Determinants of male fitness: disentangling intra- and inter-sexual selection

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Abstract

Both intra- and inter-sexual selection may crucially determine a male's fitness. Their interplay, which has rarely been experimentally investigated, determines a male's optimal reproductive strategy and thus is of fundamental importance to the understanding of a male's behaviour. Here we investigated the relative importance of intra- and inter-sexual selection for male fitness in the common lizard. We investigated which male traits predict a male's access to reproduction allowing for both selective pressures and comparing it with a staged mating experiment excluding all types of intra-sexual selection. We found that qualitatively better males were more likely to reproduce and that sexual selection was two times stronger when allowing for both selective pressures, suggesting that inter- and intra-sexual selection determines male fitness and confirming the existence of multi-factorial sexual selection. Consequently, to optimize fitness, males should trade their investment between the traits, which are important for inter- and intra-sexual selection.

Introduction

Optimal male strategy crucially depends on the mating system (Andersson, 1994; Shuster & Wade, 2003). In mating systems where intra-sexual selection through male–male competition is important, males preferentially invest in fighting abilities, for example in well-developed weapons. In contrast, in mating systems where inter-sexual selection through female choice prevails, males invest in the preferred traits, for example long tails, bright colouration or exaggerated displays. Consequently, the mating system may lead to the evolution of specific male life-history strategies (Andersson, 1994).

In polygynous mating systems where males provide females with little more than sperm, it is widely accepted that male fitness importantly depends on the number of mating partners (Bateman, 1948). It is however much less clear what determines the number of mating partners and thus how the optimal male strategy is deter-

mined. In contrast to birds, in fish, reptiles, anurans and insects, inter-sexual selection, e.g. through female choice, seems to be rare and intra-sexual selection through male–male competition is suggested to be predominant (e.g. in fish: Gross, 1985; lizards: Tokarz, 1995; anurans: Halliday, 1998; insects: Emlen, 1996; but see Andersson, 1994 for birds). In species where intra-sexual selection has been documented, it is usually unknown whether inter-sexual selection is important and vice versa. Consequently, unless experimental studies investigate, within the same species, the presence or absence of the different types of sexual selection and their interplay, the optimal male reproductive strategy will be unknown.

In this study we investigated whether intra- and inter-sexual selection coexists and their relative importance for a male's fitness. We used the common lizard (*Lacerta vivipara* Jacquin, 1787) as the model system. The common lizard is a small ovoviviparous lizard that has a polygynandrous mating system (Laloi *et al.*, 2004; Fitze *et al.*, 2005; Richard *et al.*, 2005). Earlier studies indicate that intra-sexual selection may importantly determine male reproductive success (Heulin *et al.*, 1988) and that

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inter-sexual selection may also be important (Richard *et al.*, 2005). To assess the role of intra- and inter-sexual selection, we performed three different studies. First, we assessed in six independent experimental populations the paternity of each offspring, using microsatellite genotyping. This corresponds to a situation where intra- and inter-sexual selection may contribute to male reproductive success. The use of enclosed natural habitat guaranteed unaltered social interactions (Laloi *et al.*, 2004) and allowed ascribing paternity and maternity with certainty, because the genetic profile of all animals present was known. In the second study we excluded intra-sexual selection using a staged mating experiment. By presenting a single male to a single female, we tested whether inter-sexual selection alone might cause non-random mating patterns. We then compared the selection acting on males in the two setups, to estimate the relative importance of inter- and intra-sexual selection. We applied this experimental design, because intra-sexual selection can only be indirectly measured, as it is not possible to completely exclude inter-sexual selection by mate choice, when quantifying the selection acting on fitness-relevant traits such as copulations or fertilizations (Bradbury & Davies, 1987). Thirdly, we presented three males to a female either sequentially or together. In both treatments females were presented to males for the same amount of time and male and female behaviour recorded. The third study allowed us to quantify the behaviour and to confirm the results obtained in the two previous studies.

We predicted random mating patterns in the second study if non-random mating in the first study is a consequence of intra-sexual selection only. However, the mating patterns should be similar in both studies if non-random mating is the consequence of inter-sexual selection. For the third study we predicted that females to which males were presented simultaneously copulate with less number of males compared with sequential presentation, if intra-sexual selection is present. Furthermore, male fights should be observed and winners should be more likely to copulate than losers. If only inter-sexual selection would be relevant, no differences in the number of copulation partners would be observed. To further distinguish between the different types of inter-sexual selection, we investigated whether male mate choice, female mate choice, or male sexual harassment exists.

