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BEHAVIORAL ECOLOGY –ORIGINAL RESEARCH

Experimental evidence that sperm maturation drives protandry in an ectotherm

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Abstract Protandry, i.e., the earlier arrival to breeding areas of males than females, has attracted a lot of scientific attention. However, evidence for the evolutionary hypotheses of protandry is surprisingly scarce. Here, we experimentally manipulate the time of emergence from hibernation of males, relative to females, in the common lizard, *Zootoca vivipara*. We test whether the timing of emergence affects sperm maturation and mating success, to disentangle among proposed selective advantages of protandry. Our results experimentally demonstrate that the timing of emergence affects the date of sperm presence. Moreover, the degree of protandry affected whether males had sperm upon their first encounter with females, but it did not affect the probability of copulating. Mating occurred independent of male fertility and mating during infertility was least common in early emerging males. Early emergence from hibernation by males, relative to females, thus increases the male's chance of fertilising eggs and later emergence from hibernation by females reduces the female's probability of mating with infertile males. These results point to direct reproductive benefits of protandry in males and females, where earlier emergence is predicted to increase the male's opportunities to inseminate mates, and later emergence reduces the female's probability of copulating with infertile males. This suggests that protandry evolved due to the time required for sperm maturation after emergence from hibernation.

Keywords Infertile copulations · Moulting · Sperm maturation · Waiting cost hypothesis · *Zootoca vivipara*

Introduction

Protandry, defined as the earlier arrival of males to breeding areas than females, is common in species with restricted breeding seasons. It exists in plants, arthropods, fish, birds, mammals, amphibians, and reptiles (Morbey and Ydenberg 2001). For example, in birds, males frequently come back from migration earlier than females, and some male fish appear on spawning grounds before females (Alatalo et al. 1986; Møller 2004; Rubolini et al. 2004; Morbey et al. 2012). Similarly, in many insects and spiders, males enclose or moult into adulthood (i.e., become sexually mature) earlier than females (Wiklund and Fagerström 1977; Bulmer 1983; Gunnarsson and Johnsson 1990; Wang et al. 1990; Zonneveld 1996; Lehmann 2012); in amphibians, reptiles, and mammals, males frequently emerge earlier from hibernation than females (Michener 1983; Semlitsch et al. 1993; Olsson et al. 1999); and in various angiosperms, males flower before females (Stanton 1994; Forrest 2014). At least seven hypotheses that explain why protandry may have evolved exist and can be grouped into indirect and direct selection hypotheses (Morbey and Ydenberg 2001). Indirect selection hypotheses suggest that different selective pressures act on the arrival time of males and females, or on traits correlated

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with sex-specific arrival times (e.g., constraint hypothesis; Morbey and Ydenberg 2001), and that protandry itself does not provide reproductive benefits. Direct selection hypotheses assume that protandry provides direct fitness benefits for males or females (Morbey and Ydenberg 2001), and that these maintain the difference in arrival timing between males and females. For example, the mate opportunity hypothesis posits that selection acts on males to arrive earlier than females, given that early arrival maximises mating opportunities and thus provides higher reproductive success (e.g., Kokko et al. 2006). Similarly, the waiting cost hypothesis proposes that selection acts on females to arrive later than males, given that late arrival minimises the female's time spent waiting for males to complete physiological or behavioural preparations required for reproduction, e.g., reproductive maturation and establishment of territories or dominance hierarchies (Morbey and Ydenberg 2001). Theory suggests that later female arrival is safer and energetically cheaper than waiting at the breeding area until males are ready, for example, by reducing predation risk (Morbey and Ydenberg 2001). In addition, waiting costs in females that emerge too early may result from copulation attempts by unprepared (e.g., yet to be fertile) males, which potentially imposes direct costs (e.g., through the physical act of copulation or sexual harassment), from reduced fertilisation success due to male infertility (Olsson and Madsen 1996), or from reduced reproductive success due to the termination of the female's mating window before males reach reproductive maturity (e.g., when the mating window is initiated upon encounter with males; Breedveld and Fitze 2015).

