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ORIGINAL ARTICLE

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Copulatory plug displacement and prolonged copulation in the Iberian rock lizard (*Lacerta monticola*)

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Abstract Male Iberian rock lizards (*Lacerta monticola*) produce copulatory plugs that adhere firmly inside the female cloaca and occlude both oviducts. These plugs do not prevent rival male insemination, as they neither reduce female attractiveness or receptivity to rival males nor do they function as "chastity belts". Prior to copulation, males bite various regions of the female body, including the cloacal region. A previous plug in the female cloaca is expelled only after rival male intromission. We hypothesized that: (1) such male pre-copulatory behaviours function to loosen plug adherence to the female cloaca, thereby facilitating intromission, and that (2) the hemipenis plays a role in displacing a previous plug prior to the delivery of a new plug. Neither of these hypotheses was supported. Instead, our results indicate that rival males can intromit the hemipenis past a previous plug in the female cloaca and deliver their own plug underneath it. Consequently, previous plugs are pushed away from the oviductal openings and even dislodged from the female cloaca. Copulation duration was determined both by the time used by males to deliver a plug and by the fact that males prolonged copulation beyond plug delivery. There seemed to be sexual conflict over prolonged copulation, which was resolved by the male/female head-length ratio. The adaptive value of Iberian rock lizard copulatory plugs and prolonged copulation in Iberian rock lizards is discussed in the context of sperm competition.

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Introduction

Female sexual promiscuity is a ubiquitous phenomenon among sexually reproducing organisms, and often leads to sperm competition (Birkhead and Møller 1998). Under the selective pressure of sperm competition, male traits such as copulatory plugs, prolonged copulation and sperm displacement may have evolved as paternity assurance mechanisms (Parker 1970; Birkhead 2000). Sexual selection, therefore, extends beyond male-male competition for access to females and to copulations (Møller 1998). If, on the one hand, male adaptations to sperm competition may be under constant challenge by male counter adaptations, on the other, they may also lead to sexual conflict, both arguably promoting rapid evolution of traits and an evolutionary arms race between the sexes, as they may compromise female fitness (Stockley 1997; Chapman et al. 2003). It may therefore be difficult to predict, at any point in evolutionary time, the current role of seemingly sperm competition-related traits. In the context of male and female adaptations and counter adaptations, copulatory plugs, for instance, may be acquired or lost, or they may evolve to fulfil diverse roles. Hence, it is not surprising that copulatory plugs occur in so many taxa, that so many functions have been attributed to them, and that their presence is patchily distributed within a particular taxon (see Birkhead and Møller 1998 for reviews on diverse taxa).

Copulatory plugs frequently result from the coagulation of male accessory-gland products that occlude the female reproductive tract following copulation (Birkhead and Møller 1998). In the context of sperm competition, plugs may be adaptive by increasing a male's chance of fertilization, either by preventing the sperm of rival males from reaching the fertilization site or by allowing a male to maximize sperm delivery (Parker 1970). For instance, in the decapod *Inachus phalangium* (Diesel 1990) and the butterfly *Euphydryas chaledon* (Dickinson and Rutowski 1989), plugs prevent rival male insemination by physically blocking the female genitalia, thereby functioning as “chastity belts”. In bumblebees (*Bombus terrestris*), plug components render females refractory to further copulations (Baer et al. 2001; Sauter et al. 2001). In rats (*Rattus norvegicus*), rival male pre-ejaculatory intromission dislodges plugs (Mosig and Dewsbury 1970). These plugs play an important role in the transport of sperm through the cervix to the uterus immediately after insemination (Matthews and Adler 1979; Carballada and Esponda 1993); they also prevent uterine backflow of sperm when the cervix muscles relax several hours after insemination (Carballada and Esponda 1997).

Among reptiles, copulatory plugs have been reported in 7 natricine and colubrine snakes (Devine 1984) and in 36 lacertid lizards (In den Bosch 1994). Reptilian plugs are thought to be adaptive in several ways (Olsson and Madsen 1998), but their actual role remains unclear. Gartersnake (*Thamnophis* sp.) plugs are composed of male renal sex segment (RSS) secretions, which are delivered after semen. Plugs are large and gelatinous, firmly occlude the female cloaca for a few days and have been suggested to function as “chastity belts” (Devine 1975, 1977; Shine et al. 2000b; but see Whittier and Tokarz 1992). The hypothesis that gartersnake plugs contain anti-aphrodisiac pheromones (Ross and Crews 1977, 1978) that render females unreceptive and unattractive to rival males has been refuted; those pheromones are present in fluids associated with copulation (semen, etc.) and not in the plug per se (Shine et al. 2000b).