Method

Species description

The common lizard (*L. vivipara*) is a small ovoviviparous Lacertidae that inhabits peat bogs and moist heath land (Massot *et al.*, 1992). Both males and females have nonexclusive territories (Richard *et al.*, 2005). Males emerge from hibernation between February and March,

approximately 1 month earlier than females. After female emergence the mating period starts. In this species, adult males are dominant over 1-year-old males (Lecomte *et al.*, 2004; Richard *et al.*, 2005), and fights among males can be observed (Heulin, 1988; J. Clobert personal observations). This indicates that intra-sexual selection through male–male competition for access to females exists (see also Richard *et al.*, 2005), which is believed to be the norm in reptiles (Tokarz, 1995; LeBas & Marshall, 2001; LeBas, 2002). Common lizards do not provide parental care or nuptial gifts (Heulin, 1988; Clobert *et al.*, 1994; Léna & de Fraipont, 1998) and males provide females only with sperm of low energy content (Depeiges *et al.*, 1987). Hence, the female's benefits of copulating with several males might be only indirect (but see Fitze *et al.*, 2005), reducing the scope for female mate choice. Some observations indicate that female choice may exist, as in a recent experiment the proportion of polyandrous females was found to be unaffected by the population sex ratio (Fitze *et al.*, 2005). This is in line with the three studies showing that in reptiles inter-sexual selection through female preferences for bigger sized males and thus through directional female mate choice exists (Cooper & Vitt, 1993; Censky, 1997; Shine & Mason, 2001). Recent studies further suggest that male aggression may importantly determine male and female fitness (Fitze *et al.*, 2005; Le Galliard *et al.*, 2005a). Similar to almost all except two reptile species (Orrell & Jenssen, 2002), it is not known whether common lizard males prefer specific female traits and thus whether inter-sexual selection via male mate choice exists.

Common lizard males may father offspring of up to 14 different females while females give birth to offspring of up to five different males (Laloi *et al.*, 2004; Fitze *et al.*, 2005). Copulation lasts up to several hours (Richard *et al.*, 2005) and may be quite violent, since a male first grips the female on the posterior abdomen with its mouth, thereby producing mating scars that can be seen even after several weeks (Bauwens & Verheyen, 1985; Fitze *et al.*, 2005).

Field study

Experimental setup
To measure male reproductive success under semi-natural conditions we created six independent lizard populations in July 2002 at the Ecological Research Station of Foljuif (Seine-et-Marne, France, 48°17′N, 2°41′E). Lizard populations were set up in 100 m² big enclosures, the size of which corresponds to the average female's home range. Enclosures, surrounded by plastic walls to prevent lizards from escaping (for more details, see Boudjemadi *et al.*, 1999a), contained natural vegetation, hides, rocks and two ponds (for more details see Lecomte *et al.*, 2004). Predation was avoided by using mist nets to exclude avian predators and by trapping shrews outside and inside the enclosures as to make the

two experiments comparable. This set-up allowed assessing paternity and maternity in all cases, as all potential fathers and all pregnant mothers are known. Furthermore, it ensures that the social interactions are unaltered compared with a natural population without spatial limitation (Laloi *et al.*, 2004).

In July 2002, we released in each enclosure four adult males and 14 adult females, six yearling males and six yearling females and 20–24 juveniles of each sex. The initial densities, the age structure and the adult sex ratio correspond to the values observed under natural conditions (Massot *et al.*, 1992; Le Galliard *et al.*, 2005b). In late May 2003 we recaptured all surviving lizards and ensured that all live lizards were captured by regularly surveying each enclosure during the 2 weeks following the initial capture. Subsequent to the capture we measured the body mass of all lizards and the snout–vent length (SVL). These measurements were highly repeatable as evident from two repeated measurements on 228 lizards [repeatability (*r*): SVL: $F_{227,227} = 75.698$, $P < 0.0001$, $r = 0.97$; body mass: $F_{277,277} = 96.04$, $P < 0.0001$, $r = 0.98$]. Unlike in the staged mating experiment described below, we did not recapture the lizards at the start of the mating season for two reasons. First, capturing lizards during the mating season may significantly affect the reproductive success of both males and females, as under the male–male competition scenario catching the most competitive male first, may lead to a reproductive advantage of the less competitive ones. Spring captures could potentially alter mating patterns. Secondly, in a previous study (Le Galliard *et al.*, 2005c), the characteristics of lizards captured in spring (early April) significantly correlated with those measured in May [repeatability (*r*) of individual measurements taken in April and May: SVL: $F_{223,224} = 5.35$, $P < 0.0001$, $r = 0.68$; body mass: $F_{223,224} = 10.46$, $P < 0.0001$, $r = 0.83$], and the survival within this period was high (96.2%) and not trait-dependent (SVL: $F_{1,131} < 0.001$, $P = 0.963$; body mass: $F_{1,131} < 0.001$, $P = 0.957$). This shows that the traits measured in April significantly predict those measured in May.

