies of intra- and intersexual conflict (Parker 1970; Trivers 1972; Arnqvist & Rowe 2005), copulatory plugs have increasingly been viewed as manipulative tools by which copulating males ensure their own reproductive success while compromising that of competing males and potentially that of the female (enforced chastity; Shine et al. 2000). This outcome could be achieved in three ways: first, by serving as physical plugs preventing successful hemipenis intromission and sperm transfer by other males; second, by producing pheromones that makes females unattractive to other males or suppress female re-mating; or third, by reducing the competitive ability of sperm from other males. Initial studies of garter snakes supported both the physical barrier and pheromone hypotheses (Ross & Crews 1977, 1978; Devine 1984) but subsequent work has suggested that, although pheromones from the seminal fluids reduce female attractiveness, this is largely independent of the plug itself (Shine et al. 2000).

Although logical, there are two problems with plugs as physical barriers. First, subsequent studies on garter snakes have found a high incidence of multiple paternity (Schwartz et al. 1989), suggesting that copulatory plugs are ultimately inefficient as chastity belts. However, this conclusion relies on the assumption that multiple paternity arises primarily from multiple mating within a mating season and not via sperm storage across seasons (see below). Furthermore, in *Thamnophis sirtalis*, plugs were shown to be effective for two days after

the initial mating, which may still confer sufficient benefits to be under selection (Shine et al. 2000). Second, studies of the Iberian rock lizard (*Lacerta monticola*) have shown that female re-mating probability and male intromission is not prevented by the presence of a copulatory plug (Moreira & Birkhead 2003). In fact, second mating males have a higher fertilization success when they mate 30 minutes after the first males, when the plug is still intact, than when mating 4 h after the first mating, when the plug has started to disintegrate (Moreira et al. 2007). This suggests that the mating plug contains sperm that are actively dislodged by second males and, consequently, that the plug has evolved as a means of sperm transfer rather than to prevent female remating. Furthermore, copulatory plugs may be used as a means of assessing presence or absence of sperm competition and rival quality (Olsson et al. 2004; Moreira et al. 2006), which can explain highly sophisticated patterns of ejaculate allocation by males that further increase the fertilization success of second mated males. In sand lizards, relatedness to the female predicts paternity under sperm competition (Olsson et al. 1996). Male sand lizards adaptively adjust copula duration (and hence sperm transfer) in relation to the previous male's relatedness with his female, possibly via pheromonal cues obtained from the copulatory plug (Olsson et al. 2004). Finally, using vasectomized males (that only can transfer RSS secretions and no sperm), Olsson et al. (1994b) showed that seminal fluid itself does not reduce the probability of paternity for subsequently mating rivals. Thus, there is little direct evidence that seminal fluids have evolved via postcopulatory sexual selection in reptiles.
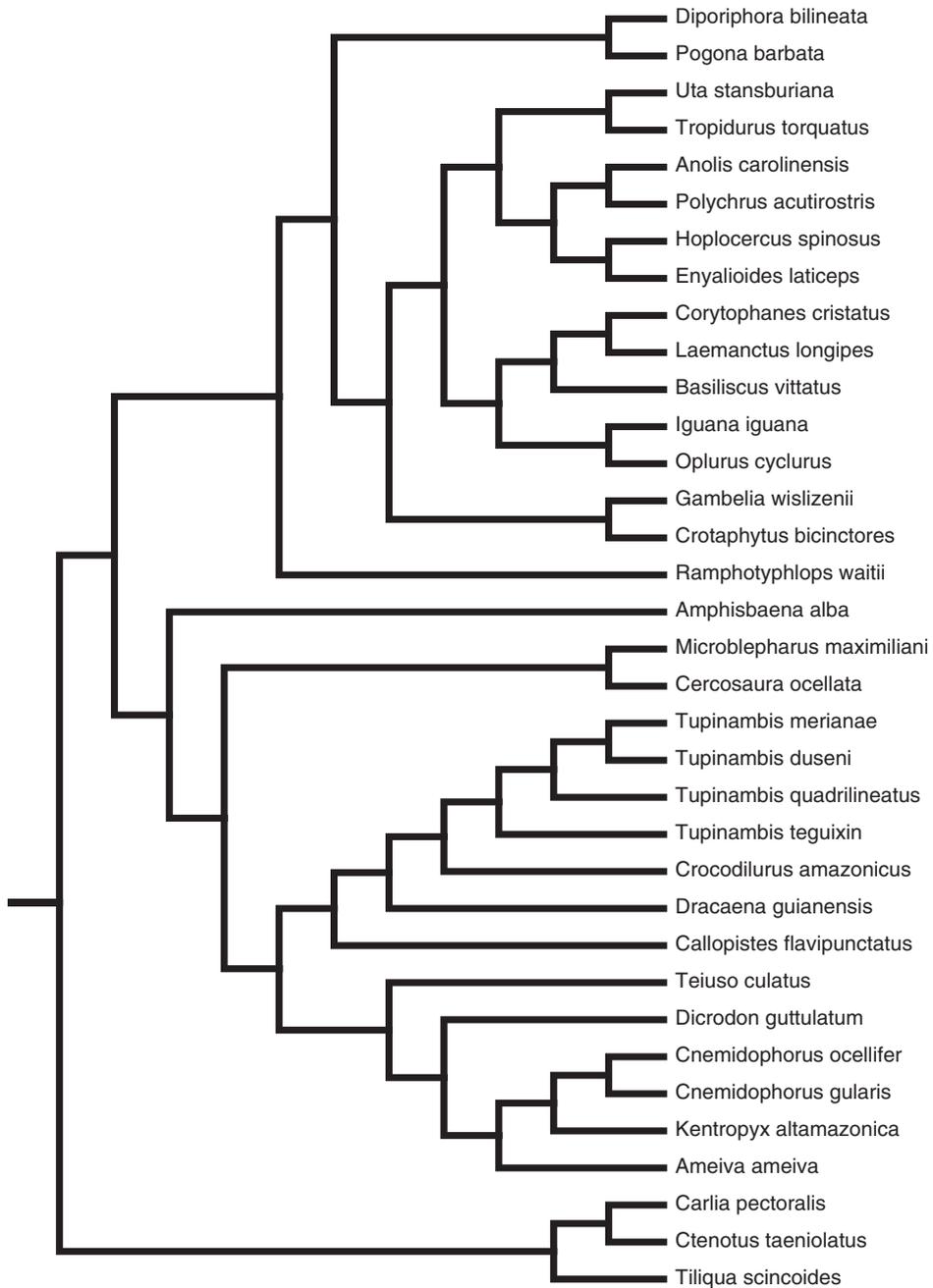
Sperm Morphology

Sperm of squamates (Furieri 1970; Jamieson 1995), turtles (Furieri 1970; Hess et al. 1991), crocodilians (Jamieson et al. 1997), and tuatara (Jamieson & Healey 1992) show a typical morphology, with a distinct head, midpiece and tail, similar to that of mammals, birds, and amphibians. There is no evidence for polymorphism in sperm morphology or the presence of non-fertilizing sperm as is commonly found in invertebrates (Swallow & Wilkinson 2002). Differences between major taxonomic groups in sperm ultrastructure mainly arise from variation in the structure of the acrosome and midpiece (Furieri 1970), but minor differences can be found also among closely related species and have

been suggested to provide important phylogenetic information (reviewed in Jamieson 1995; Vieira et al. 2007).

Sperm morphometrics (i.e., the size of different parts) may be selected both via co-evolution with female reproductive traits (e.g., the female reproductive tract; Miller & Pitnick 2002; Anderson et al. 2006), or via intrasexual selection (e.g., via sperm competition, Gage 1994; Briskie et al. 1997; Byrne et al. 2003; see Parker 1998 for theoretical overview). For example, flagellum length may be under positive selection due to selection for increased velocity (Gage 1998; Malo et al. 2006) and midpiece size may be under selection via an increase in mitochondrial number or size, thereby generating greater power output (Cardullo & Baltz 1991; Anderson et al. 2005; Immler et al. 2007). Thus, under strong sperm competition, both flagellum and midpiece length are predicted to increase, which could lead to a positive relationship between sperm length and intensity of sperm competition unless there is a trade-off between sperm size and number (Gage 1994; Byrne et al. 2003; Gomendio et al. 2007 but see Gage & Freckleton 2003; Garcia-Gonzalez & Simmons 2007). However, under prolonged sperm storage, small sperm, and in particular a small midpiece, may be favored if it enhances sperm longevity (Immler & Birkhead 2007; Immler et al. 2007).

Of the total 49 reptilian taxa for which sperm morphology could be obtained, only seven were species for which we also had information on gonadosomatic index, preventing an analysis of the relationship between sperm morphology and this index of the strength of postcopulatory sexual selection. In total, we found information on midpiece and flagellum length for 36 squamate taxa (figure 19.2; Appendix 2). Sperm lengths range from approximately 20 μm in some crododilians (Ferguson 1985) to 170 μm in the blindsnake *Ramphotyphlops waitii* (Harding et al. 1995). Within lizards, the group from which the majority of data derives, total sperm length ranges from 28.5 μm in the bearded dragon, *Pogona barbata* (Olivier et al. 1996), to 98.8 μm in the leopard lizard, *Gambelia wislizenii* (Vieira et al. 2007).

After controlling for phylogenetic relationships among species (figure 19.2; see Box 19.1), there was a significant allometric relationship between midpiece and flagellum length (table 19.1). This suggests co-evolution between kinetic (flagellum length) and energetic (midpiece size) aspects of sperm morphology among reptiles, similarly to

FIGURE 19.2 Phylogeny used for comparative analysis of sperm morphology. Sources used to construct the composite tree are given in Appendix 1. All branch lengths were set to one.

TABLE **19.1** Correlation between lengths of different parts of sperm (head, midpiece and flagellum)

Variables	N	α	PGLS		Tips	
			r	p	r	p
Head and midpiece	30	1.64	–0.18	0.33	–0.1	0.61
Head and flagellum	30	2.41	0.30	0.10	0.42	0.01
Midpiece and flagellum	35	3.82	0.60	<0.0001	0.53	<0.0001

PGLS = Phylogenetic generalized least squares, Tips = non-phylogenetic analysis. Alpha is a measure of phylogenetic conservatism (see methods). The values of alpha are low, indicating that sperm morphometrics are phylogenetically conserved. *Boa constrictor* was removed from analyses because it was an outlier (due to its unusually long midpiece).

results for mammals and birds (Gage 1998; Immler et al. 2007). However, the underlying reason for allometric relationships cannot be inferred from these data and robust conclusions regarding sperm morphology and their relation to the strength of sexual selection should await accumulation of data from more species. In an attempt to provide a starting point for further research, we document here some existing patterns of sperm morphometry in relation to reptilian mating systems.

Boa constrictor has one of the longest reptilian sperm (Tourmente et al. 2006). Furthermore, it has a greatly elongated mid-piece (approx 10 times longer than in lizards). This suggests that sperm competition is relatively intense in this species. Strong sperm competition in snakes is also supported by their mating systems (Duvall et al. 1993; Shine 2003), high levels of multiple paternity (e.g., Schwartz et al. 1989; Uller & Olsson 2008), hemipene morphology (Keough 1999), large relative testis size and long copulation times (Olsson & Madsen 1998). However, comparable data on sperm morphometrics for other snake species and the convergent legless lizards are currently lacking (Appendix 19.2; but see Hamilton & Fawcett 1968; Teixeira et al. 1999a; Tavares-Bastos et al. 2007).

In the turtle, *Chrysemys picta*, mitochondria are unusually laminated, which has been suggested to be an adaptation for prolonged survival in the female oviduct during storage (Hess et al. 1991). Prolonged sperm storage in both the female reproductive tract and in the male epididymis commonly occurs also in snakes with dissociated reproductive cycles (Saint-Girons 1982; Aldridge & Duvall 2002; see below), which makes this group well suited for addressing the role of mitochondrial reorganization in adaptation to prolonged sperm storage.