In the Iberian rock lizard (*Lacerta monticola*, Boulenger 1905), a plug consists of a portion that fills the urodaeum and of two projections inserted into both oviductal openings (In den Bosch 1994; Moreira and Birkhead 2003). The plug (i.e. the ejaculate) resembles white foam but its sperm-rich oviductal tips are more like a transparent gel. Plugs harden and adhere firmly inside the female cloaca within minutes of copulation and remain in place for 1–20 h (8 h on average) of daytime lizard activity (Moreira and Birkhead 2003). In this species, plugs do not prevent insemination by rival males; neither female attractiveness and female receptivity to rival males nor male insemination success (“chastity belt” effect) differed significantly between trials in which males were presented with females without a previous plug and with females with a plug deposited by a different male 1, 3 and 6 or more hours earlier, or between females without and with a plug (Moreira and Birkhead 2003).

The aim of the present study was to identify the mechanisms whereby Iberian rock lizard males inseminate females with a previous plug. Prior to copulation, males bite various regions of the female body, including the cloacal region. A previous plug in the female cloaca is expelled only after rival male intromission. We hypothesized that: (1) such male pre-copulatory behaviours function to loosen the adherence of a previous plug to the female cloaca, thereby facilitating intromission, and that (2) the hemipenis plays a role in displacing a previous plug prior to the delivery of a new plug. We developed general linear models to test the predictions that: (1) male pre-copulatory behaviours and (2) copulation would be longer when males are presented with females with a previous plug than with females without a plug. In addition, we aimed to identify other determinants of copulation duration.

Methods

Study species

The Iberian rock lizard is a small insectivorous lacertid endemic to the Iberian Peninsula. In Portugal, it is restricted to a single population at the Serra da Estrela mountain. There, lizards become active between March and May and enter winter hibernation between October and November. Adult males emerge from hibernation 1–2 weeks prior to adult females and the copulation season begins as early as April and as late as June, once males have shed the skin, and lasts 2–4 weeks. Male home ranges overlap those of several females, and female home ranges overlap those of several males (Aragón et al. 2001; personal observation). Both males and females are highly promiscuous (Martín and Salvador 1993). Field estimates at Serra da Estrela indicate that females copulate four to eight times per mating season, frequently with several different males (Moreira 2002). In captivity, females copulate up to 12 times in a 1-week period of sexual receptivity, and males up to 16 times over 2 weeks (personal observation). Females lay a single clutch per year 1–2 months after copulations (June-July) with 2–11 eggs. Offspring hatch between August and September. Males and females reach sexual maturity at 64–70 mm snout-vent-length (SVL), at 1–2 years of age, near the Lagoa Comprida lagoon (1,580 m) and at 3–4 years near Torre, the peak of Serra da Estrela at 1,993 m (Moreira 2002).

Breeding of lizards

Our study was conducted in 1999 on four groups of lizards breeding in captivity (for rearing conditions, see Moreira and Birkhead 2003). Lizards were captured at Serra da Estrela, measured (SVL to the nearest 1 mm, head length to the nearest 0.05 mm) and weighed (to the nearest 0.1 g). We only used adults with intact or fully regenerated tails, as tail loss reduces male and female mating success (Martín and Salvador 1993). Group 1 was captured before entering winter hibernation near Torre (10–15 October 1998) and was hibernated in the laboratory upon capture. Males were removed from hibernation on 8 February and females on 15 February 1999. Groups 2 and 3 were captured at the start of the copulation season in the field near Lagoa Comprida (9–24 May 1999) and near Torre (24 May–16 June 1999), respectively. These lizards were installed immediately in indoor breeding terraria and resumed reproduction. Group 4 was captured near Torre (29 May–11 June 1999) on sites where the snow had recently melted, and lizards had just emerged from hibernation and were still covered in mud. These animals were re-hibernated upon capture, simulating the resurgence of winter conditions, which frequently happens at Serra da Estrela. Males were removed from hibernation on 17 August and females on 24 August 1999. For all breeding groups, a single male per terrarium was kept with up to four females, thereby allowing establishment of the history of copulations of all lizards. Females were examined three to four times a day and copulations inferred (36% cases, *n*=369; others were directly observed)—including the hemipenes used by the male (contralateral to the female side bite-grasped by males during copulation; Crews 1978)—from new copulation scars and from fresh plugs in the female cloaca. After the study, the animals were released at their sites of capture.