Captured females were individually maintained in numbered terraria (terraria size: $25 \times 15 \times 15$ cm) under standardized conditions (heat, light, water and food) until parturition. Terraria were layered with soil and equipped with a small water pond and two types of hides. Every 4 days lizards were fed with moth larvae (*Pyralis* sp.) and we provided them with water *ad libitum* (for further details, see Le Galliard *et al.*, 2003). After a female gave birth we carefully searched the terrarium for live juveniles and eggs. Thereafter, females and juveniles were released into the outdoor enclosures.

Paternity assignment
We collected a small part of the tip (1 mm) of the re-growing tail of each offspring and of each lizard (before

release). Each egg without a visible embryo was collected and all genetic samples were immediately stored in 70% ethanol, until DNA extraction. We extracted DNA of all collected samples using Perfect gDNA Blood Mini Isolation kit (Eppendorf, Hamburg, Germany). Thereafter we identified the putative fathers using five highly polymorphic microsatellite DNA loci (Lv-3-19, Lv-4-72, Lv-4-alpha, Lv-4-X, and Lv-4-115; Boudjemadi *et al.*, 1999b). The exact method used for the extraction, the polymerase chain reaction (PCR), and the determination of the allelic size is described elsewhere (Laloi *et al.*, 2004). For each enclosure, separate paternity assignments were performed using Cervus 2.0 (Marshall *et al.*, 1998). Because the genetic profile of the mothers and of all potential fathers was known, the program was simply used to facilitate the attribution of the genetic father. One female (clutch size = 4) laid an unfertilized clutch and another laid one unfertilized egg. All other offspring were successfully attributed to a single father.

Staged mating experiment

Pre-experimental conditions
In July 2001 and July 2002 we introduced male and female lizards (*L. vivipara*) into empty 100-m^2 big outdoor enclosures at the Ecological Research Station of Foljuif, which were similar to those described above. Males and females were released in separate enclosures containing no individuals of the opposite sex, to prevent lizards from uncontrolled mating. In 2001, females were released into three and males into four different enclosures, and in 2002 five enclosures were used for females and six for males. We released approximately 40 adult lizards per enclosure.

Laboratory conditions
In early spring the enclosures were regularly inspected to register male and female emergence. We also monitored other enclosures, containing both male and female lizards, to determine the natural onset of the mating period. Mating activity was determined by the presence of mating scars present on the female's belly (Bauwens & Verheyen, 1985). When the first females with mating scars were detected, we started capturing the lizards from the experimental enclosures. Subsequent to the capture, we measured body mass and SVL. All captured lizards were introduced into numbered terraria and individually maintained under the same standardized conditions as described above. A same lizard stayed in the same terrarium during the entire experiment. To make sure that no interactions happened between sexes before the staged mating experiment males and females were kept on separate shelves.

Experimental method
In 2002, the mating experiments lasted from 31 March to 8 April and in 2003 from 7 to 15 April. At the start

of the experiment, we introduced randomly chosen females into escape-proof wooden boxes (2500 cm^2). Because only a limited number of boxes and females were available, not all males could be presented to a female directly after capture. For males, the mating experiments therefore started on average (± SE) 4.15 ± 0.19 days and for females, 3.36 ± 0.23 days after capture. There was no correlation between the number of days a male stayed in the laboratory before the mating experiments started and his SVL ($F_{1,198} = 0.877$, $P = 0.350$). This correlation was as well nonsignificant in females ($F_{1,94} = 0.947$, $P = 0.333$), showing that both males and females were well randomized. Each wooden box contained a shelter and a 40-W bulb, which provided light and heat. To mimic natural daylight that consists of UV light also, we illuminated the wooden boxes with a UV light source (Iguana Light 5.0 UV-B, 40 W; ZooMed Laboratories, Inc., Sacramento, CA, USA).