In addition to the large variation among species, there is also often substantial variation among males in sperm and ejaculate traits within species (e.g., Harris et al. 2007). Of particular interest is the link between mating strategies and ejaculate composition. For example, small males that are less competitive for access to females or territories may be under stronger post-copulatory sexual selection and, for example, transfer more sperm per ejaculate than larger males (Parker 1990). This was supported by a study of northern watersnakes (*Nerodia sipedon*) in which Schulte-Hostedde and Montgomerie (2006) found that smaller males produced ejaculates with a higher sperm concentration than did larger males, which may compensate for a lower mating frequency in terms of paternity success (Weatherhead et al. 2002; see also Olsson et al. 2009). Most traits (sperm velocity, spermatocrit) showed substantial variation among ejaculates. However, sperm length was largely invariable, suggesting stabilizing postcopulatory sexual selection on sperm length in *N. sipedon*. To what extent these results reflect more general intraspecific variation in ejaculate composition in snakes and other reptiles remains to be investigated (see Calhim et al. 2007 for a comparative study of passerine birds). Importantly, ejaculates can be obtained using non-invasive techniques (Schulte-Hostedde & Montgomerie 2006), which provides exciting scope for combining descriptive studies of ejaculate traits with experimental manipulations of, for example, mating opportunities.

Sperm Motility

Sperm motility could be important in scramble competition for fertilization (Birkhead et al. 1999). In ectotherms, female body temperature should have the potential to adjust sperm motility subsequent to mating, possibly serving as a behavioral mechanism for cryptic female choice via female basking. However, in the turtle *Chrysemys picta*,

sperm motility was highest at low temperatures, suggesting local adaptation of sperm motility in relation to the decreasing ambient temperatures during the timing of copulation in this species (Gist et al. 2000).

Although a high sperm motility can be favored under situations of sperm competition, low motility could be favored in species with substantial sperm storage (Gist et al. 2000). This may be supported by the lower motility of turtle sperm compared to lizards (Depeiges & Dacheux 1985; Gist et al. 2000), although the lack of data makes this hypothesis largely suggestive. However, in *C. picta*, 70% of sperm remained viable after 40 days in vitro, suggesting strong selection on sperm viability in species with prolonged storage of sperm in the male or female reproductive systems (Gist et al. 2000, 2002).

FEMALE SPERM STORAGE

Storage of sperm in the female reproductive tract is an important aspect of the reproductive biology of both invertebrates and vertebrates (Howarth 1974; Neubaum & Wolfner 1999). In reptiles, hatching or parturition is restricted to the warmer parts of the year, which means that ovulation and embryonic development normally occurs in spring and summer, respectively. However, sperm production also requires sufficiently high temperatures, which can lead to a decoupling of male and female reproductive cycles when there is insufficient time in spring for sperm production (Licht 1984; Aldridge & Duvall 2002). Despite the frequent decoupling of sperm production, mating and fertilization, long-term female sperm storage is not particularly common in reptiles (Saint-Girons 1982; Duvall et al. 1982; Schuett 1992; Aldridge & Duvall 2002; Murphy et al. 2006). Instead, sperm is commonly stored in the male reproductive tract for long periods of time (e.g., Gist et al. 2002) and mating normally occurs only weeks or a few months before fertilization.

Classification of sperm storage in female reptiles is not straightforward (see Saint-Girons 1982; Schuett 1992; Aldridge & Duvall 2002 for different schemes). For example, sperm storage could be classified according to whether it occurs within ovarian cycles or across cycles (i.e., from matings that occurred before oviposition of the previous clutch). However, for each of these two categories, sperm may be subject to either short-term sperm storage (STSS; days to weeks) or long-term sperm storage (LTSS; months to years). Furthermore, sperm storage may occur within a female ovarian cycle, but across mating periods (as is the case when there is both an autumn and a spring mating season and sperm is stored from autumn until fertilization in spring–summer; Schuett 1992; Murphy et al. 2006). Finally, very long-term sperm storage (VLTSS; many years; i.e., for a period of time longer than both the female ovarian cycle and across mating seasons) has been documented in some species. Thus, although a classification based on absolute time is artificial since the characterization of "long" and "short" may not be related to the life cycle of the species, characterizations based on reproductive cycles are also problematic as male and female cycles can be decoupled in time. Here, we will therefore be explicit with respect to the absolute time of sperm storage and its relationship to male and female reproductive cycles without attempting further classification or introduction of new terminology (see Schuett 1992; Aldridge & Duvall 2002; for useful discussions).

Short term sperm storage within ovarian cycles is probably common in many reptiles as insemination often occurs over a period of time before completion of vitellogenesis. For example, in the European adder, *Vipera berus*, sperm from spring matings is stored in the female reproductive tract for weeks or even months before fertilization occurs in late spring–early summer (Saint-Girons 1982). To some extent, such STSS should have evolved to be flexible, as the temporal dissociation of mating and egg production will vary as a result of environmental conditions, such as annual variation in temperature and food availability. Thus, even in species that do not normally have to store sperm for more than a few weeks, sperm may remain viable for longer if females are prevented from egg production.

Long-term sperm storage has been documented in many species of snakes (Duvall et al. 1982; Schuett 1992; Almeida-Santos & da Graca Salomão 2002), turtles (Pearse & Avise 2001; Pearse et al. 2001) and some lizards (Smyth & Smith 1968; Wapstra et al. 1999; Murphy et al. 2006) and is necessary when the mating season is separated from egg production for long periods of time, such as in species with autumn matings and spring vitellogenesis and ovulation. However, as pointed out by Saint-Girons (1982), most snakes with autumn

matings also mate subsequent to hibernation, suggesting that sperm storage over hibernation is not necessary to ensure fertilization. Nevertheless, at least in some American pitvipers, mating in spring may not occur at all (Schuett 1992; Almeida-Santos & da Graca Salomão 2002). Long-term sperm storage resulting from temporal separation of mating and fertilization seems to be rarer in lizards, maybe as a result of the generally shorter reproductive cycle of lizards compared to snakes (Licht 1984; but see Wapstra et al. 1999; Murphy et al. 2006).

Very long term sperm storage (> 1 year) is known from snakes and turtles. The longest reported storage of viable sperm in the female reproductive tract is seven years in a file snake (*Achrochordus arafurae;* Magnusson 1979) and storage for more than three years has repeatedly been recorded in turtles (Sever & Hamlett 2002). Importantly, sperm storage for such long periods of time will extend across mating seasons and, hence, mating opportunities and, in most cases, also extend across female reproductive cycles. However, although data on female fertility in the absence of males suggests VLTSS, previous anecdotal reports of facultative virgin births in squamates have recently been substantiated using molecular verification of offspring genotype (Groot et al. 2003; Watts et al. 2006). Thus, reports of VLTSS in female reptiles should be treated with caution until facultative parthenogenesis can be ruled out (Schuett 1992). However, VLTSS in snakes does not seem unlikely, given that females of many species reproduce infrequently (e.g., Bull & Shine 1979).

Natural and Sexual Selection on Female Sperm Storage

As noted above, STSS and LTSS within ovarian cycles are common in many reptiles and have probably initially evolved via natural selection to ensure fertilization. Nevertheless, presence of sperm storage will generate strong post-copulatory sexual selection on males as it increases the intensity of sperm competition (provided that females mate multiply; Olsson & Madsen 1998). In some species, sperm competition has led to male strategies to prevent female remating, such as mate guarding (Olsson et al. 1996), whereas in others it has led to changes in ejaculate composition (see above). More importantly from the female perspective, however, is that an increase in the number of ejaculates

present in the reproductive tract at the timing of fertilization would select for mechanisms that ensure fertilization of sperm that is 'optimal' with respect to genetic composition (either because of high genetic quality or complementarity; Madsen et al. 1992; Olsson et al. 1996; Birkhead & Pizzari 2002).

Although sperm storage from mating to fertilization is obligate in species with temporal separation between the two, it is more difficult to explain why females store sperm across ovarian cycles or across mating seasons (table 19.2). Surprisingly, very few studies have addressed the role of sperm storage across ovarian cycles for male and female reproductive success, although evidence from two species (*Ctenophorus pictus* and *Uta stansburiana*), suggest that it can lead to significant changes in selection on male mating strategies (Zamudio & Sinervo 2000; Olsson et al. 2007a, 2009). Early workers suggested that female sperm storage across reproductive events has evolved as a strategy to ensure fertilization when access to new sperm is uncertain (e.g., Connor & Crews 1980). At low population densities, for example, mate encounter rates may not be sufficiently high to ensure mating during each ovarian cycle. Storage of sperm would thereby prevent females from being sperm limited. Furthermore, some species can have a two-phased mating season, one in autumn and one in spring (e.g., Schuett 1992; Wapstra et al. 1999; Murphy et al. 2006). Storage of sperm from autumn matings may ensure that high-quality females can progress rapidly through ovulation in spring, without wasting time and energy on mating, in particular under high male harassment (see Løvlie & Pizzari 2007 for a similar scenario). Thus, it could allow earlier oviposition or parturition, traits that are likely to be favored in temperate-zone reptiles (e.g., Olsson & Shine 1997a; Warner & Shine 2007). Despite these two widely inferred selective pressures, there is virtually no evidence that female sperm storage has evolved because it ensures fertilization of eggs in the absence of males. Such evidence would require showing that (i) mate encounter rates are sufficiently low to cause sperm depletion under natural conditions and (ii) females that store sperm have a higher fitness (or at least fertilization success) than female that do not store sperm. Species with both autumn and spring matings would be ideal candidates for an experimental approach to address this issue. For example, manipulation of the operational sex ratio, perceived and actual

TABLE **19.2** Hypotheses for the evolution of female sperm storage in reptiles

Hypothesis	Comment	References
Consequence of selection for optimal timing of birth or parturition	Likely in many snakes and turtles with selection against mating in spring, but cannot explain sperm storage across ovarian cycles.	Saint Girons 1982; Schuett 1992
Consequence of selection for optimal timing of spermiogenesis	Could lead to female sperm storage if selection favors summer or autumn mating, but is unlikely to explain storage across ovarian cycles.	Saint-Girons 1982
Fertilization insurance under low mating encounter rate	Possible in some turtles and snakes with low population densities. Unlikely in most lizards.	Phillipp 1979; Conner & Crews 1980
Fertilization insurance under risk of male infertility	Unlikely as male infertility should be highest in early emerging males with sperm that is not yet mature.	Olsson & Madsen 1996
Reduction in copulation frequency when copulation is costly	Unlikely in species with annual mating seasons. Little evidence that females reject males when receptive.	Conner & Crews 1980
Allowing cryptic female choice of viable sperm via passive sperm loss	Possible, but no direct evidence.	Olsson & Madsen 1998
Allowing cryptic female choice via manipulation of ejaculates	Possible but no direct evidence. May be unlikely considering that sperm storage organs are relatively undifferentiated compared to, for example, insects.	Olsson & Madsen 1998
Selection on sperm to remain viable across ovarian cycles	Possible. Evidence that stored sperm contribute to male reproductive success in two short-lived species with multiple clutches.	Zamudio & Sinervo 2000; Olsson et al. 2007

mate encounter, and monitoring of female reproductive behavior, sperm storage and paternity could shed light on the extent to which female sperm storage is flexible and contingent upon mate encounter rates.

If sperm storage across reproductive cycles has not evolved in response to sperm depletion, what can explain its presence in so many reptiles? One explanation that has been favored by many authors is that sperm storage facilitates cryptic female choice (Olsson and Madsen 1998). For example, in some insects, females may have control over the entry of sperm into sperm storage organs via muscular contractions (Simmons 2001) and, in redback spiders, sperm storage organs facilitate discrimination among ejaculates (Snow & Andrade 2005). Thus, the morphology and position of sperm storage organs may yield important information regarding the potential for female control.