Experimental procedures

Males were presented with females in $50 \times 25 \times 25$ cm^3 trial terraria with a temperature gradient that covered the species' selected body temperature (see detailed methods in Moreira and Birkhead 2003). In order to study the function of Iberian rock lizard plugs (reported in Moreira and Birkhead 2003) and address the mechanisms whereby males inseminate females with a previous plug, relatively large males (78–82 mm SVL) were presented with females without a previous plug in the cloaca and with females with a plug deposited by a different male 1, 3 and 6 or more hours earlier. Previous plugs in the female cloaca were marked before the trials by applying a vital stain (Cresil blue) to the emergent face with a cotton bud. In order to complement the information on the determinants of copulation duration, males outside the above SVL range (all but one male were below it) were presented with females without a previous plug. Males were placed in trial terraria 15 min before females and trials were terminated whenever males did not attempt to copulate within 15 min. Trials were filmed from above with an 8-mm video camera and copulations were simultaneously observed from below (trial terraria were provided with a plastic mesh for substrate). We dictated to the camera's audio system the moments when cloacal contact was established and broken, and when plugs became visible outside of the female cloaca. After copulations, females were examined and inseminations were considered to have been successful if plugs were well inserted in the cloaca. In copulations involving females with a previous plug, a new (unstained) plug (with projections into both oviductal openings) was deposited underneath the previous (stained) plug. This was confirmed by extracting new plugs with forceps immediately after copulation, which also allowed determination of the position of the previous plug.

Statistical methods

The description of copulatory behaviour was based on 174 male copulation attempts (10 additional copulation attempts were excluded from this analysis due to incomplete filming) involving 57 males and 57 females, which covered the entire SVL range of adult males and females. The duration of copulatory events is presented as mean ±1 SD (range). The study of the mechanisms whereby males inseminate females with a previous plug was based on 85 trials in which relatively large males (78–82 mm SVL) copulated with females with (40 trials) and without (45 trials) a previous plug. These trials involved 31 males and 31 females. We developed general linear models (GLMs) in order to test for the effect of the presence of a plug in the female cloaca on the durations of male biting of the female, male biting on the female cloacal region prior to copulation, and of copulation. To identify other determinants of copulation duration, we developed GLMs using data for all 127 inseminations obtained (10 were excluded due to incomplete filming), thus covering the entire SVL range of adult males and females. Since there were no significant differences between plugs deposited 1, 3 and 6 or more hours earlier for any of the studied variables ($P>0.20$ in all cases), the data were pooled together.

GLMs were developed in a backward stepwise manner (Grafen and Hails 2002) using the software package Statistica 6.0, and non-significant terms at $P>0.10$ were removed from final models. As both males and females were sometimes repeated within and between treatments, and to control for pseudoreplication, we used mean values per male for all variables and covariates in the models. The covariates were the following: (1) previous plug present or absent in the female cloaca; (2) right or left hemipenis used by the male; (3) morphometric relationship between male and female: male/female ratio for SVL, head length, and body mass; (4) prior history of male inseminations: number of plugs that a male had previously produced using the same hemipenis, number of different females with which a male had previously produced plugs, and number of times that a male had previously produced plugs with the same female; (5) prior history of female inseminations: number of times that a female had previously been inseminated, number of different males with which a female had previously been inseminated, and number of times that a female had been previously inseminated by the same male; (6) experimental conditions: breeding group (categorical covariate with four levels corresponding to groups 1, 2, 3 and 4) and time of the trial. In order to satisfy the assumption of normality, durations of male biting of the female prior to copulation and of copulation were ln-transformed (Grafen and Hails 2002). GLMs for copulation duration considering data for all 127 inseminations followed the same method as before, but plug presence or absence in the female cloaca was no longer used as a covariate.