We first released a female in the wooden boxes and 2–4 min later a randomly selected male was introduced in each box. Mating experiments started at 09:00 hours and the last experiment started no later than 17:00 hours. After introduction, we observed the lizards for 1 h to determine the start, the end and the number of copulations. These data allowed the measuring of copulation duration with a precision of 3 min. A male–female encounter was defined as copulation when the male gripped the female with the mouth on the posterior abdomen, when he successfully twisted his body around her, and when his hemipenis penetrated the cloaca. If copulations were not yet finished after 1 h, we waited until they ended. After 1 h, or 5 min after the end of the copulation, males were removed from the female's box. Males were replaced in their terraria and they were presented to a new unknown female on average 1.3 ± 0.05 days later (range: 26 min to 6 days). If males did not copulate with any female after presenting at least five different females (on average 5.2 ± 0.2 females), we stopped presenting them to new females. A male was never presented twice to the same female and he was allowed to copulate with maximally three different females, because males sire offspring of up to three different females (see female-biased populations, Fitze *et al.*, 2005). The first successful copulation of a male is hereafter referred to as 'first copulation'. If a male thereafter copulated with a second female, this copulation is referred to as 'second copulation'. After the experiments all lizards were released into the outdoor enclosures where they were captured previously. To determine fertilization success we recaptured all surviving females in late May 2003. The capture and all procedures applied thereafter were the same as those applied to the females of the field study.

Combined mating experiment

In April 2007, we regularly inspected female lizards to determine the onset of the mating activity as in the staged mating experiment. On 18 April we found the first female with mating scars. Thereafter, on 18 April and 19 April 2007 we captured 22 females and 66 males in Roncesvalles (Navarra, Spain). All captured lizards were introduced into numbered terraria and individually maintained under the same standardized conditions as described above.

The combined mating experiment lasted from 23 to 30 April 23 and was conducted at the Instituto Pirenaico de Ecología in Jaca (Huesca). At the start of the experiment, we introduced randomly chosen females into escape-proof wooden boxes as for the staged mating experiment. Females were attributed to two different treatment groups. Either we presented to a female three randomly chosen males at once for 3 h, hereafter referred to as 'grouped' treatment, or sequentially presented three randomly chosen males, hereafter referred to as 'sequential' treatment. In the sequential treatment, we first presented a male to a female for 1 h. Thereafter we replaced the male by a second male, which remained for another hour in the mating arena before he was replaced by a third male. The third male remained as well for 1 h in the mating arena. Consequently, there were no differences between treatments in the amount of time a female spent with males and there were also no differences between females attributed to the sequential or grouped treatment (SVL: $F_{1,20} = 0.046$, $P = 0.832$; body mass: $F_{1,20} = 0.045$, $P = 0.835$; body condition: $F_{1,19} = 0.002$, $P = 0.963$). There were no significant differences in SVL or body condition between males presented to females sequentially or grouped (mean SVL: $F_{1,20} < 0.001$, $P = 1$; mean body mass: $F_{1,20} < 0.001$, $P = 1$). To avoid pseudoreplication, each female and each male was used only once. The presentation protocol, the timing and the other experimental parts were the same as for the staged mating experiment.

During the entire experiment we recorded the male's dominance and copulation behaviour. We recorded for each male the number of times he chased other lizards. Chasing was defined as an approach towards another individual, which resulted in fleeing of the other individual. This measure includes three levels of interactions. First, a male approaches another lizard and the other lizard flees as a consequence. Secondly, a male approaches another male. The other male flees and the approaching male pursues it. Thirdly, a male approaches another male and bites him. As a consequence the bitten male flees. We also recorded for each male the number of times he bit another lizard. These measurements were taken before the first copulation and after the first or second copulation, depending on whether a second copulation occurred.

Statistical analysis

Field study

Eighty-five males were recaptured at the end of May 2003. Paternity analysis revealed that all except 10 males that fathered offspring were recaptured. These 10 males must have died between the copulation and the end of May, because we searched for live lizards for 2 weeks, and because all live lizards were captured within the first 2 days. Consequently, no spring measurements could be obtained and thus only the 85 recaptured males whose traits could be measured, were included in our analyses.

Staged mating experiment

For the second experiment we used a total of 200 different males and 96 different females. As in the first experiment the probability of copulating was modelled using the PROC GLIMMIX procedure. The covariates (SVL and body mass) were simultaneously introduced and the enclosure of origin, the year and their interactions with the covariates were included as random effects.