Morphology of Sperm Storage and its Implications

The reptilian oviduct consists of the vagina, uterus, isthmus, tuba (or uterine tube) and infundibulum (Girling 2002). The main difference between squamates, turtles and crocodilians is that the latter two have a much longer tuba region containing tubular glands, which release albumen during egg formation (Gist & Jones 1987). During copulation, sperm is deposited in the cloaca and subsequently reaches the vagina. Sperm is eventually transferred to the tuba or infundibulum where fertilization takes place (Saint-Girons 1975; Girling 2002). Whether sperm transport occurs via muscular contraction of the oviduct or via sperm movement is unknown, although both hypotheses are plausible (Saint-Girons 1975; Halpert et al. 1982).

Sperm storage occurs in receptacles (tubules) in the vagina and infundibulum in squamates, whereas it is confined to the tuba and uterus in chelonians (Fox 1956, 1963; Gist & Jones 1987, 1989; Gist & Congdon 1998; Girling 2002; Sever & Hamlett 2002). Over-winter sperm storage has also been reported to occur via uterine muscular twisting (Yamanouye et al. 2004). The ancestral state for squamates is infundibular sperm storage tubules, and vaginal sperm storage is less common (Sever & Hamlett 2002; Sever & Hopkins 2004). Regardless of the position of the sperm storage

tubules, they seem to be unspecialized structures that show little cytological difference from those that lack sperm (e.g., Gist & Fischer 1993; Sever & Ryan 1999; Gist & Jones 1987; Girling 2002; Sever & Hamlett 2002). Sperm are present in bundles or arranged head-first towards the luminal wall. In squamates, sperm heads are in close contact with the epithelium (Cuellar 1966), whereas this does not seem to be the case in turtles (Gist & Jones 1987). Many authors have suggested that sperm receive nourishment from the oviduct (in particular in squamates; Cuellar 1966; Conner & Crews 1980; Halpert et al. 1982), but there is little direct evidence for this (Gist & Jones 1987; Olsson & Madsen 1998; Girling 2002; Sever & Hamlett 2002).

The available data on morphology of sperm storage tubules in reptiles suggest a much lower level of sophistication than that of insects and birds (Bakst 1987; Pitnick et al. 1999; Simmons 2001). Indeed, Gist & Jones (1987) concluded that the receptacles containing sperm are indistinguishable from normal glands in the area and recent evidence support this conclusion (Sever & Hamlett 2002). A notable exception, however, is that sperm storage tubules in *Anolis sagrei* lack cilia and secretory products at the place of sperm concentration (Sever & Hamlett 2002; see also Conner & Crews 1980), an anatomy similar to that in birds (Bakst 1987), suggesting selection on female sperm storage. Interestingly, in contrast to other lizards but similar to birds, Anoles produce one egg at a time but have a prolonged period of egg laying (Smith et al. 1972), which may select for sperm storage to avoid the need for remating between eggs (Sever & Hamlett 2002). Indeed, sperm storage for up to two months has been shown in *Anolis sagrei* (Calsbeek et al. 2007; see also Conner & Crews 1980) and variation in paternity among eggs is common (Calsbeek et al. 2007).

Given that specialized sperm storage tubules are not found in most reptiles, selection on female sperm storage across reproductive events may be weak in most reptiles. Consequently, it is unknown whether sperm storage can contribute to, or is a result of selection for, cryptic female choice. Furthermore, there is no current evidence for co-evolution of sperm length and size of sperm receptacles (as may be the case in birds; Briskie & Montgomerie 1993) although this may simply be due to lack of attention. Instead, prolonged sperm

survival in the female reproductive tract may be a (near) neutral trait for female fitness, suggesting that it may evolve simply because of strong selection on male sperm viability, in particular in species with a rapid turn-over of ovarian cycles. If so, males may differ in their ability to produce sperm that survive in the oviduct of females and, hence, variation in this trait and the timing of mating may strongly affect reproductive success in species with multiple clutches (Oring et al. 1992; Zamudio & Sinervo 2000; Olsson et al. 2009), with potential consequences for offspring development (e.g., offspring sex: Olsson et al. 2007b). Documenting the anatomy and physiology of sperm storage together with patterns of parentage in (related) species that differ in key aspects of life history (such as associated vs. dissociated reproductive cycles and clutch interval) should be a research priority for the future.

CLUTCH SIZE AND OVULATION PATTERNS

Follicular growth in single-clutched reptiles either begins in early spring with ovulation in late spring or starts in summer or autumn (directly subsequent to oviposition or birth) and is completed the following spring (with the exception of a few viviparous species; Licht 1984). The first situation is common in lizards and snakes and the second in turtles. Ovulation is autochronic (i.e., synchronous among follicles) in the vast majority of reptile species (Licht 1984). However, in anoles, ovulation of one egg alternates between the ovaries (allochrony), leading to the presence of multiple eggs at different stages in the female reproductive tract at a given point in time, similar to the situation in birds (Smith et al. 1973). The ultimate reason for this peculiar reproductive system is unknown. Nevertheless, it may increase the scope for female control of sex-specific resource allocation (Uller 2006; Uller et al. 2007) and cryptic female choice of sperm (Calsbeek et al. 2007). Anoles would therefore be a suitable model system in which to address how an evolutionary shift in ovulation patterns may lead to changes in the direction and strength of sexual selection on both primary and secondary sexual traits.

Variation in clutch size is normally addressed from a perspective of natural selection. Indeed, there are a number of studies on reptiles that show

that clutch size is under selection to maximize lifetime reproductive output via the trade-off between offspring size and number and a negative effect of reproductive effort on female survival (e.g., Schwartzkopf 1994; Bonnet et al. 2002). However, evidence from other taxa suggests that sexual selection may also be important, primarily via two routes: First, males of some insects may be able to increase female reproductive output either via resource provisioning (e.g., nuptial gifts, Vahed 1998) or via manipulation of female oviposition rate by transfer of proteins in seminal fluids (Ram & Wolfner 2007). Second, increased sexual dimorphism can select for reduced clutch size because of increased demands on production of the larger sex (Carranza 1996) or to reduce the temporal and spatial overlap between offspring of different resource demands (Uller 2006, Badyaev et al. 2006; Kuhl et al. 2007). The latter is perhaps particularly likely to occur in viviparous animals (Uller 2003, 2006). In a comparative study of 106 species of mammals, Carranza (1996) found a negative relationship between litter size and sexual size dimorphism. We suggest that similar patterns also exist in squamate reptiles. The large number of independent evolutionary shifts from oviparity to viviparity provides further opportunities to test whether the relationship is stronger in species with a higher degree of developmental overlap between the sexes.

Nuptial gifts do not occur in reptiles and male manipulation of female clutch size has not been documented and may be unlikely considering that females normally oviposit all their eggs simultaneously. Furthermore, in some species (primarily capital breeders), reproductive allocation decisions may be inflexible at the time of mating (e.g., Bonnet et al. 2001). In contrast, a study of the sand lizard, *Lacerta agilis*, suggested that females adjust their reproductive investment in relation to partner quality by increasing their reproductive output when mated to males with large sexual ornaments (Olsson et al. 2005). As this study manipulated male ornamentation independently of male quality, it suggests that females are able to differentially allocate resources in relation to perceived male quality (Sheldon 2000).

CONSTRAINTS AND CO-EVOLUTION

Current research in sexual selection on primary sexual traits emphasizes the dynamic role of co-evolution between males and females (Arnqvist & Rowe 2005; Andersson & Simmons 2006; Parker 2006). Thus, an evolutionary response to selection in one of the sexes is likely to generate a correlated response in the other (even in the absence of genetic correlations). For example, the evolution of female sperm storage creates an arena for sperm competition and may select for increased sperm transfer, changes in seminal fluid composition, sperm morphology, and sperm longevity. Reptiles with female sperm storage are therefore key candidates for studies of sperm competition and the evolution of male primary reproductive traits. Furthermore, sperm storage has the potential to have a strong impact on selection on mating strategies and secondary sexual traits in males, for example, by increasing the benefits of copulations early in the breeding season in species with multiple clutches (Olsson et al. 2007a).

We argue that an understanding of the evolution of male and female primary sexual characteristics in reptiles is facilitated by an appreciation of the importance of environmental constraints on reproduction and the co-evolution of male and female traits (figure 19.3). Strong selection on matching embryonic development and spermiogenesis to benign environmental conditions has frequently led to a decoupling of male and female reproductive cycles (Licht 1984; Aldridge & Duvall 2002), which dictates the framework in which post-copulatory sexual selection can occur (figure 19.3). For example, it may lead to differences in the timing of emergence from hibernation (Olsson & Madsen 1996; Olsson et al. 1999), with potential impact on selection on male–male competition, female choice, and fitness of both sexes via changes in the operational sex ratio. Risk of mating with infertile males (with immature sperm) may also partly explain high levels of female promiscuity in some species (Olsson & Madsen 1996; Olsson & Shine 1997b; Uller & Olsson 2006). Second, the decoupling of sperm production from mating activities and fertilization reduces or even eliminates the link between peak testosterone levels, maximum testis size, sperm number and quality, and male phenotype at mating (Licht 1984; Duvall et al. 1982; Olsson & Madsen 1998; Murphy et al. 2006). This modifies the cost of reproduction in males and could have important consequences for the application of certain pre- and post-copulatory sexual selection scenarios (Crews 1984; Birkhead & Møller 1993), such as the immunocompetence handicap hypothesis,
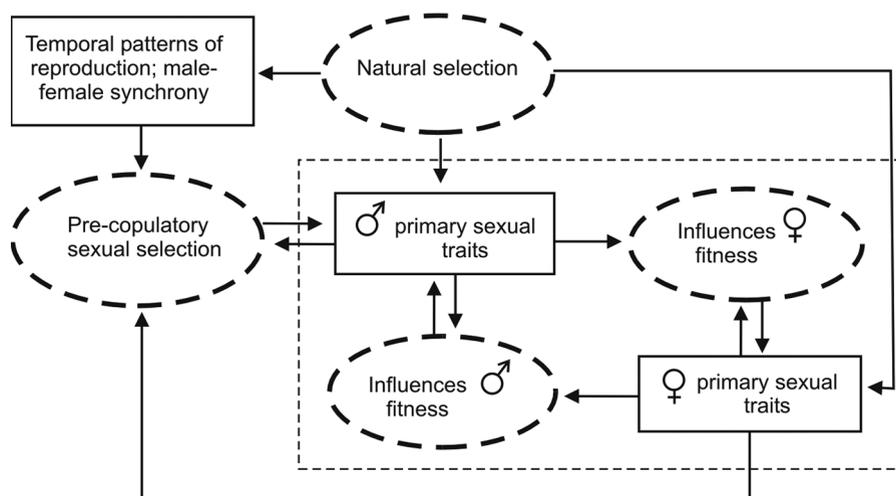
FIGURE 19.3 Evolutionary processes (dashed ovals) influencing male and female primary sexual traits (black rectangles). Male primary sexual traits include testis size, sperm morphology, seminal fluids and copulatory plugs. Female primary sexual traits include characteristics of the female reproductive tract, especially in relation to sperm storage as well as clutch size and ovulation patterns. Thin black dashed rectangle indicates processes of post-copulatory sexual selection and male–female coevolution.

which proposes a trade-off between testosterone-dependent sexual traits and immune function. Furthermore, environmental effects on mating systems may explain why peak testis mass is larger in associate temperate zone reptiles. Specifically, a prolonged or dissociated mating season may change the rules by which ejaculates compete for fertilization, for example, by reducing the importance of rapid and high sperm production in favor of other aspects of ejaculate composition and sperm longevity (Snook 2005; Dean et al. 2007; Pizzari et al. 2008). Finally, sperm is frequently stored for several weeks and storage across ovarian cycles in short-lived species with multiple clutches has been documented (Olsson et al. 2007a,b; Calsbeek et al. 2007). However, the lack of evolution of sophisticated sperm storage organs despite prolonged storage of sperm (Sever & Hamlett 2002) suggests a relatively minor role of the female reproductive tract for sperm survival and viability. This could imply that sperm storage serves as a filter to ensure fertilization of males that are able to produce highly viable and long-lived sperm, perhaps thereby accruing genetic benefits for the offspring. Furthermore, the presence of a simple sperm storage system suggests that cryptic female choice for genetically compatible males may occur primarily via gamete

interactions (Swanson & Vacquier 2002; see Madsen et al. 1992; Olsson et al. 1994a, 1996; Olsson & Madsen 2001 for discussion in reptiles). Ultimately, disentangling the selective causes for the observed variation among species will require studies of the fitness consequences of variation in male and female primary sexual characters in natural populations. Furthermore, as primary sexual traits are unlikely to evolve in isolation of secondary traits, we advocate an integrative whole organism approach, something for which many reptiles are uniquely suited (Lailvaux & Irschick 2006; Irschick et al. 2007).