Although protandry has received a lot of scientific attention, surprisingly little evidence exists for its selective advantages. Comparative and non-manipulative studies do not allow disentangling among the proposed evolutionary hypotheses responsible for the existence and maintenance of protandry (Morbey et al. 2012), since in these studies, the causes of the observed relationships are unknown. To identify the selective factors that shape differential timing of arrival, individual-based intraspecific experiments need to compare the relative fitness among males and females in response to the degree of protandry, i.e., the arrival date of individuals relative to their mates (Morbey et al. 2012). Furthermore, current results are inconsistent and experimental studies cannot exclude alternative hypotheses (Morbey and Ydenberg 2001; Morbey et al. 2012). Thus, the selective advantages of protandry remain elusive.

Here, we use an experimental approach to disentangle whether direct selective advantages of protandry exist, and discuss the potential hypotheses explaining the evolution of protandry in our model species, the common lizard, *Zootoca vivipara* (Lichtenstein, 1823). Protandry has been documented in northern and southern populations of common lizards; males emerge from hibernation in spring and females emerge from several days up to several weeks later, marking the onset of the mating season (van Nuland and Strijbosch 1981; Bauwens and Verheyen 1985; Van Damme et al. 1987; Roig et al. 2000). In males, sperm maturation and moulting start after emergence, and an association between the completion of the first annual moult cycle and the onset of male mating behaviour has been proposed (Bauwens et al. 1989; Gavaud 1991b; Roig et al. 2000). This suggests that the temporal difference in emergence allows common lizard males to undergo physiological preparations (sperm maturation, moult completion) before females arrive, indicating that protandry may lead to increased male mating opportunities (mate opportunity hypothesis) or may allow females to avoid waiting for preparing males (waiting cost hypothesis). In addition, if males mate independent of whether they have completed sperm maturation, protandry may decrease costs associated with infertile copulations (Olsson and Madsen 1996).

We experimentally manipulated protandry by entering males into hibernation on three different dates and all females on the same date as the earliest protandry group. In early spring, males of all treatments were emerged after the exact same number of days in hibernation, and females were emerged on the same date as the latest protandry group. Treatment effects (early, intermediate, and late emerged) were tested on the timing of moulting and sperm maturation, and on mating propensity during mating trials. We predicted a directional effect of the protandry treatment on male physiological state, given that both moulting and sperm maturation happen after emergence from hibernation. Specifically, we predicted: (1) earliest moult completion in early emerging males, intermediate moult completion in intermediate emerging males, and latest moult completion in late emerging males, and (2) earliest presence of sperm in early emerging males, later presence in intermediate emerging males, and latest presence of sperm in late emerging males. Under the waiting cost hypothesis and given the suggested association between moulting (and/or sperm maturation) and mating (Bauwens et al. 1989; Gavaud 1991b; Roig et al. 2000), we predicted (3) treatment effects on male mating propensity. Specifically, that early emerged males would mate earlier than intermediate males and that late emerged males would mate latest (in line with delayed physiological preparations in the latter group) and thus that early emerging females would need to wait until males are ready to reproduce. If waiting costs arise due to mating attempts of unprepared (i.e., infertile) males, we predicted (4) reduced mating costs for females presented to early emerged males.

Materials and methods

Species description

The common lizard, *Z. vivipara*, is a small ground-dwelling Lacertidae that occurs in cool, temperate climates, and is widely distributed throughout Eurasia, where it inhabits habitats such as peat bogs and moist heath land (Massot et al. 1992; Peñalver Alcázar et al. 2016). In natural populations, the hibernation period varies between and within locations; hibernation starts between late September and late October, and emergence from hibernation occurs around February–April (Bauwens and Verheyen 1985; Roig et al. 2000; Horváthová et al. 2013; Breedveld and Fitze 2015). Males commonly emerge up to one month before females. However, under prolonged snow cover (i.e., snow cover until late April—mid May), where lizards already appear on small uncovered patches rather than after the snowmelt, females and males emerge almost simultaneously (PS Fitze, personal observations from Roncesvalles and Somport populations). Emergence dates are related to local air temperatures (Horváthová et al. 2013). Specifically, in females, important predictors of emergence are maximum and minimum daily temperatures (Breedveld and Fitze 2015). The mating system is polygynandrous; females lay clutches that are fathered by 1–5 different males and on average by 2 males, and males sire clutches of 1–14 different females and on average of 4 females (Fitze et al. 2005, 2010). During the mating season, males, which are non-territorial, move around to search for females. Females exhibit mate choice with respect to male size, body condition, tail length, and male colour morph, and can fend off unwanted males (Fitze et al. 2010, 2014; Huyghe et al. 2013; San-Jose et al. 2014). Copulations can last up to several hours (mean = 36.7 min ± 3.3 SE; Fitze et al. 2010; Breedveld and Fitze 2016). No female sperm storage occurs from one reproductive season to the next (Bleu et al. 2011). Within-season female sperm storage does occur; second seasonal clutches can be fertilised without re-mating and sperm obtained through re-mating displaces stored sperm (Breedveld et al., unpublished data).