For the analyses of body condition in nonparametric tests (e.g. Spearman's rank correlations) we used the residuals of the regression with SVL as independent and body mass as the dependent variable. In several analyses dealing with the male's body mass the degrees of freedom are reduced because the measurements of four males were missing. For some models the assumptions were not met even after transformation. Consequently, we applied Spearman's rank-order correlations (SRC) instead of parametric regression analyses, or Wilcoxon's signed-ranks test (WSR) for the analysis of paired samples. In WSR, sample sizes may considerably vary among tests because of pair-wise differences equalling zero (Siegel & Castellan, 1988). The repeatability (r) of the mate partner's traits was calculated according to Lessells & Boag (1987).

Statistical analysis used in all three studies

In the field study the probability of reproducing and in the laboratory mating experiments the probability of copulating were modelled using the PROC GLIMMIX procedure in SAS with a binomial error distribution and a logit link (Littell *et al.*, 1996). In the field study and the staged mating experiment the starting model included SVL and body mass as covariates, enclosure and year as random factors, as well as the interactions between the enclosure and the covariates, and the interactions between year and the covariates. In the combined mating experiment the model included SVL and body mass as covariates, female as random factor, and the interactions between female and the covariates. Nonsignificant interactions and covariates were backward eliminated. To check for stabilizing or disruptive selection we modelled the different covariates as well as quadratic terms (Lande & Arnold, 1983). Standardized logistic selection gradients were calculated according to

Janzen & Stern (1998). Body size and body mass were usually positively correlated (e.g. in the field study: SVL and body mass were positively correlated with each other; $F_{1,83} = 321.831$ $P < 0.001$). Consequently, the final model obtained by backward elimination could simply have arisen because of collinearity (Quinn & Keough, 2002). However, if the final model derived using forward selection coincides with that one given by backward selection, there will be no risk that the results arose because of collinearity (Quinn & Keough, 2002). We therefore derived models from forward selection and state for each model whether forward selection led to the same results. The assumptions of the statistical models were verified in all cases (Quinn & Keough, 2002).

Results

Field experiment

In 2003, we recaptured 85 males: 48 males (56.5%) fertilized eggs and the remaining 37 males did not fertilize a single egg. The probability of fertilizing eggs increased with increasing male body mass (Table 1, Fig. 1). Male body size did not significantly predict the probability of fertilizing eggs (Table 1) and the enclosure effect and interactions were not significant (all interactions $z < 0.001$, $P > 0.99$). A model using forward selection led to the same final model, showing that there existed no collinearity problem (for more details, see section Method). Quadratic terms were not significant (SVL^2: $F_{1,72} = 0.25$, $P = 0.618$; body $mass^2$: $F_{1,73} = 0.48$, $P = 0.491$) and also their interactions with enclosure were not significant (all interactions $z < 0.001$, $P > 0.99$).

Staged mating experiment

Probability of copulating and fertilization success

Of the 200 males used during this experiment 121 (60.5%) copulated with a female. The probability that a male copulated with a female increased with increasing male body mass (Table 2a; Fig. 2). Male body size, year and enclosure did not significantly affect the probability of copulating (Table 2a). Similarly, the interactions between year or enclosure and the covariates were all

Table 1 Probability that a male reproduced in relation to its body size and body condition.

Trait	Test statistic	*P*-value	Estimates (± SE)	Selection gradient (± SE)
Body mass	$F_{1,83} = 25.58$	<0.001	2.427 ± 0.480	0.467 ± 0.095
Body size (SVL)	$F_{1,82} = 0.93$	0.338	0.164 ± 0.170	0.079 ± 0.154
Enclosure	$z = 0$	1		