SUMMARY: SEXUAL SELECTION AND PRIMARY SEXUAL TRAITS IN REPTILES

Evidence for sexual selection on male and female primary reproductive traits is strong in many taxa as evident from the contributions to this volume. In reptiles, however, the evidence is currently more circumstantial than direct. The scarcity of studies directly addressing this issue makes it difficult to evaluate to what extent this reflects a relatively minor effect or simply a lack of attention. However, the

ubiquitous occurrence of multiple mating and multiple paternity strongly suggests that postcopulatory sexual selection should be strong in general in reptiles (Olsson & Madsen 1998; Uller & Olsson 2008). Our analyses of relative testis size found a significant relationship with climate, testis size being larger in temperate zone species. This may be an effect of a more intense sperm competition or a reduced cost of sperm production compared to tropical species with a prolonged breeding season.

In snakes, the available data suggest that copulatory plugs prevent intromission by foreign males or, alternatively, reduce female attractiveness via pheromonal cues. In lizards, however, there is no evidence that copulatory plugs prevent remating and plugs are more likely to serve as a source of sperm being taken up by females subsequently to mating. To what extent this allows cryptic female choice via biased uptake or ejection of sperm as has been described in fowl (Pizzari & Birkhead 2000), remains to be addressed.

Postcopulatory sexual selection on sperm should also be strong in reptiles. Comparative data do not yet allow direct tests of theory but the variation among investigated species suggests that this is a promising research approach. Within-species, among-individual variation in ejaculate and sperm traits and their relation to fertilization success under sperm competition have rarely been investigated and likewise deserve greater attention. The documented non-random variation in fertilization success (e.g., in relation to genetic similarity, Olsson et al. 1996) and wide distribution of sperm storage, suggest that sperm–female co-evolution (Miller & Pitnick 2002) should occur.

Sperm storage over periods extending the time from mating to fertilization seems to be common in female reptiles and is unlikely to be explained by selection to ensure fertilization. Surprisingly, however, there are virtually no studies that directly address the extent to which sperm storage facilitates cryptic female choice or its consequences for fertilization success among males (but see Zamudio & Sinervo 2000; Calsbeek & Sinervo 2004; Olsson et al. 2007a, b; 2009). However, evidence from an agamid lizard that stored sperm gives rise to a larger proportion of sons compared to recently inseminated sperm suggests that sperm storage could relate to sex allocation strategies (Olsson et al. 2007b).

TABLE 19.3 Suggested questions that could be addressed in future work on reptiles using a combination of field and laboratory experiments, molecular paternity assignment, and comparative methods

Testis size and sperm production/allocation

- What are the relative importance of sperm number, sperm morphology, and sperm longevity for male reproductive success under sperm competition? Does this differ for associate and dissociate breeders?
- Is sperm allocation facultative in relation to the level of sperm competition and environmental conditions (e.g., mate availability) and are there consistent differences between males (e.g., sneaks versus territorial males)?
- What is the cost of sperm production and are the relatively smaller testes of tropical species due to higher costs in prolonged breeders?

Sperm and ejaculates

- Is sperm competition important for the evolution of sperm morphology and longevity?
- Do ejaculates and female reproductive systems co-evolve, for example, does midpiece morphology and sperm aging relate to presence of sperm storage at the interspecific level?
- To what extent does dissociate mating systems select for changes in ejaculates and their relation to primary sexual characters?

Sperm storage

- Can sperm storage facilitate cryptic female choice via biased use of sperm from different males?
- Does sperm storage lead to differences in the strength and direction of post-copulatory sexual selection, for example by favoring increased sperm longevity over sperm numbers?
- What are the consequences of sperm storage for the strength of pre- and post-copulatory sexual selection and male mating strategies?

Clutch size and ovulation patterns

- Do females adjust their reproductive effort in relation to male traits and to what extent does capital breeding constrain adaptive allocation?
- Does sequential ovulation facilitate cryptic female choice of sperm and differential allocation?

Furthermore, the resulting selective pressures on male sexual traits, including ejaculate composition, sperm number and morphology, suggest that studies of species with sperm storage could generate valuable insights into the evolution of ejaculates and male and female co-evolution.

CONCLUDING REMARKS

Our survey of the literature on primary sexual traits in reptiles shows that there is an exciting scope for addressing key issues in sexual selection using reptilian model systems, both via within- and among-species studies. However, it also reveals our ignorance of the proximate mechanisms and selective forces behind the observed variation. We therefore outline some of the routes that seem to us the most interesting and feasible to take over the next ten years (figure 19.3; table 19.3).

Reptiles generated important insights in the early days of sexual selection studies (Noble & Bradley 1933; Trivers 1976; Stamps 1977) and have continued to generate insights into postcopulatory phenomena (e.g., Madsen et al. 1992; Olsson et al. 1996). However, we would argue that reptilian systems are being underutilized with respect to our understanding of the evolution of primary sexual traits. The field has been invigorated with novel conceptual and experimental approaches regarding both inter- and intraspecific variation over the last two decades (e.g., Arnqvist & Rowe 2005; Snook 2005). Thus, a concerted research agenda using reptilian model systems could add to our understanding of the evolution of gametes and their storage and provide important steps towards an integration of proximate and ultimate levels of explanation in post-copulatory sexual selection.

REFERENCES

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

Almeida-Santos, S. M. & Graça Salomão, M. 2002. Reproduction in Neotropical Pivipers, with Emphasis on Species of the Genus *Bothrops*. In: Biology of Vipers. (Ed. by G. W. Schuett, M. Höggren, M. E. Douglas & H. W. Greene), pp. 445–462, Eagle Mountain, UT: Eagle Mountain Publishing.

Anderson, M. J., Nyholt, J. & Dixson, A. F. 2005. Sperm competition and the evolution of sperm midpiece volume in mammals. Journal of Zoology, 267, 135–142.

Anderson, M. J., Dixson, A. S. & Dixson, A. F. 2006. Mammalian sperm and oviducts are sexually selected: evidence for co-evolution. Journal of Zoology, 270, 682–686.

Andersson, M. 1994. Sexual Selection. Princeton: Princeton University Press.

Andersson, M. & Simmons, L. W. 2006. Sexual selection and mate choice. Trends in Ecology & Evolution, 21, 296–302.

Arnqvist, G. & Rowe, L. 2005. Sexual Conflict. Princeton: Princeton University Press.

Badyaev, A. V., Seaman, D. A., Navara, K. J., Hill, G. E. & Mendonca, M. T. 2006. Evolution of sex-biased maternal effects in birds: III. Adjustment of ovulation order can enable sex-specific allocation of hormones, carotenoids, and vitamins. Journal of Evolutionary Biology, 19, 1044–1057.

Bakst, M. R. 1987. Anatomical basis of sperm-storage in the avian oviduct. Scanning Microscopy, 1, 1257–1266.

Birkhead, T. R. & Møller, A. P. 1993. Sexual selection and the temporal separation of reproductive events—sperm storage data from reptiles, birds and mammals. Biological Journal of the Linnean Society, 50, 295–311.

Birkhead, T. R. & Møller, A. P. 1998. Sperm Competition and Sexual Selection. New York: Academic Press.

Birkhead, T. & Pizzari, T. 2002. Postcopulatory sexual selection. Nature Reviews Genetics, 3, 262–273.

Birkhead, T. R., Martinez, J. G., Burke, T. & Froman, D. P. 1999. Sperm mobility determines the outcome of sperm competition in the domestic fowl. Proceedings of the Royal Society of London, Series B, 266, 1759–1764.

Bonnet, X., Naulleau, G., Shine, R. & Lourdais, O. 2001. Short-term versus long-term effects of food intake on reproductive output in a

viviparous snake (*Vipera aspis*). Oikos, 92, 297–308.

Bonnet, X., Lourdais, O., Shine, R. & Naulleau, G. 2002. Reproduction in a typical capital breeder: Costs, currencies, and complications in the aspic viper. Ecology, 83, 2124–2135.

Breque, C., Surai, P. & Brillard, J. P. 2003. Roles of antioxidants on prolonged storage of avian spermatozoa in vivo and in vitro. Molecular Reproduction and Development, 66, 314–323.

Briskie, J. V. & Montgomerie, R. 1993. Patterns of sperm storage in relation to sperm competition in passerine birds. Condor, 95, 442–454.

Briskie, J. V., Montgomerie, R. & Birkhead, T. R. 1997. The evolution of sperm size in birds. Evolution, 51, 937–945.

Bull, J. J. & Shine, R. 1979. Iteroparous animals that skip opportunities for reproduction. American Naturalist, 114, 296–303.

Byrne, P. G., Roberts, J. D. & Simmons, L. W. 2002. Sperm competition selects for increased testes mass in Australian frogs. Journal of Evolutionary Biology, 15, 347–355.

Byrne P. G., Simmons, L. W. & Roberts, J. D. 2003. Sperm competition and the evolution of gamete morphology in frogs. Proceedings of the Royal Society of London, Series B, 270, 2079–2086.

Calhim, S. & Birkhead, T. R. 2007. Testes size in birds: quality versus quantity—assumptions, errors, and estimates. Behavioral Ecology, 18, 271–275.

Calhim, S., Immler, S. & Birkhead, T. R. 2007. Postcopulatory sexual selection is associated with reduced variation in sperm morphology. PLoS ONE, 5, e413.

Calsbeek, R. & Sinervo, B. 2004. Within-clutch variation in offspring sex determined by differences in sire body size: cryptic mate choice in the wild. Journal of Evolutionary Biology, 17, 464–470.

Calsbeek, R., Bonneaud, C., Prabhu, S., Manoukis, N. & Smith, T. B. 2007. Multiple paternity and sperm storage lead to increased genetic diversity in Anolis lizards. Evolutionary Ecology Research, 9, 495–503.

Cardullo, R. A. & Baltz, J. M. 1991. Metabolic-regulation in mammalian sperm—mitochondrial volume determines sperm length and flagellar beat frequency. Cell Motility and the Cytoskeleton, 19, 180–188.

Carranza, J. 1996. Sexual selection for male body mass and the evolution of litter size in mammals. American Naturalist, 148, 81–100.

Cogger, H. G. 1978. Reproductive-cycles, fat-body cycles and socio sexual behavior in the mallee dragon, Amphibolurus-fordi (Lacertilia-Agamidae). Australian Journal of Zoology, 26, 653–672.

Colli, G. R., Teixeira, R. D., Scheltinga, D. M., Mesquita, D. O., Wiederhecker, H. C. & Bao, S. N. 2007. Comparative study of sperm ultrastructure of five species of teiid lizards (Teiidae, Squamata), and Cercosaura ocellata (Gymnophthalmidae, Squamata). Tissue & Cell, 39, 59–78.

Conner, J. & Crews, D. 1980. Sperm Transfer and Storage in the Lizard, *Anolis-carolinensis*. Journal of Morphology, 163, 331–348.

Crews, D. 1984. Gamete production, sex hormone secretion, and mating behavior uncoupled. Hormones and Behavior, 18, 22–28.

Cuellar, O. 1966. Oviducal anatomy and sperm storage structures in lizards. Journal of Morphology, 119, 7–19.