Annual gonadal cycles of *Z. vivipara* are under thermoperiodic regulation (Licht 1972; Gavaud 1991a, b). Spermatogenesis starts after the mating period, develops during summer, and is inhibited by hibernation (Gavaud 1991b). The last step of spermatogenesis, spermiogenesis, is completed after emergence from hibernation, and depends on heat input during daily basking (Gavaud 1991b). Spermatozoa are, therefore, already present in the testis upon emergence; however, they are not mature until they pass to the epididymis, about 2 weeks after emergence, when males become fully fertile (Courty and Dufaure 1980; Gavaud 1991b). The ovarian cycle is induced by the change in thermal conditions from hibernation to spring, i.e., following female emergence (Gavaud 1991a). The ovulation of matured oocytes occurs about 3–4 weeks after mating (Bauwens and Verheyen 1985).

Hibernation

Adult males ($N = 54$) and females ($N = 28$) were captured in the Pyrenees in northern Spain during late September and early October of 2009, close to the end of their activity period. All lizards were transported to the laboratory (Instituto Pirenaico de Ecología, Jaca, Huesca), where body mass, body size (SVL: snout-to-vent-length), and tail length were measured. Lizards were housed in individual terrariums (25 × 15 × 15 cm), males and females assigned to separate shelves, and randomly distributed with respect to the previously measured traits (all $P \geq 0.5$). Lizards were kept under standardised conditions (Fitze et al. 2010), fed every 3 days with moth larvae (*Galleria mellonella,* Pyralidae) and provided with water ad libitum. Light and heat were provided by a 40-W light bulb between 9.00 a.m. and 6.00 p.m., and between 1.00 p.m. and 3.00 p.m. ultraviolet (UV)-B light was provided.

Males were randomly assigned to three protandry treatments: "early emerging males" were entered into hibernation on 14 October, "intermediate emerging males" on 23 October, and "late emerging males" on 27 October 2009. Early, intermediate, and late emerging males were emerged from hibernation on 11 March, 20 March, and 24 March 2010, respectively. Thus, all males hibernated during exactly 148 days. There were no differences in male traits between treatments (all $P \geq 0.3$; mean body mass = 3.34 g ± 0.07 SE, range 2.54–4.07; mean SVL = 52.41 mm ± 0.39 SE, range 47–58; mean tail length = 68.27 mm ± 2.12 SE, range 39–94). All females were entered into hibernation on the same day as the early emerging males, and emerged on the same day as the late emerging males. The 13-day difference in emergence dates between males and females is conservative with respect to natural populations, where males have been observed up to 1 month before females (personal observation).

All individuals were prepared for experimental induction of hibernation by fasting them for 1 week and gradually acclimatising them to dark and cool (15 °C) conditions. Food was last provided on the first morning of the acclimatising week, and heating (by means of a heating cable) was provided for 4.5 h during the first 3 days. This duration corresponds to *Z. vivipara*'s gut passage time (Van Damme et al. 1991), and allowed them to complete digestion and empty the digestive tract prior to hibernation. On the day of entry into hibernation, individuals were given a warm water bath and an abdominal massage to expel any faeces remaining in the digestive system. They were then placed in individual hibernation containers containing humid soil and air holes. Containers were introduced into a dark incubator, where they were maintained at a temperature of 4 °C throughout hibernation. At the end of the hibernation period, lizards were removed from the incubator, returned to standardised laboratory conditions, and body mass, SVL, and tail length, were measured. In summary, all lizards were hibernated under exactly the same conditions and only emergence date varied between male treatment groups.