Results

Copulatory behaviour

Copulatory behaviour in captivity followed the same sequential events as observed in the field. Male copulation attempts do not involve any obvious courtship. They are initiated when the male bite-grasps the female and are followed by a pre-copulatory period during which males bite on various regions of the female body. Copulation starts when males establish cloacal contact by grasping females on the pelvic region and by assuming a contorted copulatory posture termed "dough-nut". Copulation terminates when males lose their bite-grasp on the female pelvic region and invariably also cloacal contact, most often as a result of females escaping and/or biting the male.

Males attempted copulation in 174 trials. They approached or rushed up to females, sometimes tongue-flicked briefly over their body, and bite-grasped them. Males bit females continuously on various parts of their body, quickly shifting the position of the bites, before they got hold on the pelvic region and assumed the "dough-nut". During this pre-copulatory period, females were frequently agitated and moved very actively, dragging the males. Females appeared to be unreceptive to copulation in at least 20% of the trials (35/174), as they bit males repeatedly. Nonetheless, males maintained their grasp and assumed the "dough-nut" in 40% of those trials (14/35), in what seemed to be forced copulations. Overall, males assumed the "dough-nut" (even if briefly) in 88% of the copulation attempts (153/174). Biting of the female during the pre-copulatory period in these 153 trials lasted an average of 1.28±0.38 min (0.58–2.92 min). Males bit on the female cloacal region in 97% of the trials (148/153) for an average of 0.82±0.40 min (0.08–2.13 min). They used (or attempted to use) the right hemipenis (52% of trials, 80/153) as frequently as the left one (48% of trials, 73/153) (chi-square test: $\chi^2=0.12$, *df*=1, $P=0.73$). There were no significant differences in the use of hemipenes according to whether males were presented with females with and without a previous plug (chi-square test: $\chi^2=0.02$, *df*=1, $P=0.90$). In 29% of the trials (45/153), females moved very actively (dragging males grasped to them) soon after they assumed the "dough-nut", causing cloacal contact to be broken in 9% of the trials (14/153). In 2 of these 14 cases, males maintained their grasp on the female pelvic region and produced plugs. Male

Table 1 Results of GLM analyses on how the durations of male biting of the female (ln-transformed) and male biting on the female cloacal region prior to copulation, and copulation (ln-transformed) were affected by the presence of a previous plug in the female cloaca, while controlling for the morphometric relationship between male and female, the hemipenis used by the male, prior history of male and female inseminations, breeding group, and time of trial. Data were obtained from an experiment in which 78- to 82-mm SVL males were presented with females without a previous plug and females with a plug deposited earlier by a different male. GLMs were developed in a backward stepwise manner and terms at $P>0.10$ were removed from final models. Adjusted R^2 of final models were 0.28, 0.43 and 0.64, respectively

Variable source	SS	*F*	*P*	*df*	Coeff.
Male biting of the female					
Corrected model	15.4	8.6	<0.001	3	
Intercept	477.8	799.7	<0.001	1	9.99
Female no. of previous inseminations	10.1	16.9	<0.001	1	−0.31
Breeding group	5.8	4.9	0.02	2	
Group 2					0.63
Group 3					0.05
Group 4					−0.68
Error	16.1			27	
Male biting on the female cloacal region					
Corrected model	2544.9	4.8	0.01	3	
Intercept	20026.6	114.2	<0.001	1	64.69
Female no. of previous inseminations	831.1	4.7	0.04	1	−2.80
Breeding group	1792.4	5.1	0.01	2	
Group 2					11.19
Group 3					−1.17
Group 4					−10.02
Error	4735.9			27	
Copulation					
Corrected model	1.8	10.0	<0.001	6	
Intercept	1.0	32.8	<0.001	1	4.16
Male/female SVL ratio	0.65	22.3	<0.001	1	2.78
Plug presence in the female cloaca	0.19	6.4	0.02	1	−0.26
Hemipenis used by male	0.11	3.7	0.07	1	0.18
No. of plugs previously produced with same hemipenis	0.09	3.0	0.10	1	0.04
Female no. of previous inseminations with same male	0.24	8.0	0.01	1	−0.26
Time of trial	0.16	5.5	0.03	1	−1.29
Error	0.71			24	

hemipenes were everted outside the female cloaca and plugs started oozing out of the hemipenes 1.80 min and 1.53 min after cloacal contact had been first established.