Data from the field study are shown.
The results of a GLIMMIX model, the logistic estimates and the standardized selection gradients are given.
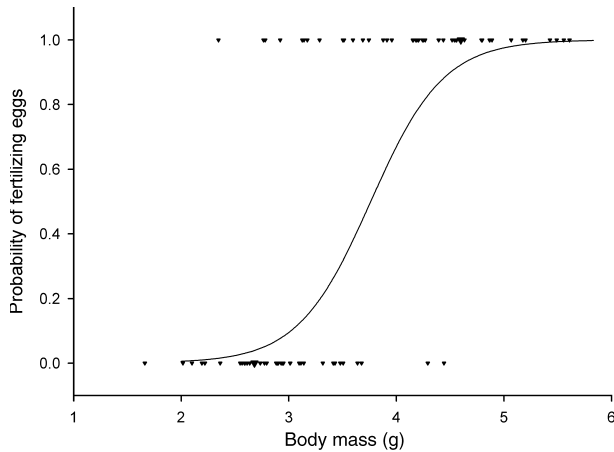
Fig. 1 Probability of fertilizing at least one egg in relation to body mass. The dot size corresponds to the sample size (small dots $n = 1$; large dots $n = 2$). The line corresponds to the predicted relationship between body mass and the probability of copulating.
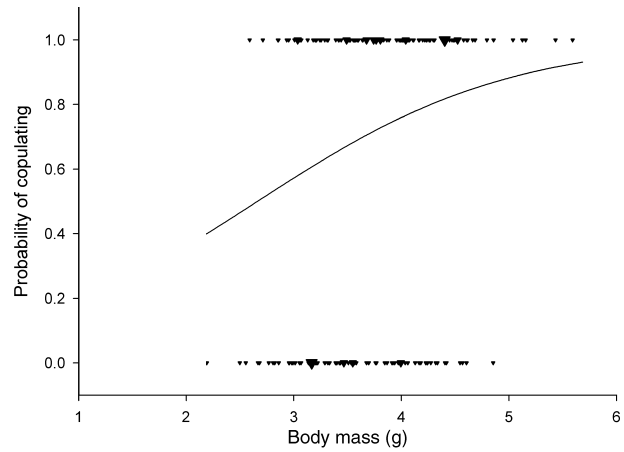

Fig. 2 Probability of copulating with a female in relation to body mass. The dot size corresponds to the sample size (smallest dot $n = 1$; biggest dot $n = 3$). The line corresponds to the estimated relationship between the body mass and the probability of copulating.

not significant (enclosure × SVL: $z < 0.001$, $P > 0.99$; enclosure × body mass: $z = 0.91$, $P > 0.180$; year × SVL: $z < 0.001$, $P > 0.99$; year × body mass: $z < 0.001$, $P > 0.99$). A model using forward selection led to the same final model, showing that there existed no collinearity problem. Quadratic terms were not significant (SVL²: $F_{1,188} = 0.12$, $P = 0.727$; body mass²: $F_{1,186} = 0.02$, $P = 0.882$) and also their interactions with enclosure or year were not significant (all $P > 0.1$). The probability that a male fertilized eggs increased with its body mass ($F_{1,194} = 4.23$, $P = 0.041$; selection gradient 0.219 ± 0.108). Similar to that for the probability of copulating, body size ($F_{1,186} = 0.01$, $P = 0.970$; selection gradient 0.048 ± 0.164), year ($z = 0.52$, $P = 0.301$) and enclosure ($z = 0.72$, $P = 0.235$) did not significantly affect the probability of fertilizing eggs.

A statistical model including both the data from the field study and the fertilization data from the staged mating experiment shows that the two slopes were significantly different [interaction body mass × treatment: $F_{1,277} = 11.29$, $P = 0.0009$, estimate (field study) = 1.882 ± 0.560].

First copulation

Sixty-seven males (55.4%) mated with the first presented female. On average we had to present 1.86 ± 0.12 females to a male until he copulated (Fig. 3). To investigate whether inter-sexual selection through male mate choice may exist, we analysed the 54 males, which did not copulate with the first presented female and which thus might have been choosy. We found that the SVL of the female with which a male copulated was

Table 2 Probability that a male copulated in relation to its body size and body mass. Data from the staged mating experiment (a) and the combined mating experiment (b) are shown.

Trait	Test statistic	P-value	Estimates ($\pm$ SE)	Selection gradient ($\pm$ SE)
(a)				
Body mass	$F_{1,194} = 15.02$	0.0001	1.076 ± 0.278	0.231 ± 0.059
Body size (SVL)	$F_{1,192} = 0.58$	0.448	0.059 ± 0.078	0.037 ± 0.086
Year	$z = 0.60$	0.376		
Enclosure	$z = 0$	1		
(b)				
Grouped treatment				
Body mass	$F_{1,31} = 5.80,$	0.022	2.253 ± 0.935	0.482 ± 0.189
Body size	$F_{1,30} = 0.41$	0.528	0.215 ± 0.336	0.172 ± 0.291
Female	$z = 0$	1		
Sequential treatment				
Body mass	$F_{1,31} = 1.62$	0.213	0.999 ± 0.786	0.143 ± 0.113
Body size	$F_{1,30} = 0.34$	0.564	0.204 ± 0.349	0.165 ± 0.220
Female	$z = 0$	1		