Davis, L. M., Glenn, T. C., Elsey, R. M., Dessauer, H. C. & Sawyer, R. H. 2001. Multiple paternity and mating patterns in the American alligator, *Alligator mississippiensis*. Molecular Ecology, 10, 1011–1024.

Dean, R., Bonsall, M. B. & Pizzari, T. 2007. Aging and sexual conflict. Science, 316, 383–384.

Depeiges, A. & Dacheux, J. L. 1985. Acquisition of sperm motility and its maintenance during storage in the lizard, Lacerta-vivipara. Journal of Reproduction and Fertility, 74, 23–27.

Devine, M. C. 1975. Copulatory plugs in snakes: Enforced chastity. Science, 187, 844–845.

Devine, M. C. 1977. Copulatory plugs, restricted mating opportunities, and reproductive competition among male garter snakes. Nature, 267, 345–346.

Devine, M. C. 1984. Potential for sperm competition in reptiles: Behavioral and physiological consequences. In: Sperm Competition and the Evolution of Mating Systems (Ed. by R. L. Smith), pp. 509–521. New York: Academic Press.

Duvall, D., Guillette L. J. & Jones, R. E. 1982. Environmental control of reptilian reproductive cycles. In: Physiology and Physiological Ecology: Biology of the Reptilia, (Ed. by

C. Gans & F. H. Pough), pp. 201–231. New York: Wiley.

Duvall, D., Schuett, G. W. & Arnold, S. J. 1993. Ecology and evolution of snake mating systems. In: Snakes: Ecology and Behavior (Ed. by R. A. Seigel & J. T. Collins), pp. 165–200. New York: McGraw-Hill.

Eberhard, W. G. 1996. Femal Control: Sexual Selection by Cryptic Female Choice. Princeton: Princeton University Press.

Felsenstein, J. 1985. Phylogenies and the comparative method. American Naturalist, 125, 1–15.

Ferguson, M. W. J. 1985. Reproductive biology and embryology of the Crocodilians. In: Biology of the Reptilia, Vol 14. Development A (Ed. by A. F. Billett & P. F. A. Madserson), pp. 329–491. New York: John Wiley & Sons.

Fitch, H. S. 1970. Reproductive Cycles in Lizards and Snakes. Miscellaneous Publication 52, University of Kansas Museum of Natural History.

Fox, W. 1956. Seminal receptacles in snakes. The Anatomical Record, 124, 415–450.

Fox, W. 1963. Special tubules for sperm storage in female lizards. Nature, 198, 500–501.

Fox, H. 1977. The urogenital system of reptiles. In: Biology of the Reptilia. Vol. 6, Morphology (Ed. by C. Gans & T. S. Parsons), pp. 1–157. New York: Academic Press.

Furieri, P. 1970. Sperm morphology of some reptiles: Squamata and Chelonia. In: Comparative Spermatology (Ed. by B. Baccetti), pp. 115–131. New York: Academic Press.

Gage, M. J. G. 1994. Associations between body-size, mating pattern, testis size and sperm lengths across butterflies. Proceedings of the Royal Society of London, Series B, 258, 247–254.

Gage, M. J. G. 1998. Influences of sex, size, and symmetry on ejaculate expenditure in a moth. Behavioral Ecology, 9, 592–597.

Gage, M. J. G. & Freckleton, R. P. 2003. Relative testis size and sperm morphometry across mammals: No evidence for an association between sperm competition and sperm length. Proceedings of the Royal Society of London, Series B, 270, 625–632.

Garcia-Gonzalez, F. & Simmons, L. W. 2007. Shorter sperm confer higher competitive fertilization success. Evolution, 61, 816–824.

Gillott, C. 2003. Male accessory gland secretions: Modulators of female reproductive physiology and behavior. Annual Review of Entomology, 48, 163–184.

Girling, J. E. 2002. The reptilian oviduct: A review of structure and function and directions for future research. Journal of Experimental Zoology, 293, 141–170.

Gist, D. H. & Congdon, J. D. 1998. Oviductal sperm storage as a reproductive tactic of turtles. Journal of Experimental Zoology, 282, 526–534.

Gist, D. H. & Fischer, E. N. 1993. Fine-structure of the sperm storage tubules in the box turtle oviduct. Journal of Reproduction and Fertility, 97, 463–468.

Gist, D. H. & Jones, J. M. 1987. Storage of sperm in the reptilian oviduct. Scanning Microscopy, 1, 1839–1849.

Gist, D. H. & Jones, J. M. 1989. Sperm storage within the oviduct of turtles. Journal of Morphology, 199, 379–384.

Gist, D. H., Turner, T. W. & Congdon, J. D. 2000. Chemical and thermal effects on the viability and motility of spermatozoa from the turtle epididymis. Journal of Reproduction and Fertility, 119, 271–277.

Gist, D. H., Dawes, S. M., Turner, T. W., Sheldon, S. & Congdon, J. D. 2002. Sperm storage in turtles: A male perspective. Journal of Experimental Zoology, 292, 180–186.

Giugliano, L. G., Teixeira, R. D., Colli, G. R. & Bao, S. N. 2002. Ultrastructure of spermatozoa of the lizard Ameiva ameiva, with considerations on polymorphism within the family Teiidae (Squamata). Journal of Morphology, 253, 264–271.

Gomendio, M., Malo, A. F., Garde, J. & Roldan, E. R. S. 2007. Sperm traits and male fertility in natural populations. Reproduction, 134, 19–29.

Greer, A. E. 1975. Clutch size in crocodilians. Journal of Herpetology, 9, 319–322.

Groot, T. V. M., Bruins, E. & Breeuwer, J. A. J. 2003. Molecular genetic evidence for parthenogenesis in the Burmese python, *Python molurus bivittatus*. Heredity, 90, 130–135.

Halpert, A. P., Garstka, W. R. & Crews, D. 1982. Sperm transport and storage and its relation to the annual sexual cycle of the female red-sided garter snake, *Thamnophis sirtalis parietalis*. Journal of Morphology, 174, 149–159.

Hamilton, D. W. & Fawcett, D. W. 1968. Unusual features of neck and middle-piece of snake spermatozoa. Journal of Ultrastructure Research, 23, 81–97.

Harding, H. R., Aplin, K. P. & Mazur, M. 1995. Ultrastructure of spermatozoa of Australian blindsnakes, Ramphotyphlops spp. (Typhlopidae, Squamata): first observations on the mature spermatozoon of scolecophidian snakes. In: Advances in Spermatozoal Phylogeny and Taxonomy (Ed. by Jamieson, B. G. M., Ausio, J. & Justine, J. L.) Mém. Mus. Natn. Hist. Nat., 166, 385–396. Paris

Harris, W. E., Moore, A. J. & Moore, P. J. 2007. Variation in sperm size within and between ejaculates in a cockroach. Functional Ecology, 21, 598–602.

Hedges, S. B. & Poling, L. L. 1999. A molecular phylogeny of reptiles. Science, 283, 998–1001.

Herczeg, G., Saarikivi, J., Abigel, G., Perälä, J., Toumola, A. & Merilä, J. 2007. Suboptimal thermoregulation in male adders (*Vipera berus*) after hibernation imposed by spermiogenesis. Biological Journal of the Linnean Society, 92, 19–27.

Hess, R. A., Thurston, R. J. & Gist, D. H. 1991. Ultrastructure of the turtle Spermatozoon. The Anatomical Record, 229, 473–481.

Hoggren, M. 1995. Mating strategies and sperm competition in the adder (*Vipera berus*). PhD Thesis, Uppsala University.

Hosken, D. J. 1998. Testes mass in megachiropteran bats varies in accordance with sperm competition theory. Behavioral Ecology and Sociobiology, 44, 169–177.

Hosken, D. J. & Ward, P. I. 2001. Experimental evidence for testis size evolution via sperm competition. Ecology Letters, 4, 10–13.

Howarth, B. 1974. Sperm storage: as a function of the female reproductive tract. In: The Oviduct and its Functions (Ed. by A. D. Johnson & C. W. Foley), pp. 237–270. New York: Academic Press.

Immler, S. & Birkhead, T. R. 2007. Sperm competition and sperm midpiece size: no consistent pattern in passerine birds. Proceedings of the Royal Society of London, Series B, 274, 561–568.

Immler, S., Saint-Jalme, M., Lesobre, L., Sorci, G., Roman, Y. & Birkhead, T. R. 2007. The evolution of sperm morphometry in pheasants. Journal of Evolutionary Biology, 20, 1008–1014.

in den Bosch, H. A. J. 1994. First record of mating plugs in lizards. Amphibia-Reptilia, 15, 89–93.

Irschick, D. J., Herrel, A., Vanhooydonck, B. & Van Damme, R. 2007. A functional approach to sexual selection. Functional Ecology, 21, 621–626.

James, C. & Shine, R. 1985. The seasonal timing of reproduction - a tropical-temperate comparison in Australian lizards. Oecologia, 67, 464–474.

Jamieson, B. G. M. 1995. The ultrastructure of spermatozoa of the Squamata (Reptilia) with phylogenetic considerations. In: Advances in Spermatozoal Phylogeny and Taxonomy (Ed. by Jamieson, B. G. M., Ausio, J. & Justine, J. L.). Mem. Mus. Natn. Hist. Nat. 166, pp. 359–383.

Jamieson, B. G. M. & Healy, J. M. 1992. The phylogenetic position of the Tuatara, Sphenodon (Sphenodontida, Amniota), as indicated by cladistic-analysis of the ultrastructure of spermatozoa. Philosophical Transactions of the Royal Society of London, Series B, 335, 207–219.

Jamieson, B. G. M. & Scheltinga, D. M. 1994. The ultrastructure of spermatozoa of the Australian skinks, *Ctenotus taeniolatus*, *Carlia pectoralis* and *Tiliqua scincoides scincoides* (Scincidae, Reptilia). Memoirs of the Queensland Museum, 37, 181–193.

Jamieson, B. G. M., Scheltinga, D. M. & Tucker, A. D. 1997. The ultrastructure of spermatozoa of the Australian freshwater crocodile, *Crocodylus johnstoni* Krefft, 1873 (Crocodylidae, Reptilia). Journal of Submicroscopic Cytology and Pathology, 29, 265–274.

Jensen, M. P., Abreu-Grobois, F. A., Frydenberg, J. & Loeschcke, V. 2006. Microsatellites provide insight into contrasting mating patterns in arribada vs. non-arribada olive ridley sea turtle rookeries. Molecular Ecology, 15, 2567–2575.

Keogh, J. S. 1999. Evolutionary implications of hemipenial morphology in the terrestrial Australian elapid snakes. Zoological Journal of the Linnean Society, 125, 239–278.

Krohmer, R. W. 2004. Variation in seasonal ultrastructure of sexual granules in the renal sexual segment of the Northern Water Snake, *Nerodia sipedon sipedon*. Journal of Morphology, 261, 70–80.

Kuhl, A., Mysterud, A., Erdnenov, G. I., Lushchekina, A. A., Grachev, I. A., Bekenov, A. B. & Milner-Gulland, E. J. 2007. The 'big spenders' of the steppe: sex-specific maternal allocation and twinning in the saiga antelope. Proceedings of the Royal Society London, Series B, 274, 1293–1299.

Lailvaux, S. P. & Irschick, D. J. 2006. A functional perspective on sexual selection: insights and future prospects. Animal Behaviour, 72, 263–273.

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

Licht, P. 1984. Reproductive cycles of vertebrates. Reptiles. In: Marshall's Physiology of Reproduction vol 1 (Ed. by G. E. Lamming), pp 206–282. New York: Churchill Livingstone.

Lopez, P., Aragon, P. & Martin, J. 2003. Responses of female lizards, *Lacerta monticola*, to males' chemical cues reflect their mating preference for older males. Behavioral Ecology and Sociobiology, 55, 73–79.

Lovlie, H. & Pizzari, T. 2007. Sex in the morning or in the evening? Females adjust daily mating patterns to the intensity of sexual harassment. American Naturalist, 170, E1–E13.