Moult completion

To determine when males completed their first annual moult (Bauwens et al. 1989), males were continuously examined from emergence until the end of June. The end of moult (hereafter: day of moult completion) was determined when a male had recently shed all large skin flakes of the old epidermal layer, at most small skin flakes remained on extremities of the body, and a bright and shiny colouration of the new epidermal layer could be detected.

Sperm presence

As soon as all males were emerged from hibernation, completion of sperm maturation was determined by the presence of spermatozoa in the distal region of the epididymis (Courty and Dufaure 1980; Depeiges and Dacheux 1985). For this purpose, sperm presence was determined every week during a period of 1 month, and 1 and 2 months after the mating trials had ended (dates: 27 March, 3 April, 10 April, 17 April, 25 April, 29 May, 19 June 2010), by gently pressing on the sides of the cloaca to extract a droplet that was microscopically analysed. The extracted droplet was collected with a micropipette and directly suspended at a 1:10 ratio in Tyrode's medium (136 mM NaCl, 2.6 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 12 mM $NaHCO_3$ 0.4 mM NaH_2PO_4, 5.5 mM glucose). The suspension was gently mixed, aliquots were examined under a microscope, and sperm presence or absence was recorded. This non-invasive method was used to avoid sacrificing animals. Its accuracy has been validated in 12 non-experimental male *Z. vivipara*. In each of these 12 males, the non-invasive method was first performed, and directly afterwards, the male was sacrificed, and presence/absence of sperm in the epididymis determined following dissection. A highly significant positive correlation between sperm presence/absence as determined through the two methods existed ($r_s = 0.837$, $P < 0.001$; MC Breedveld, unpublished result). The day of first sperm presence was defined as the first day on which sperm presence was detected. The duration of sperm presence was defined as the number of days passed between the first and the last time sperm was detected.

Mating experiment

To determine the effect of protandry on mating, mating trials were performed with a subset of 30 randomly selected males and all surviving females. Males were distributed into 10 triplets, each consisting of one early, one intermediate, and one late emerged male. Males of the subset did not differ from the not included males in any of the measured traits ($P > 0.2$). Within triplets, males of the 3 protandry treatments differed only in the time of emergence from hibernation, but not in other traits (triplet modelled as random effect, all treatment effects $P > 0.3$). 20 females were distributed into 10 female pairs, and within pairs females were of similar SVL (average range (biggest to smallest) = 1.5 mm; minimum = 0 mm; and maximum = 3 mm). Female pairs were assigned to a male triplet size-assortatively ($r_s = 0.976$, $P < 0.001$; big females were presented to big males, and small females to small males; Heulin 1988). Mating trials started 1 day after female emergence, which corresponds to the timing of copulation observed in natura (Fitze et al. 2010), and 1, 5, and 14 day(s) after the emergence of late, intermediate, and early males, respectively. Mating trials were conducted every 5 days (dates: 25 March, 30 March, 4 April, 9 April, 14 April, 19 April, 24 April, 29 April 2010) between 9.00 a.m. and 4.00 p.m.

During each mating trial, males of a triplet were presented sequentially to one single female. At the first mating trial, the female was randomly selected, and thereafter, females were presented alternately. Triplets were assigned to one of the six possible male presentation orders (e.g., early, intermediate, and late emerged; or late, early, and intermediate), and each male presentation order was used for one (2 presentation orders) or two (4 presentation orders) triplets. Within triplets, the male presentation order and the time of presentation were the same in all mating trials. Each triplet was presented to both females four times, i.e., within 36 days, 8 mating trials were conducted. Thus, mating trials spanned the pre-ovulatory, ovulatory, and post-ovulatory periods (Bauwens and Verheyen 1985), and the whole period during which maturation and completion of sperm maturation should happen in late emerging males.