For the staged mating experiment the GLIMMIX model included year and the enclosure as random effects and for the combined mating experiment the female.
The logistic estimates and the standardized selection gradients are given.
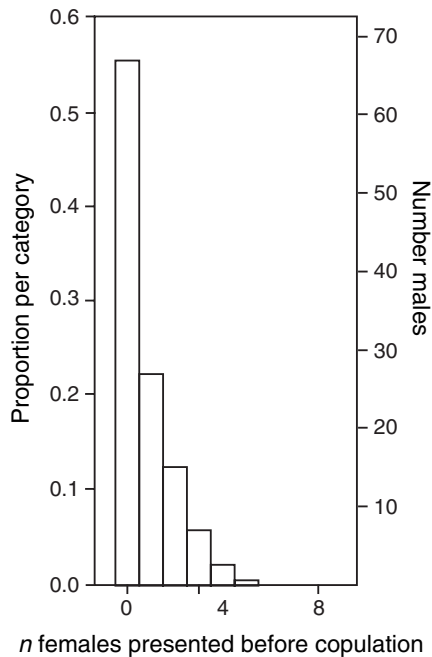
Fig. 3 Distribution of the number of females a male encountered before copulating with the first female.

bigger and showed a higher body condition than the average SVL or body condition of the females with which he was not copulating (Table 3a). The interactions between year and the repeated measures were not significant (SVL: $F_{1,52} = 1.689$, $P = 0.199$; body mass: $F_{1,51} = 2.360$, $P = 0.131$).

Second copulation
Fifty-two of the 121 copulating males copulated with a second female. Males that copulated a second time showed significantly better body mass [$F_{1,115} = 10.385$, $P = 0.0002$, estimate (males copulating a second time): 0.169 ± 0.05] and body condition [$F_{1,114} = 6.907$, $P = 0.010$, estimate (males copulating a second time): 0.102 ± 0.038] compared with males that copulated only once. There were no differences in SVL ($F_{1,118} = 1.963$, $P = 0.164$). Year was not significant in all cases (year in SVL analysis: $F_{1,117} < 0.001$, $P = 0.999$; year in body

condition analysis: $F_{1,113} = 0.760$, $P = 0.385$) and there were no significant interactions between the year and the number of times a male copulated (once vs. more than once copulated $\times$ SVL: $F_{1,116} = 0.021$, $P = 0.886$; once vs. more than once copulated $\times$ body condition: $F_{1,112} = 0.204$, $P = 0.653$).

After the first copulation we had to present on average 1.4 ± 0.1 females (maximum: 4th; 90th quantile: 2.7th female) to a male until he copulated for the second time. Thirty-nine males (of the 52 males) copulated with the first female presented after his first copulation. Like in the first copulation, the SVL of the female with which a male copulated was bigger than the average SVL of the females with which he did not copulate (Table 3b). There were no significant difference in body condition between copulating and noncopulating females (Table 3b) and interactions between year and repeated measures were not significant (year $\times$ SVL difference: $F_{1,11} = 1.569$, $P = 0.236$; year $\times$ body condition difference: $F_{1,10} = 0.427$, $P = 0.527$). Additionally, the duration of the first copulation did not help predict how many females had to be presented to a male until he copulated a second time (SRC: $\rho = 0.217$, $P = 0.477$).

Repeatability of a male's mate partner characteristics
Males that mated with two or three different females did not copulate with females of similar SVL [ANOVA: $F_{51,56} = 0.805$, $P = 0.782$, repeatability $(r) = -0.103$] and body condition $(F_{52,55} = 1.162$, $P = 0.291$, $r = 0.072$). Similarly, the number of females that had to be presented to a male before he copulated was not repeatable ($F_{51,56} = 0.875$, $P = 0.684$, $r = -0.064$).

Combined mating experiment

Females to which we presented three males sequentially copulated on average with more males (2.09 ± 0.21) than females to which we presented three males at the same time (1.46 ± 0.16; $F_{1,20} = 5.833$, $P = 0.025$, $R^2 = 0.23$). In all of the 11 grouped trials, at least one male bit the other lizards before copulating. The copulating male bit on average 8.82 ± 3.09 times, while the other two males bit on average 1.59 ± 0.60 times before copulating. In 10 of the 11 trials the male that bit the most, thereafter copulated with the female (sign test:

Table 3 Differences between females with which a male did or did not copulate during (a) the first and (b) the second copulation.