Lutz, P. L. & Musick, J. A. 1996. The Biology of Sea Turtles, vol I. Boca Raton, FL: CRC Press.

Madsen, T., Shine, R., Loman, J. & Håkansson, T. 1992. Why do female adders copulate so frequently? Nature, 355, 440–441.

Magnusson, W. E. 1979. Production of an embryo by an *Achrochordus javanicus* isolated for seven years. Copiea, 1979, 744–745.

Malo, A. F., Gomendio, M., Garde, J., Lang-Lenton, B., Soler, A. J. & Roldan, E. R. S. 2006. Sperm design and sperm function. Biology Letters, 2, 246–249.

Martins, E. P. 1994. Phylogenetic perspectives on the evolution of lizard territoriality. In: Lizard Ecology—Historical and Experimental Perspectives (Ed. by L. J. Vitt & E. R. Pianka), pp. 117–144. Princeton: Princeton University Press.

Martins, E. P. 2004. COMPARE 4.6: statistical analysis of comparative data, version 4.6. Department of Biology, Indiana University, Bloomington.

Martins, E. P. & Hansen, T. F. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. American Naturalist, 149, 646–667.

Miller, G. T. & Pitnick, S. 2002. Sperm–female coevolution in *Drosophila*. Science, 298, 1230–1233.

Møller, A. P. & Briskie, J. V. 1995. Extra-pair paternity, sperm competition and the evolution of testis size in birds. Behavioral Ecology and Sociobiology, 36, 357–365.

Moreira, P. L. & Birkhead, T. R. 2003. Copulatory plugs in the Iberian Rock Lizard do not prevent insemination by rival males. Functional Ecology, 17, 796–802.

Moreira, P. L., Lopez, P. & Martin, J. 2006. Femoral secretions and copulatory plugs convey chemical information about male identity and dominance status in Iberian rock lizards (*Lacerta monticola*). Behavioral Ecology and Sociobiology, 60, 166–174.

Moreira, P., Nunes, V., Martin, J. & Paulo, O. S. 2007. Copulatory plugs do not assure high first male fertilisation success: sperm displacement in a lizard. Behavioral Ecology and Sociobiology, 62, 281–288.

Murphy, K., Hudson, S. & Shea, G. 2006. Reproductive seasonality of three cold-temperate viviparous skinks from southeastern Australia. Journal of Herpetology, 40, 454–464.

Neubaum, D. M. & Wolfner, M. F. 1999. Wise, winsome, or weird? Mechanisms of sperm storage in female animals. Current Topics in Developmental Biology, Vol 41, 41, 67–97.

Newton, W. D. & Trauth, S. E. 1992. Ultrastructure of the spermatozoon of the lizard *Cnemidophorus sexlineatus* (Saura: Teiidae). Herpetologica, 48, 330–343.

Noble, G. K. & Bradley, H. T. 1933. The mating behavior of lizards: Its bearing on the theory of sexual selection. Annals of the New York Academy of Sciences, 35, 25–100.

Olivier, S. C., Jamieson, B. G. M. & Scheltinga, D. M. 1996. The ultrastructure of spermatozoa of squamata. II. Agamidae, varanidae, colubridae, elapidae, and boidae (Reptilia). Herpetologica, 52, 216–241.

Olsson, M. & Madsen, T. 1995. Female choice on male quantitative traits in lizards—why is it

so rare? Behavioral Ecology Sociobiology, 36, 179–184.

Olsson, M. & Madsen, T. 1996. Costs of mating with infertile males selects for late emergence in female sand lizards. Copeia, 2, 462–464.

Olsson, M. & Madsen, T. 1998. Sexual selection and sperm competition in reptiles. In: Sperm Competition and Sexual Selection (Ed. by T. R. Birkhead & A. P. Møller), pp. 503–578. Cambridge: Academic Press.

Olsson, M. & Madsen, T. 2001. Promiscuity in sand lizards (*Lacerta agilis*) and adder snakes (*Vipera berus*): Causes and consequences. Journal of Heredity, 92, 190–197.

Olsson, M. & Shine, R. 1997a. The seasonal timing of oviposition in sand lizards: why early clutches are better. Journal of Evolutionary Biology, 10, 369–381.

Olsson, M. & Shine, R. 1997b. Advantages of multiple matings to females: a test of the infertility hypothesis using lizards. Evolution, 51, 1684–1688.

Olsson, M., Gullberg, A., Tegelström, H., Madsen, T. & Shine, R. 1994a. Can female adders multiply? Nature 369, 528.

Olsson, M., Gullberg, A. & Tegelström, H. 1994b. Sperm competition in the sand lizard. Animal Behaviour, 48, 193–200.

Olsson, M., Gullberg, A. & Tegelstrom, H. 1996. Mate guarding in male sand lizards (*Lacerta agilis*). Behaviour, 133, 367–386.

Olsson, M., Shine, R., Madsen, T., Gullberg, A. & Tegelström, H. 1996. Sperm selection by females. Nature, 383, 585–585.

Olsson, M., Madsen, T. & Shine, R. 1997. Is sperm really so cheap? Costs of reproduction in male adders, *Vipera berus*. Proceedings of the Royal Society of London, Series B, 264, 455–459.

Olsson, M., Birkhead, T. & Shine, R. 1999. Can relaxed time constraints on sperm production eliminate protandry in an ectotherm? Biological Journal of the Linnean Society, 66, 159–170.

Olsson, M., Madsen T., Ujvari, B. & Wapstra, E. 2004. Fecundity and MHC affects ejaculation tactics and paternity bias in sand lizards. Evolution, 58, 906–909.

Olsson, M., Wapstra, E. & Uller, T. 2005. Differential sex allocation in sand lizards: bright males induce daughter production in a species with heteromorphic sex chromosomes. Biology Letters, 1, 378–380.

Olsson, M., Healey, M., Wapstra, E., Schwartz, T., LeBas, N. & Uller, T. 2007a. Mating system variation and morph fluctuations in a polymorphic lizard. Molecular Ecology, 16, 5307–5315.

Olsson, M., Schwartz, T., Uller, T. & Healey, M. 2007b. Sons are made from old stores: sperm storage effects on sex ratio in a lizard. Biology Letters, 3, 491–493.

Olsson, M., Schwartz, T., Uller, T. & Healey, M. 2009. Effects of sperm storage and male colour on probability of paternity in a polychromatic lizard. Animal Behaviour, 77: 419–424.

Ord, T. J. & Stuart-Fox, D. 2006. Ornament evolution in dragon lizards: multiple gains and widespread losses reveal a complex history of evolutionary change. Journal of Evolutionary Biology, 19, 797–808.

Oring, L. W., Fleischer, R. C., Reed, J. M. & Marsden, K. E. 1992. Cuckoldry through stored sperm in the sequentially polyandrous spotted sandpiper. Nature, 359, 631–633.

Parker, G. A. 1970. Sperm competition and its evolutionary consequences in insects. Biological Reviews of the Cambridge Philosophical Society, 45, 525–567.

Parker, G. A. 1990. Sperm competition games—raffles and roles. Proceedings of the Royal Society of London, Series B, 242, 120–126.

Parker, G. A. 1998. Sperm competition and the evolution of ejaculates: towards a theory base. In: Sperm Competition and Sexual Selection (Ed. by T. R. Birkhead & A. P. Møller), pp. 3–55. Cambridge: Academic Press.

Parker, G. A. 2006. Sexual conflict over mating and fertilization: an overview. Philosophical Transactions of the Royal Society of London, Series B, 361, 235–259.

Parker, G. A., Ball, M. A., Stockley, P. & Gage, M. J. G. 1997. Sperm competition games: a prospective analysis of risk assessment. Proceedings of the Royal Society of London, Series B, 264, 1793–1802.

Pearse, D. E. & Avise, J. C. 2001. Turtle mating systems: Behavior, sperm storage, and genetic paternity. Journal of Heredity, 92, 206–211.

Pearse, D. E., Janzen, F. J. & Avise, J. C. 2001. Genetic markers substantiate long–term storage and utilization of sperm by female painted turtles. Heredity, 86, 378–384.

Pearse, D. E., Janzen, F. J. & Avise, J. C. 2002. Multiple paternity, sperm storage, and reproductive success of female and male painted turtles (*Chrysemys picta*) in nature.

Behavioral Ecology and Sociobiology, 51, 164–171.

Pitnick, S., Markow, T. & Spicer, G. S. 1999. Evolution of multiple kinds of female sperm-storage organs in *Drosophila*. Evolution, 53, 1804–1822.

Pizzari, T. & Birkhead, T. R. 2000. Female feral fowl eject sperm of subdominant males. Nature, 405, 787–789.

Pizzari, T., Dean, R., Pacey, A., Moore, H. & Bonsall, M. B. 2008. The evolutionary ecology of pre- and post-meiotic sperm senescence. Trends in Ecololgy & Evolution, 23, 131–140.

Poiani, A. 2006. Complexity of seminal fluid: A review. Behavioral Ecology and Sociobiology, 60, 289–310.

Prosser, M. R., Weatherhead, P. J., Gibbs, H. L. & Brown, G. P. 2002. Genetic analysis of the mating system and opportunity for sexual selection in northern water snakes (*Nerodia sipedon*). Behavioral Ecology, 13, 800–807.

Purvis, A., Gittleman, J. L., Cowlishaw, G. & Mace, G. M. 2000. Predicting extinction risk in declining species. Proceedings of the Royal Society of London, Series B, 267, 1947–1952.

Ram, K. R. & Wolfner, M. F. 2007. Seminal influences: *Drosophila* Acps and the molecular interplay between males and females during reproduction. Integrative and Comparative Biology, 47, 427–445.

Ross, P. & Crews, D. 1977. Influence of seminal plug on mating-behavior in garter snake. Nature, 267, 344–345.

Ross, P. & Crews, D. 1978. Stimuli influencing mating-behavior in garter snake, *Thamnophis-radix*. Behavioral Ecology and Sociobiology, 4, 133–142.

Saint-Girons, H. 1975. Sperm survival and transport in the female genital tract of reptiles. In: The Biology of the Spermatozoa, (Ed. by E. Hafez & C. Thibault), pp. 105–113. Basel: Karger.

Saint-Girons, H. 1982. Reproductive cycles of male snakes and their relationship with climate and female reproductive cycles. Herpetologica, 38, 5–16.

Scheltinga, D. M., Jamieson, B. G. M., Trauth, S. E. & Mcallister, C. T. 2000. Morphology of the spermatozoa of the iguanian lizards *Uta stansburiana* and *Urosaurus ornatus* (Squamata, Phrynosomatidae). Journal of Submicroscopic Cytology and Pathology, 32, 261–271.

Scheltinga, D. M., Jamieson, B. G. M., Espinoza, R. E. & Orrell, K. S. 2001. Descriptions of the mature spermatozoa of the lizards *Crotaphytus bicinctores*, *Gambelia wislizenii* (Crotaphytidae), and *Anolis carolinensis* (Polychrotidae) (Reptilia, Squamata, Iguania). Journal of Morphology, 247, 160–171.

Schuett, G. W. 1992. Is long-term sperm storage an important component of the reproductive biology of temperate pitvipers? In: Biology of the Pitvipers (Ed. by J. A. Campbell & E. D. Brodie III), pp. 169–184. Tyler, TX: Selva.

Schulte-Hostedde, A. I. & Montgomerie, R. 2006. Intraspecific variation in ejaculate traits of the northern watersnake (*Nerodia sipedon*). Journal of Zoology, 270, 147–152.

Schwartz, J. M., McCracken, G. F. & Burghardt, G. M. 1989. Multiple paternity in wild populations of the garter snake, *Thamnophis sirtalis*. Behavioral Ecology and Sociobiology, 25, 269–273.

Schwarzkopf, L. 1994. Measuring trade-offs: a review of studies of costs of reproduction in lizards. In: Lizard Ecology: Historical and Experimental Perspectives (Ed. by L. J. Vitt & E. R. Pianka), pp. 7–29. Princeton: Princeton University Press.