All mating trials were performed in escape-proof wooden boxes (2500 cm^2), equipped with two cardboard shelters, a heating rock, a small drinking pond, and light (Fitze et al. 2010). Females were released into the box first and, thereafter, the male was introduced. Males were allowed to interact during one hour, and if lizards were not mating, they were replaced with the subsequent male, corresponding to the average rate of natural mating attempts ($1.1\ h^{-1} \pm 0.9$ SE; Heulin 1988). If individuals were still mating after 1 h, they were allowed to complete copulation, and removed 5 min after copulation ended. Video cameras allowed constant observation without disturbing the lizards and provided recordings of all copulations, from which the following variables were derived. To analyse protandry effects on mating opportunities, the day when a male's first copulation occurred (hereafter: day of first mating) and the number of mating trials during which a male copulated (hereafter: number of times copulated) were determined. To analyse potential mating costs for females, sperm presence during copulation and protandry effects on sperm presence were analysed. Sperm presence and sperm absence during copulation were defined using a conservative approach: sperm presence was defined as sperm presence before and after a mating trial, while sperm absence was defined as sperm absence before and/or after a trial. Note that sperm presence before and sperm absence after the trial did not occur. For the first mating trial, sperm presence was only determined after the trial.

Statistics

Day 1 was defined as the calendar day on which the first lizards emerged from hibernation (11 March 2010 = day 1). One male did not moult during the entire experiment and was, therefore, excluded from the analyses. The day of moult completion and the time from emergence until moult completion were analysed using generalized linear models (GLMs) with a Poisson error and a log link function, including treatment as a factor. Ordered heterogeneity (OH) analyses (Rice and Gaines 1994) were performed, here as well as in the following analyses, to test the predicted directional hypotheses; namely, that early emerging males were earliest, intermediate males later, and late emerging males latest.

The day of first sperm presence, the time from emergence until first sperm presence, and the duration of sperm presence were analysed using GLMs with a Poisson error and a log link function, including treatment as a factor and the day of moult completion as a covariate. The probability of sperm presence during the first mating trials was analysed using a GLM with binomial error and a logit link function, with treatment as a factor. The probability of mating (i.e., whether a male did or did not mate at least once) was analysed using a GLM with a binomial error and a logit link function. Treatment was included as a factor, and day of first sperm presence and day of moult completion were included as covariates. The day of first mating and the time from emergence until the first mating were analysed using GLMs with a Poisson error and a log link function, including treatment as a factor and the day of moult completion and the day of sperm presence as covariates. The probability of mating before moult completion and the probability of mating before sperm presence were analysed using GLMs with binomial error and a logit link function, with treatment as a factor and the day of moult completion as a covariate. The number of times a male copulated was analysed using GLMs with a Poisson error and a log link function, including treatment as a factor and the day of moult completion as a covariate.

All two-way interactions between treatment and covariates were included in the initial models. Quasi-Poisson or quasi-binomial GLMs were used in the presence of overdispersion. Model selection was performed using likelihood ratio tests and type III SS, and non-significant terms were backward eliminated ($P > 0.05$). Model assumptions were verified in all cases, including collinearity, which did not exist in any of the models. Data were analysed in R 3.0.0 (R Core Team 2013).

Results

Moult completion

The day of moult completion differed significantly among treatments ($F_{2,40} = 8.620$, $P < 0.001$, Fig. 1a), and it significantly followed the predicted order (OH: $r_sP_c = 0.999$, $P < 0.001$). Early emerging males moulted earlier than intermediate emerging males and late emerging males moulted latest (Fig. 1a). Differences were significant between late and intermediate males (individual contrast: $P = 0.012$) and late and early emerging males ($P < 0.001$). The time from emergence from hibernation until moult completion did not significantly differ among treatment groups ($F_{2,40} = 2.008$, $P = 0.148$).