Males secured the “dough-nut” (i.e. cloacal contact was maintained and copulation occurred) in 80% of the trials (139/174). Females usually became calmer and did not move once males secured the “dough-nut” posture. In only 5 (3.6%) of 139 copulations did females move throughout copulation. However, in all but 2 (1.5%) of the remaining 134 copulations, females became agitated again and first tried moving an average of 4.32±1.80 min (0.92–11.45 min) after the start of copulation, and when a new plug had been delivered (see below). In 57% of the copulations (79/139), females also attempted to terminate copulation by biting males repeatedly in the head/neck. In these cases, females’ first bite occurred an average of 5.42±2.27 min (0.83–12.35 min) after copulation had started. Males lost their grasp on females and the cloacal contact almost simultaneously in 74% copulations (103/139) as a consequence of females moving and/or biting males. However, in 26% of the copulations (36/139), males maintained their grasp after cloacal contact was broken for an average of 1.82±1.57 min (0.17–7.33 min). The 139 copulations lasted an average of 6.98±2.72 min (2.33–16.18 min). The scar produced by the male bite-grasp on the female pelvic region bled in 36% of the copulations (50/139).

Plug displacement

Neither male biting of the female nor male biting on the female cloacal region prior to copulation appeared to function to loosen the adherence of a previous plug to the female cloaca. These pre-copulatory behaviours did not last longer when males copulated with females with a previous plug than with females without a plug (Table 1).

Displacement of a previous plug in the female cloaca seemed to result from rival males depositing a new plug underneath it. In 40 trials that males copulated with females with a previous plug, these were never expelled from the female cloaca prior to rival male intromission. Rival males successfully inseminated females with a previous plug in 90% of the copulations (36/40). In the other four trials, new plugs were found outside of the female cloaca after copulation. As a result of the 36 successful inseminations, previous plugs (oviductal tips were also observed to be displaced) were totally (20 cases) or partially (10 cases) removed from the female cloaca in 83% of the cases. The other six (17%) plugs, as well as the portion of the ten plugs that were only partially

Table 2 Results of GLM analyses on how the duration of copulation (ln-transformed) was determined by the morphometric relationship between male and female, hemipenis used by the male, prior history of male and female inseminations, breeding group, and time of trial. A total of 127 inseminations were considered, covering the entire SVL range of adult males and females. Presence or absence of a previous plug in the female cloaca was not taken into consideration, as we only presented relatively large males with females with a previous plug. GLMs were developed in a backward stepwise manner and terms at $P>0.10$ were removed from final models. Adjusted R^2 of final model was 0.49

Variable source	SS	*F*	*P*	*df*	Coeff.
Copulation					
Corrected model	2.3	7.3	<0.001	8	
Intercept	2.7	68.6	<0.001	1	4.65
Male/female head length ratio	0.81	20.6	<0.001	1	2.00
Hemipenis used by the male	0.18	4.7	0.04	1	0.17
No. of previous plugs produced with same hemipenis	0.13	3.4	0.07	1	0.04
Female no. of previous inseminations	0.13	3.4	0.07	1	−0.03
Time of trial	0.76	19.2	<0.001	1	−1.68
Breeding group	1.3	11.4	<0.001	3	
Group 1					−0.32
Group 2					0.20
Group 3					0.24
Group 4					−0.12
Error	1.7			44	

removed, remained in the female cloaca (on top of the new plug), but were pushed away from the oviductal openings. In 93% of the trials (28/30) in which previous plugs were totally or partially removed, they were seen being expelled from the female cloaca during copulation. In 25 trials (those that were entirely filmed), previous plugs were expelled an average of 4.00±0.62 min (3.00–5.28 min) after rival male intromission. The two plugs that were removed from the female cloaca but were not seen being expelled were found at the tip of the hemipenis after copulation. They appeared to have simply adhered to the hemipenis after being dislodged.