Seigel, R. A. & Ford, N. B. 1987. Reproductive ecology. In: Snakes: Ecology and Evolutionary Biology (Ed. by R. A. Seigel, J. T. Collins & S. S. Novak), pp. 210-252. New York: Macmillan.

Sever, D. M. & Hamlett, W. C. 2002. Female sperm storage in reptiles. Journal of Experimental Zoology, 292, 187–199.

Sever, D. M. & Hopkins, W. A. 2004. Oviductal sperm storage in the ground skink *Scincella laterale holbrook* (Reptilia : Scincidae). Journal of Experimental Zoology Part a—Comparative Experimental Biology, 301A, 599–611.

Sever, D. M. & Hopkins, W. A. 2005. Renal sexual segment of the ground skink, *Scincella laterale* (Reptilia, Squamata, Scincidae). Journal of Morphology, 266, 46–59.

Sever, D. M. & Ryan, T. J. 1999. Ultrastructure of the reproductive system of the black swamp snake (*Seminatrix pygaea*): Part I. Evidence for oviducal sperm storage. Journal of Morphology, 241, 1–18.

Sheldon, B. 2000. Differential allocation: tests, mechanisms and implications. Trends in Ecology & Evolution, 15, 397–402.

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

Shine, R. 1988. Parental care in reptiles. In: Biology of the Reptilian, vol 16 (Ed. by C. Gans & R. B. Huey), pp. 275–330. New York: Alan R. Liss Inc.

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

Shine, R. 2005. Life-history evolution in reptiles. Annual Review of Ecology Evolution and Systematics, 36, 23–46.

Shine, R., Olsson, M. M. & Mason, R. T. 2000. Chastity belts in gartersnakes: the functional significance of mating plugs. Biological Journal of the Linnean Society, 70, 377–390.

Short, R. V. 1979. Sexual selection and its component parts, somatic and genital selection, as illustrated by man and the great apes. Advances in the Study of Behavior, 9, 131–158.

Simmons, L. W. 2001. Sperm Competition and its Evolutionary Consequences in the Insects. Princeton: Princeton University Press.

Simmons, L. W. & Emlen, D. J. 2006. Evolutionary trade-off between weapons and testes. Proceedings of the National Academy of Sciences of the United States of America, 103, 16346–16351.

Smith, H. M., Sinelnik, G., Fawcett, J. D. & Jones, R. E. 1972. A survey of the chronology of ovulation in Anoline lizard genera. Transactions of the Kansas Academy of Sciences, 75, 107–121.

Smyth, M. & Smith, M. J. 1968. Obligatory sperm storage in skink *Hemiergis peronii*. Science, 161, 575–576.

Snook, R. R. 2005. Sperm in competition: not playing by the numbers. Trends in Ecology & Evolution, 20, 46–53.

Snow, L. S. E. & Andrade, M. C. B. 2005. Multiple sperm storage organs facilitate female control of paternity. Proceedings of the Royal Society of London, Series B, 272, 1139–1144.

Stamps, J. A. 1977. Social behavior and spacing patterns in lizards. In: Biology of the Reptilia Vol 7. Ecology and Behavior. (Ed. by C. Gans & D. W. Tinkle), pp. 265–334. New York: Academic Press.

Stuart-Fox, D. & Moussalli, A. 2007. Sex-specific ecomorphological variation and the evolution of sexual dimorphism in dwarf chameleons (Bradypodion spp.). Journal of Evolutionary Biology, 20, 1073–1081.

Swain, R. & Jones, S. M. 1994. Annual cycle of plasma testosterone and other reproductive parameters in the Tasmanian skink, *Niveoscincus metallicus*. Herpetologica, 50, 502–509.

Swallow, J. G. & Wilkinson, G. S. 2002. The long and short of sperm polymorphisms in insects. Biological Reviews, 77, 153–182.

Swanson, W. J. & Vacquier, V. D. 2002. Reproductive protein evolution. Annual Review of Ecology and Systematics, 33, 161–179.

Tavares-Bastos, L., Teixeira, R. D., Colli, G. R. & Bao, S. N. 2002. Polymorphism in the sperm ultrastructure among four species of lizards in the genus *Tupinambis* (Squamata: Teiidae). Acta Zoologica, 83, 297–307.

Tavares-Bastos, L., Cunha, L. D., Colli, G. R. & Bao, S. N. 2007. Ultrastructure of spermatozoa of scolecophidian snakes (Lepidosauria, Squamata). Acta Zoologica, 88, 189–197.

Teixeira, R. D., Colli, G. R. & Bao, S. N. 1999a. The ultrastructure of the spermatozoa of the worm lizard *Amphisbaena alba* (Squamata, Amphisbaenidae) and the phylogenetic relationships of amphisbaenians. Canadian Journal of Zoology, 77, 1254–1264.

Teixeira, R. D., Colli, G. R. & Bao, S. N. 1999b. The ultrastructure of the spermatozoa of the lizard *Micrablepharus maximiliani* (Squamata, Gymnophthalmidae), with considerations on the use of sperm ultrastructure characters in phylogenetic reconstruction. Acta Zoologica, 80, 47–59.

Teixeira, R. D., Colli, G. R. & Bao, S. N. 1999c. The ultrastructure of the spermatozoa of the lizard *Polychrus acutirostris* (Squamata, Ploychrotidae). Journal of Submicroscopic Cytology and Pathology, 31, 387–395.

Teixeira, R. D., Vieira, G. H. C., Colli, G. R. & Bao, S. N. 1999d. Ultrastructural study of spermatozoa of the neotropical lizards, *Tropidurus semitaeniatus* and *Tropidurus torquatus* (Squamata, Tropiduridae). Tissue & Cell, 31, 308–317.

Teixeira, R. D., Scheltinga, D. M., Trauth, S. E., Colli, G. R. & Bao, S. N. 2002. A comparative ultrastructural study of spermatozoa of the teiid lizards *Cnemidophorus gularis gularis*, *Cnemidophorus ocellifer*, and *Kentropyx altamazonica* (Reptilia,

Squamata, Teiidae). Tissue & Cell, 34, 135–142.

Tokarz, R. 1995. Mate choice in lizards: a review. Herpetological Monographs, 9, 17–40.

Tourmente, M., Cardozo, G., Bertona, M., Guidobaldi, A., Giojalas, L. & Chiaraviglio, M. 2006. The ultrastructure of the spermatozoa of *Boa constrictor occidentalis*, with considerations on its mating system and sperm competition theories. Acta Zoologica, 87, 25–32.

Townsend, T. M., Larson, A., Louis, E. & Macey, J. R. 2004. Molecular phylogenetics of Squamata: The position of snakes, Amphisbaenians, and Dibamids, and the root of the Squamate tree. Systematic Biology, 53, 735–757.

Trivers, R. L. 1972. Mother-offspring conflict. American Zoologist, 12, 648–648.

Trivers, R. L. 1976. Sexual selection and resource-accruing abilities in *Anolis garmani*. Evolution, 30, 253–269.

Uller, T. 2003. Viviparity as a constraint on sex-ratio evolution. Evolution, 57, 927–931.

Uller, T. 2006. Sex-specific sibling interactions and offspring fitness in vertebrates: patterns and implications for maternal sex ratios. Biological Reviews, 81, 207–217.

Uller, T. & Olsson, M. 2006. Multiple copulations in natural populations of lizards: evidence for the fertility assurance hypothesis. Behaviour, 142, 45–56.

Uller, T. & Olsson, M. 2008. Multiple paternity in reptiles: patterns and processes. Molecular Ecology, 17, 2566–2580.

Uller, T., Astheimer, L. & Olsson, M. 2007. Consequences of egg yolk testosterone for offspring development and survival: experimental test in a lizard. Functional Ecology, 21, 544–561.

Vahed, K. 1998. The function of nuptial feeding in insects: review of empirical studies. Biological Reviews, 73, 43–78.

Vieira, G. H. C., Colli, G. R. & Bao, S. N. 2004. The ultrastructure of the spermatozoon of the lizard *Iguana iguana* (Reptilia, Squamata, Iguanidae) and the variability of sperm morphology among iguanian lizards. Journal of Anatomy, 204, 451–464.

Vieira, G. H. C., Colli, G. R. & Bao, S. N. 2005. Phylogenetic relationships of corytophanid lizards (Iguania, Squamata, Reptilia) based on partitioned and total evidence analyses of sperm morphology, gross morphology,

and DNA data. Zoologica Scripta, 34, 605–625.

Vieira, G. H. C., Cunha, L. D., Scheltinga, D. M., Glaw, F., Colli, G. R. & Bao, S. N. 2007. Sperm ultrastructure of hoplocercid and oplurid lizards (Sauropsida, Squamata, Iguania) and the phylogeny of Iguania. Journal of Zoological Systematics and Evolutionary Research, 45, 230–241.

Volsøe, H. 1944. Structure and seasonal variation of the male reproductive organs of *Vipera berus* (L.). Spolia Zoologica Musei Heuni Ensis V, 5, 1–157.

Wapstra, E., Swain, R., Jones, S. M. & O'Reilly, J. 1999. Geographic and annual variation in reproductive cycles in the Tasmanian spotted snow skink, *Niveoscincus ocellatus* (Squamata : Scincidae). Australian Journal of Zoology, 47, 539–550.

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

Watts, P. C., Buley, K. R., Boardman, W., Ciofi, C. & Gibson O. R. 2006. Parthenogenesis in komodo dragons. Nature, 444, 1021–1022.

Weatherhead, P. J., Prosser, M. R., Gibbs, H. L. & Brown, G. P. 2002. Male reproductive success and sexual selection in northern water snakes determined by microsatellite DNA analysis. Behavioral Ecology, 13, 808–815.

Yamanouye, N., Silveira, P., Abdalla, F. M. F., Almeida-Santos, S. M., Breno, M. C. & Salomao, M. G. 2004. Reproductive cycle of the Neotropical *Crotalus durissus terriicus*: II. Establishment and maintenance of the uterine muscular twisting, a strategy for long-term sperm storage. General and Comparative Endocrinology, 139, 151–157.

Zamudio, K. R. & Sinervo, E. 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.

APPENDIX 1. SOURCES USED TO CONSTRUCT PHYLOGENY

Brandley, M. C., Schmitz, A. & Reeder, T. W. 2005. Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of

scincid lizards. Systematic Biology, 54, 373–390.

Carranza, S. & Arnold, E. N. 2006. Systematics, biogeography, and evolution of *Hemidactylus* geckos (Reptilia: Gekkonidae) elucidated using mitochondrial DNA sequences. Molecular Phylogenetics and Evolution, 38, 531–545.

Castoe, T. A. & Parkinson, C. L. 2006. Bayesian mixed models and the phylogeny of pitvipers (Viperidae: Serpentes). Molecular Phylogenetics and Evolution, 39, 91–110.

de Queiroz, A. Lawson, R. & Lemos-Espinal, J. A. 2002. Phylogenetic relationships of north American garter snakes (*Thamnophis*) based on four mitochondrial genes: how much DNA sequence is enough? Molecular Phylogenetics and Evolution, 22, 315–329.

Fitch, A. J., Goodman, A. E. & Donnellan, S. C. 2006. A molecular phylogeny of the Australian monitor lizards (Squamata:Varanidae) inferred from mitochondrial DNA sequences. Australian Journal of Zoology, 54, 253–269.

Flores-Villela, O., Kjer, K. M., Benabib, M. & Sites Jr, J. W. 2000. Multiple data sets, congruence, and hypothesis testing for the phylogeny of basal groups of the lizard genus *Sceloporus* (Squamata, Phrynosomatidae). Systematic Biology, 49, 713–739.

Giugliano, L. G., Collevatti, R. G. & Colli, G. R. 2007. Molecular dating and phylogenetic relationships among Teiidae (Squamata) inferred by molecular and morphological data. Molecular Phylogenetics and Evolution, 45, 168–179.