Sperm presence

The day of first sperm presence differed significantly among treatments ($F_{2,34} = 4.841$, $P = 0.014$, Fig. 1b), and it significantly followed the predicted order (OH: $r_sP_c = 0.986$, $P < 0.010$). In early emerging males, the day when sperm was present for the first time was earlier than in intermediate emerging males and late emerging males had sperm present latest (Fig. 1b). In late emerging males, the day of first sperm presence was significantly later than in early emerging males ($P = 0.010$), and it tended to be later than in intermediate emerging males ($P = 0.092$). There was no significant effect of the day of moult completion ($F_{1,33} = 0.891$, $P = 0.352$) and no significant interaction between treatment and the day of moult completion ($F_{2,31} = 0.221$, $P = 0.803$). The time from emergence from hibernation until the day of first sperm presence did not significantly differ among treatment groups ($F_{2,33} = 0.430$, $P = 0.654$), and day of moult completion and its interaction with treatment were not significant ($P \geq 0.2$). On the day that males were presented to a female for the first

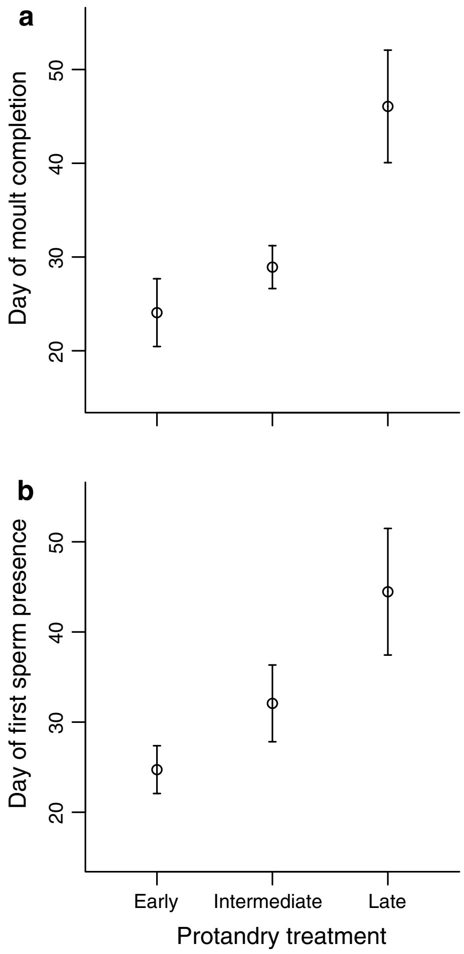

Fig. 1 **a** Average ± SE day of moult completion and **b** average ± SE day of first sperm presence in *Zootoca vivipara* males, for each of the three treatment groups. Day 1 refers to 11 March 2010

time, the probability of sperm presence was significantly affected by treatment ($\chi^2_2 = 10.1$, $P = 0.006$). 40 % of the early males had sperm present, while none of the intermediate and late males had sperm present. The duration of sperm presence (mean = 23 days ± 3 SE, range 1–78) was not significantly affected by treatment ($F_{2,34} = 1.382$, $P = 0.265$), or the day of moult completion ($F_{1,33} = 2.260$, $P = 0.142$), and there was no significant interaction ($F_{2,31} = 1.291$, $P = 0.289$).

Mating

The probability of mating was significantly and negatively affected by the day of moult completion ($\chi^2_1 = 4.618$, $P = 0.032$, Fig. 2). However, when removing two outliers (males that moulted very late; see Fig. 2), the effect was no longer significant ($P = 0.343$), indicating that mating did not depend on moult completion. Treatment ($\chi^2_2 = 4.234$, $P = 0.120$), the day of first sperm presence ($\chi^2_1 = 0.257$, $P = 0.612$), and the interactions ($P \geq 0.2$) were not significant. The day of first mating was not significantly affected by treatment ($F_{2,8} = 0.336$, $P = 0.724$), the day of moult completion ($F_{1,10} = 0.018$, $P = 0.897$), or the day of first sperm presence ($F_{1,11} = 2.376$, $P = 0.152$). The time from emergence from hibernation until the day of first mating was not significantly affected by treatment ($F_{2,10} = 2.896$, $P = 0.102$), the day of moult completion ($F_{1,8} = 0.331$, $P = 0.581$), or the day of first sperm presence ($F_{1,9} = 3.159$, $P = 0.109$).