Copulation duration

The hypothesis that the hemipenis plays a role in displacing a previous plug in the female cloaca prior to the delivery of a new plug was not supported. In contrast to our prediction, copulation duration was shorter when males inseminated females with a previous plug than females without a plug (Table 1). Copulation duration was also determined by several other factors, including the hemipenis used by the male and the male/female head-length ratio (Table 2).

Longer copulations when males used the right rather than the left hemipenis (Table 2) seemed to result from a slower rate of plug delivery. In the 25 trials in which males inseminated females with a previous plug, these were observed being expelled (a new plug had already been delivered underneath it) from the female cloaca an average of 4.23±0.56 min (3.33–5.28 min, *n*=12) and 3.77±0.58 min (3.00–4.98 min, *n*=13) after right and left hemipenis intromission, respectively. These differences were statistically significant, considering averages per male for the time elapsed between intromission and plug expulsion (*t*-test: $t=2.4$, $df=17$, $P=0.03$. Right: 4.23±0.60 min, $n=10$. Left: 3.65±0.45 min, $n=9$).

Increasing copulation duration with increasing male/female head-length ratio (Table 2) supports behavioural observations that suggest sexual conflict over prolonged copulation. When males were presented with females with a previous plug, females attempted to terminate copulations soon after a new plug was delivered (as indicated by the expulsion of the previous plug). In contrast, males maintained their grasp on females beyond the time used to deliver a plug and, frequently, even after losing cloacal contact. Of the 25 trials in which males inseminated females with a previous plug, females became agitated and moved actively only after previous plugs were observed being expelled in 21 (84%) trials (1.13±1.10 min; 0.15–4.33 min), simultaneously with plug expulsion in 2 trials, and before plug expulsion in another 2 (0.10 min and 3.33 min). When females also bit males, the females' first bite occurred after the previous plug was expelled in 12 trials (2.03±2.17 min; 0.15–7.78 min) and only 0.10 min before plug expulsion in 1 (8%) trial.

Discussion

We did not find evidence in support of the hypotheses that: (1) male biting of the female and male biting on the female cloacal region prior to copulation function to loosen the adherence of a previous plug to the female cloaca, and that (2) the hemipenis plays a role in displacing a previous plug prior to the delivery of a new plug. Instead, male Iberian rock lizards were able to intromit the hemipenis past a previous plug in the female cloaca and deliver their own plug underneath it. As a new plug was delivered, it seemed to be capable of pushing the previous plug (including its sperm-rich oviductal tips) away from the oviductal openings and, frequently, out of the female cloaca. These observations suggest that copulatory plugs function as sperm-displacement devices. Renal sex segment secretions may increase the volume and viscosity of the ejaculate, allowing it to displace more efficiently a previous ejaculate by volume displacement. RSS secretions may also serve as a barrier to the movement of sperm, thereby preventing the displaced sperm still remaining in the female cloaca from reaching the oviducts and competing for fertilization of the eggs.

Our results also show that males prolonged copulation beyond the time used to deliver a plug, while females attempted to terminate copulation soon after plug delivery. The resulting conflict over prolonged copulation seemed to be resolved by the male/female head-length ratio. In lizards, bite strength correlates positively with head size (Verwaijen et al. 2002); a larger head may increase both the male's ability to bite-grasp the female during copulation and the female's ability to terminate copulation by biting the male. Male Iberian rock lizards have larger heads than females (Braña 1996) and they may therefore be able to obtain and prolong copulations by force. In Lake Eyre dragons (*Ctenophorus maculosos*), relatively larger (SVL) males were more successful at obtaining forced copulations and copulated longer by force (Olsson 1995).