Han, D., Zhou, K. & Bauer, A. M. 2004. Phylogenetic relationships among gekkotan lizards inferred from Cmos nuclear DNA sequences and a new classification of the Gekkota. Biological Journal of the Linnean Society, 83, 353–368.

Lawson, R., Slowinski, J. B., Crother, B. I. & Burbrink, F. T. 2005. Phylogeny of the Colubroidea (Serpentes): new evidence from mitochondrial and nuclear genes. Molecular Phylogenetics and Evolution, 37, 581–601.

Macey, J. R., Schulte II, J. A., Larson, A., Ananjeva, N. B., Wang, Y., Pethiyagoda, R., Rastegar-Pouyani, N. & Papenfuss, T. J. 2000. Evaluating trans-Tethys migration: an example using acrodont lizard phylogenetics. Systematic Biology, 49, 233–256.

Nagy, Z. T., Lawson, R., Joger, U. & Wink, M. 2004. Molecular systematics of racers, whipsnakes and relatives (Reptilia: Colubridae) using mitochondrial and nuclear markers. Journal of Zoological Systematics and Evolutionary Research, 42, 223–233.

Odierna, G., Canapa, A., Andreone, F., Aprea, G., Barucca, M., Capriglione, T. & Olmo, E. 2002. A phylogenetic analysis of Cordyliformes (Reptilia: Squamata): comparison of molecular and karyological data. *Molecular Phylogenetics and Evolution*, 23, 37–42.

Poe, S. 2004. Phylogeny of anoles. Herpetological Monographs, 18, 37–89.

Reeder, T. W. 2003. A phylogeny of the Australian Sphenomorphus group (Scincidae: Squamata) and the phylogenetic placement of the crocodile skinks (*Tribolonotus*): Bayesian approaches to assessing congruence and obtaining confidence in maximum likelihood likelihood inferred relationships. Molecular Phylogenetics and Evolution, 27, 384–397.

Reeder, T. W., Cole, C. J. & Dessauer, H. C. 2002. Phylogenetic relationships of whiptail lizards of the genus *Cnemidophorus* (Squamata: Teiidae): a test of monophyly, reevaluation of karyotypic evolution, and review of hybrid origins. American Museum Novitates, 3365, 1–61.

Schulte II, J. A., Melville, J. & Larson, A. 2003a. Molecular phylogenetic evidence for ancient divergence of lizard taxa on either side of Wallace's Line. Proceedings of the Royal Society of London Series B, 270, 597–603.

Schulte II, J. A., Valladares, J. P. & Larson, A. 2003b. Phylogenetic relationships within Iguanidae inferred using molecular and morphological data and a phylogenetic taxonomy of iguanian lizards. Herpetologica, 59, 399–419.

Slowinski, J. B. & Keogh, J. S. 2000. Phylogenetic relationships of elapid snakes based on cytochrome b mtDNA sequences. Molecular Phylogenetics and Evolution, 15, 157–164.

Townsend, T., Larson, A., Louis, E. & Macey, J. R. 2004. Molecular phylogenetics of Squamata: the position of snakes,

amphisbaenians, and dibamids, and the root of the squamate tree. Systematic Biology, 53, 735–757.

Vidal, N. & Hedges, S. B. 2005. The phylogeny of squamate reptiles (lizards, snakes, and amphisbaenians) inferred from nine nuclear protein-coding genes. Comptes Rendus Biologies, 328, 1000–1008.

Vidal, N., Delmas, A-S., David, P., Cruaud, C., Couloux, A. & Hedges, S. B. 2007. The phylogeny and classification of caenophidian snakes inferred from seven nuclear protein-coding genes. Comptes Rendus Biologies, 330, 182–187.

Wiens, J. J. 2000. Decoupled evolution of display morphology and display behaviour in phrynosomatid lizards. Biological Journal of the Linnean Society, 70, 597–612.

Wiens, J. J. & Hollingsworth, B. D. 2000. War of the iguanas: conflicting molecular and morphological phylogenies and long-branch attraction in iguanid lizards. Systematic Biology, 49, 143–159.

APPENDIX 2. Data on sperm morphology in reptiles

Family	Genus	Species	Sperm length	Head length	Midpiece length	Flagellum length	Reference
Agamidae	Diporiphora	bilineata	68.27	12.1	4.05	50.36	Vieira et al. 2007
Agamidae	Pogona	barbata	28.5	-	3.7	18.4	Olivier et al. 1996
Amphisbaenidae	Amphisbaena	alba	84.3	14.3	4.3	65.7	Teixeira et al. 1999a
Chamaeleonidae	Bradypodion	karrooicum	-	.	9.13	.	Vieira et al. 2007
Gymnophthalmidae	Microblepharus	maximiliani	60	11	2.5	46.5	Teixeira et al. 1999b
Gymnophthalmidae	Cercosaura	ocellata	67.49	14.75	2.18	50.57	Colli et al. 2007
Iguanidae	Anolis	carolinensis	83.2	16.5	4.6	63.3	Scheltinga et al. 2001
Iguanidae	Anolis	carolinensis	83.22	16.99	4.38	63.24	Vieira et al. 2007
Iguanidae	Basiliscus	vittatus	90.31	18.31	2.91	68.17	Vieira et al. 2005
Iguanidae	Corytophanes	cristatus	96.11	18.43	3.02	68.17	Vieira et al. 2005
Iguanidae	Crotaphytus	bicinctores	95.22	19.88	4.06	72.5	Vieira et al. 2007
Iguanidae	Crotaphytus	bicinctores	85.5	20	4	57.2	Scheltinga et al. 2001
Iguanidae	Enyalioides	laticeps	102.76	19.31	3.73	78.53	Vieira et al. 2007
Iguanidae	Gambelia	wislizenii	99.33	20.15	5.07	77.52	Vieira et al. 2007
Iguanidae	Gambelia	wislizenii	98.8	20.2	5	76.8	Scheltinga et al. 2001
Iguanidae	Hoplocercus	spinosus	110.58	27.61	4.75	74.32	Vieira et al. 2007
Iguanidae	Iguana	iguana	71.7	18.2	3.4	53.5	Viera et al. 2004
Iguanidae	Iguana	iguana	71.69	18.22	3.36	49.5	Vieira et al. 2005
Iguanidae	Laemanctus	longipes	97.65	18.37	3.02	75.7	Vieira et al. 2005
Iguanidae	Oplurus	cyclurus	85.85	22.36	3.83	60.41	Vieira et al. 2007
Iguanidae	Polychrus	acutirostris	-	-	7.5	-	Teixiera et al. 1999c
Iguanidae	Polychrus	acutirostris	83.7	17.15	3.84	62.94	Vieira et al. 2005
Iguanidae	Tropidurus	semitaeniatus	-	-	2.8	-	Teixeira et al. 1999d
Iguanidae	Tropidurus	semitaeniatus	-	.	2.52	.	Vieira et al. 2007
Iguanidae	Tropidurus	torquatus	-		2.8	-	Teixeira et al. 1999
Iguanidae	Tropidurus	torquatus	93.17	19.53	2.63	70.67	Vieira et al. 2005
Iguanidae	Urosaurus	ornatus	-	-	3.9	-	Scheltinga et al. 2000
Iguanidae	Uta	stansburiana	88.2	18.2	3.8	67.5	Scheltinga et al. 2000
Scincidae	Carlia	pectoralis	96.5	-	11.5	79.8	Jamieson et al. 1994
Scincidae	Ctenotus	taeniolatus	84	-	7.1	64.8	Jamieson et al. 1994
Scincidae	Tiliqua	scincoides	-	5.5	5.5	42.8	Jamieson et al. 1994
Teiidae	Ameiva	ameiva	68	15.4	4.6	48	Guigliano et al. 2002
Teiidae	Callopistes	flavipunctatus	104.98	16.81	1.82	85.56	Colli et al. 2007
Teiidae	Cnemidophorus	gularis	54.5	10.8	3.5	40.25	Teixeira et al. 2002
Teiidae	Cnemidophorus	ocellifer	56.4	13.3	3.35	40.1	Teixeira et al. 2002
Teiidae	Cnemidophorus	sexlineatus	-	-	4	-	Newton & Trauth 1992
Teiidae	Crocodilurus	amazonicus	84.98	16.6	3.7	63.85	Colli et al. 2007
Teiidae	Dicrodon	guttulatum	78.34	15.6	3.52	54.01	Colli et al. 2007
Teiidae	Dracaena	guianensis	90.5	18.08	3.82	66.66	Colli et al. 2007
Teiidae	Kentropyx	altamazonica	75.6	14.7	7.55	53.6	Teixeira et al. 2002
Teiidae	Teius	oculatus	74.74	18.91	3.54	53.2	Vieira et al. 2005
Teiidae	Tupinambis	duseni	82.3	23.5	4.2	55.5	Tavarez-Bastos et al. 2002
Teiidae	Tupinambis	merianae	82.3	23.5	4.2	55.5	Tavarez-Bastos et al. 2002
Teiidae	Tupinambis	quadrilineatus	82.3	23.5	3.3	55.5	Tavarez-Bastos et al. 2002
Teiidae	Tupinambis	teguixin	82.3	23.5	3.3	55.5	Tavarez-Bastos et al. 2002
Varanidae	Varanus	gouldii	-	-	3.1	-	Olivier et al. 1996
Boidae	Boa	constrictor	97.1	12.5	40.5	44.1	Tourmente et al. 2006
Colubridae	Nerodia	sipedon	112	-	-	-	Schulte-Hostedde & Montgomerie 2006
Typhlopidae	Ramphotyphlops	waitii	179	-	55	108	Harding et al. 1995

20

Sexual Conflict and the Intromittent Organs of Male Birds

ROBERT MONTGOMERIE

INTRODUCTION

Birds are unique in the animal kingdom in that internal fertilization is the rule but male intromittent organs (IOs) are the exception. The otherwise almost universal association between IOs and internal fertilization in animals (see table 1 in Briskie & Montgomerie 1997) led many early authors to suggest that IOs were necessary to facilitate insemination and were thus typical primary sexual traits shaped by natural selection. Thus interspecific variation in IO structure was often thought to reduce hybridization and its costs. As a result, complex male and female genitalia were presumed to fit together in a lock–and–key fashion specifically to facilitate within-species matings and reduce the efficacy of interspecific coupling. Eberhard (1985, 1990) and others (see Hosken & Stockley 2004), however, suggested that male genitalia might serve other, more cryptic, functions, including male advertisement, internal courtship, sexual coercion, and sperm competition, all of which are mechanisms of sexual selection. These insights blurred the distinction between primary and secondary sexual traits such that that dichotomy is no longer particularly useful, as Darwin (1871, pp. 253–254) had insightfully intimated.

The study of bird IOs has made some useful contributions to our modern understanding of the function of male external genitalia in general, even though both the structure and, especially, the function of bird IOs are notoriously difficult to study. This difficulty stems from their relative rarity (occurring in only 3% of bird species), their soft tissue, their erectile anatomy, and their internal location in the male except at the moment of insertion into the female (Brennan et al. 2010). Thus although some early anatomists examined the genitalia of a few bird species (Müller 1836; Eckhard 1876; Owen 1879; Müller 1908; see figure 20.1), only recently has there been renewed interest in studying and quantifying phallic structures of non–domestic species (Oliveira & Mahecha 2000; Brennan et al. 2007, 2008), and the position and behavior of bird IOs during copulation has only just been determined (Brennan et al. 2010). From today's perspective, it seems as if lack of interest, or possibly interesting theory to test, is partly responsible for the dearth of knowledge about the male genitalia of birds. Thus, even though we expect, for phylogenetic reasons, that about 300 bird species have IOs, we have convincing evidence for the presence of IOs from only about 25% of those species, and important discoveries are still being made (Brennan et al. 2010).

For a long time, ornithologists believed that the interesting thing about the male phallus in birds was that it is present in so few species. Recent work, however, has pointed out that the absence of IOs in almost 10,000 species of obligate internal fertilizers is really the mystery (Briskie & Montgomerie 1997). Thus it is the loss of IOs in so many lineages