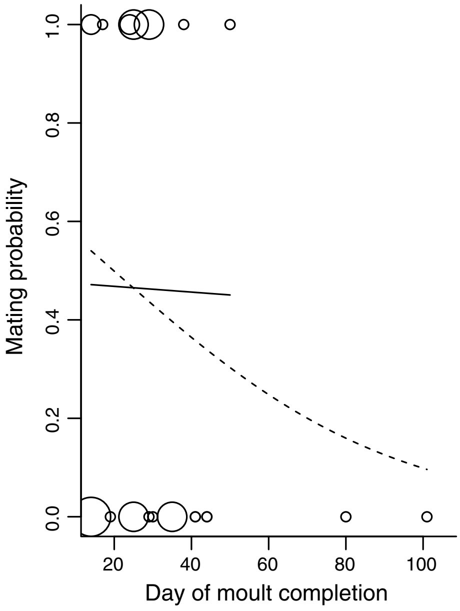

Fig. 2 Probability of mating in relation to the day of moult completion. The *hatched line* corresponds to the prediction when including and the *solid line* to the prediction when excluding the two males with extreme values for day of moult completion and *dot size* corresponds to sample size. Day 1 refers to 11 March 2010

31 % of males copulated before they completed moult. The probability of mating before moult completion was positively related with the day of moult completion ($\chi^2_1 = 12.229$, $P = 0.001$, Fig. 3). Early completing males did not copulate before moult completion, while males that completed moult late copulated with the females, independent of moult completion. Treatment and the interaction with day of moult completion were not significant ($P \geq 0.9$). 69 % of males copulated before they first had sperm present, i.e., during infertility. The probability of copulating before sperm presence was independent of treatment, day of moult completion, or their interaction ($P \geq 0.1$). The number of times a male copulated did not significantly differ between treatment groups ($F_{2,9} = 1.794$, $P = 0.221$), and day of moult completion and the interaction were not significant ($P \geq 0.5$).

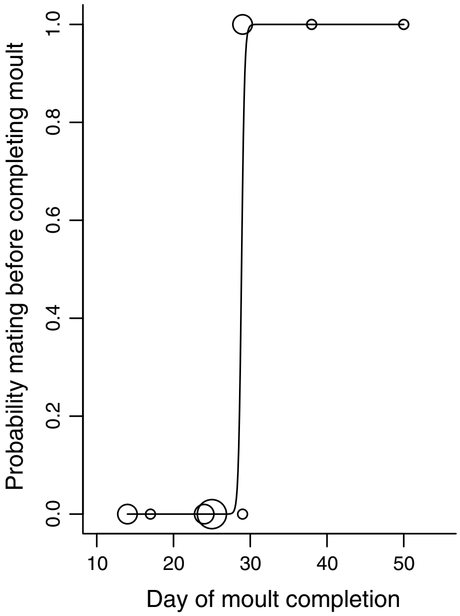

Fig. 3 Probability of mating before moult completion in relation to the day of moult completion. The *line* corresponds to the model prediction and *dot* size corresponds to sample size. Day 1 refers to 11 March 2010

Discussion

Protandry is very common and observed in a wide range of species covering large parts of the tree of life [reviewed in Morbey and Ydenberg (2001)]. As a result, it has attracted a lot of interest and theoretical models have tackled its evolution (e.g., Wiklund and Fagerström 1977; Bulmer 1983; Iwasa et al. 1983; Parker and Courtney 1983; Zonneveld and Metz 1991; Rubolini et al. 2004; Kokko et al. 2006; Spottiswoode et al. 2006). Several hypotheses exist and direct selective advantages have been put forward. However, conclusive experimental evidence remains elusive (Morbey and Ydenberg 2001; Morbey et al. 2012). Here, we provide experimental evidence for direct selective advantages of protandry. Our experimental manipulation affected the date of moult completion and the date of first sperm presence. In early males, moult was completed first and sperm was present first, and in late males, both events occurred last (Fig. 1). These findings corroborate the directional predictions that moulting (prediction 1) and sperm maturation (prediction 2) are temporally related to the date of emergence from hibernation. Moreover, there was no significant difference between protandry treatments in the time from emergence from hibernation until moult completion or until first sperm presence. In other words, the speed of physiological preparation was not plastic, and thus, males were unable to compensate for late emergence, consistent with the presence of an endogenous program that determines moult and sperm maturation, which is initiated at emergence (Gavaud 1991b). This result and the fact that part of the early, but no intermediate and late males had sperm present when presented to females for the first time, shows that the earlier males emerge from hibernation the more likely they are physiologically prepared when females emerge and mating starts. This physiological advantage may result in increased mating opportunities and higher reproductive success of early compared to late males, given that the latter may have reduced access to reproduction due to the female's short mating window (Breedveld and Fitze 2015) and may face higher sperm competition due to the increased number of reproductively active males.