Prolonged copulation has been reported in several reptiles and has been suggested to result in multiple fitness benefits for males, including (1) prevention of sperm leakage, (2) prevention of rival copulations, and (3) the transfer of relatively more sperm and/or RSS secretions (Olsson and Madsen 1998). However, these hypotheses do not seem to apply to Iberian rock lizards, as (1) plugs block the female cloaca for several hours, (2) males exhibit post-copulatory mate guarding (personal observation), and (3) males maintain the copulatory posture after plug delivery and, frequently, even after losing cloacal contact. Although there is ample evidence that copulation duration can affect the amounts of sperm and/or seminal fluids transferred to females in other taxa (Birkhead and Møller 1998), this is still unclear in reptiles. In dragon lizards (*C. fordi*), the ejaculate volume correlated positively with copulation duration (Olsson 2001; sperm and RSS secretions were not told apart), but neither sperm number in brown anoles (*Anolis sagrei*) (Tokarz 1999; males were sampled across different breeding seasons in the year) nor plug mass in gartersnakes (Shine et al. 2000a) correlated with copulation duration. In fact, there are few reptile studies (but see Wikelski and Bäurle 1996) where it is possible to distinguish between the time used by males to deliver sperm and/or RSS secretions and the copulation period beyond it. We suggest that prolonged copulation beyond the time used to deliver sperm and/or RSS secretions may have important methodological implications for the study of the mechanisms of sperm competition in squamate reptiles. In fact, it may mask the eventual relationship between the amounts of sperm and/or RSS secretions transferred during copulation and the copulation duration.

Several hypotheses may be put forward for the adaptive value of male Iberian rock lizards prolonging copulation against female interests. Prolonged copulation may be a mechanism for preventing females from ejecting sperm, as it allows for plugs to harden and adhere to the female cloacal walls before males separate from females. Sperm ejection following forced copulation has been reported in birds (Birkhead and Møller 1992), and in female feral fowl (*Gallus gallus*) it is mediated by male social status (Pizzari and Birkhead 2000). In kittiwakes (*Rissa tridactyla*), males reduce the risk of sperm ejection by remaining longer on their mates' backs (Helfenstein et al. 2003). Female sperm ejection might also be expected to evolve in Iberian rock lizards. While females show a preference for dominant older males with lower asymmetry in their femoral pores (Martín and López 2000; López et al. 2002, 2003), males are able to force copulations and this tactic appears to be preferentially used by subordinate males (López et al. 2003).

Prolonged copulation may also increase physical damage to females, in the form of bleeding copulation scars, thereby discouraging females from remating (Johnstone and Keller 2000). Physical damage may also influence female use of sperm in ways that increase a male's chance of fertilization. Promiscuous female reptiles have been shown to be fitter than monogamous ones, since offspring genetic quality may be increased through intrauterine selection of sperm (Olsson and Madsen 2001). Immunological processes have been suggested to mediate such cryptic sperm choice (Olsson and Madsen 2001). Accordingly, males might force females to shift their immunological responses so as to heal bleeding wounds and avoid infection, thereby preventing females from selecting against their sperm.

Our study indicates that the paired reproductive organ of male Iberian rock lizards is asymmetrical, as previously documented for some other reptiles (Olsson and Madsen 1998; Shine et al. 2000a, 2000b). In this species, the male's right-hand side seems to have smaller sperm and/or smaller RSS secretion storages. First, copulation duration was longer when males used the right hemipenis, which seemed to be related to a slower rate of plug delivery. Second, copulation duration increased (just short of statistical significance) with increasing number of plugs that a male had previously produced using the same hemipenis, that is, when males were supposedly more depleted of sperm, RSS secretions or both. In other reptiles, both sperm count (Tokarz and Slowinski 1990) and plug weight (Shine et al. 2000a) decreased between consecutive copulations. Taken together, these observations suggest that the transfer of lower amounts of sperm and/or lower RSS secretion results in slower plug delivery, and that differences in rates of plug delivery between hemipenes arise from differences in sperm and/or RSS secretion storages between males' left- and right hand side. Copulation duration was also shorter when males inseminated females with a previous plug rather than females without a plug, and it decreased (just short of statistical significance) with increasing number of times that a female had been previously inseminated. These observations seem to be consistent with the hypothesis that males strategically allocate their ejaculate in response to the risk of sperm competition (see Olsson 2001). Male Iberian rock lizards, upon detecting a plug in the female cloaca, or when copulating with females that had previously copulated several times, may react to the increased risk of sperm competition by increasing the amounts of sperm and/or RSS secretions transferred during copulation. This would result in faster plug delivery (and, consequently, shorter copulation) if higher sperm and/or RSS secretions investment results in faster plug delivery, as argued above. In fact, both increased sperm number (as argued by Olsson 2001) and increased RSS secretion volume (as suggested by plug displacement observations) may be adaptive in terms of sperm competition.

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