Interestingly, the day that a male first mated as well as the probability that a male mated were independent of treatment, the day of moult completion, and the day of first sperm presence. This shows that no association between male physiological state and mating propensity existed, in contrast with prediction 3. This and the absence of a treatment effect on the time interval between emergence from hibernation and first copulation is in line with the occurrence of mating without functional sperm. Indeed, 80 % of copulating males of both the late and intermediate group, and 33 % of the males of the early group, had no sperm present during at least one copulation. These findings show that early emerging females (i.e., females emerging at the same time as males) run the risk of mating with infertile males, pointing to waiting costs due to mating attempts of unprepared males (prediction 4). Early emerging females that mate with infertile males may not produce fertilised eggs, given their relatively short copulation-induced window of receptivity (Breedveld and Fitze 2015), which potentially refrains females from mating once fertile males become available. These results are congruent with findings in sand lizards (*Lacerta agilis*), where females mated with early compared to late emerged males exhibited higher fertilisation success (Olsson and Madsen 1996), and suggest that advanced sperm maturation in early males was likely responsible for these findings. Moreover, early emerging females may also be exposed to prolonged copulation attempts or increased sexual harassment (Fitze et al. 2005; Le Galliard et al. 2005), potentially imposing additional waiting costs. We found no association between mating and sperm presence, indicating that female common lizards either cannot detect male fertility (and do not adjust their expression of receptive behaviour) or that they cannot resist copulation attempts by males with immature sperm. In contrast, sand lizard females courted by early compared to late emerged males became receptive sooner (Olsson and Madsen 1996), suggesting that sand lizard females, but not common lizard females, may be able to assess sperm maturity.

An association between the completion of the first annual moult and the onset of male mating behaviour has been suggested (Bauwens et al. 1989). Our results show that moult completion takes time, but no significant effect of moult completion on either mating probability or the day of first mating was found. Moreover, the probability of mating before moult completion depended on the date of moult completion (Fig. 3). Males that moulted early did not copulate before moult completion and almost 100 % of males that moulted late copulated before completing moult. Therefore, our results indicate that even though moulting may usually occur before mating in the field, moult completion is not a requirement for copulation.

In summary, the here-presented results provide experimental evidence for direct fitness consequences of protandry in males and in females, and are in line with protandry resulting from time constraints of reproductive maturation in males. On the one hand, early male emergence, relative to female emergence, can be beneficial to males. This is because early emerged males that mate can benefit from an advanced stage of sperm maturation, resulting from an earlier onset of spermiogenesis (Olsson et al. 1999). Earlier emergence, thereby, increases the probability of providing viable sperm, i.e., of fathering offspring. At the same time, late female emergence, relative to male emergence, can be beneficial to females. The probability of mating with infertile males will be smaller in late emerging females, and costs resulting from the physical act of copulation or sexual harassment will be reduced. This suggests that different selective forces may operate simultaneously and independently in each sex. Reduced male fertility or temporal infertility, and evidence for the occurrence of mating with infertile males, exists in many species exhibiting protandry; e.g., birds (Sheldon 1994), mammals (Gomendio et al. 2007), reptiles (Olsson and Shine 1997), and insects (García-González 2004). Moreover, in a species where sperm production starts and copulations happen months after emergence, the Tasmanian snow skink, *Niveoscincus microlepidotum*, no protandry has been detected (Olsson et al. 1999). This suggests that the time constraints imposed by sperm maturation may be an important reason for the evolution of protandry.

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Author contribution statement MCB and PSF designed the experiment, MCB performed theexperiments, MCB and PSF performed the statistical analyses and wrote the manuscript.

Compliance with ethical standards

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