Trait	Copulating (mean ± SE)	Noncopulating (mean ± SE)	Test statistic	P-value
(a)				
SVL (mm)	64.5 ± 0.5	62.7 ± 0.4	$F_{1,53} = 7.881$	0.007
Body condition	0.103 ± 0.061	−0.143 ± 0.059	$F_{1,52} = 8.571$	0.005
(b)				
SVL (mm)	66.5 ± 1.2	63.5 ± 1.2	$F_{1,12} = 5.970$	0.031
Body condition	0.174 ± 0.291	0.023 ± 0.204	$F_{1,11} = 0.284$	0.604

Repeated measures analysis, with the female traits of copulating and the mean traits of the noncopulating females as repeated measures.

$N = 11$, $N_{exceptions} = 1$, $P = 0.012$). Similarly, in eight of the nine trials where one lizard was chasing the others, the male that was chasing most, thereafter copulated with the female (sign test: $N = 9$, $N_{exceptions} = 1$, $P = 0.04$).

Between the last copulation and the end of the trial, males were still aggressive in 10 of the 11 trials. In nine of the 10 trials the previously copulating males were more aggressive than the non-copulating males (copulating males: 9.23 ± 1.53 times biting, noncopulating males: 1.86 ± 1.40; sign test: $N = 10$, $N_{exceptions} = 1$, $P = 0.02$). These results show that before and after the copulations males were fighting among each other for dominance over access to females and suggest that intra-sexual selection was responsible for the reduced number of males that copulated with females in the grouped treatment.

In the grouped treatment the probability that a male copulated with a female increased with increasing body mass (Table 2b) and the covariate SVL and the random factor female were not significant. In the sequential treatment the probability that a male copulated with a female was not significantly affected by body mass (Table 2b). These results show that the intensity of sexual selection was 3.4 times higher in the grouped treatment than in the sequential treatment.

Discussion

Our results reveal that the mating patterns observed in the field experiment and the staged mating experiment were similar. First, both the probability of fathering offspring in the first study and the probability of copulating in the second study, increased with male body condition. Secondly, body size and the enclosure of origin did not predict the probability of reproducing. Thirdly, no stabilizing or disruptive sexual selection could be observed in either of the two studies, and fourthly in both experiments many males did not mate with a single female. The results thus indicate that in both studies positive directional sexual selection acted on male quality (in this study being represented by body condition), which is consistent with the mating patterns found in other taxa (e.g. in insects, amphibians, fish, birds and mammals, Andersson, 1994; Shuster & Wade, 2003; and in reptiles, see Olsson & Madsen, 1998 for a review). However, in most of these taxa it is not known whether the observed patterns are imposed by intra- and/or inter-sexual selection. Thus behavioural, morphological and life-history adaptations of both males and females can hardly be understood (Andersson, 1994; Shuster & Wade, 2003). In contrast, our study allows distinguishing between intra- and inter-sexual selection. The fact that in the staged mating experiment, which excludes all types of intra-sexual selection, the observed patterns were similar to the patterns observed in the field study, which includes all types of intra- and inter-sexual selection,

clearly demonstrates that in this species inter-sexual selection imposes an important selective pressure on male reproduction. The strength of the sexual selection acting on males was twice as high in the field study compared with the staged mating experiment. This suggests that both inter-sexual selection and intra-sexual selection contributed to a male's fitness. Indeed, our combined mating experiment shows that males fight for access to females both before and after the copulation, which is in line with earlier studies suggesting that intra-sexual selection (Lecomte *et al.*, 2004; Richard *et al.*, 2005) is important in the common lizard. Given that sexual selection was stronger in the grouped treatment, our study further indicates that intra-sexual selection was the cause of the stronger sexual selection observed in the field experiment. Male body mass significantly predicted the copulation success in the staged mating experiment, but not in the sequential treatment of the combined experiment. This difference is most likely the consequence of reduced power because of lower sample size ($N = 33$ vs. $N = 196$), given that both estimates were very similar [0.999 ± 0.786 for the sequential mating experiment (Table 2b) and 1.076 ± 0.278 for the staged mating experiment (Table 2a)] and given that the standard error in the sequential mating experiment was much larger.

The inter-sexual selection acting on male quality observed in the staged mating experiment might be imposed by at least four different selective pressures. First, male mate choice might be present (Olsson, 1993) because a male's mate partners were bigger and in better body condition, compared with those with which a male was not mating. The male's mate partner characteristics were not repeatable and male mate choice cannot explain why small males in the staged mating experiment were less likely to copulate, as not to copulate with a female is a bad strategy in species where males provide females with little more than sperm (Bateman, 1948). Secondly, inter-sexual selection imposed by female mate choice may explain why better-quality males were more likely to reproduce. However, female mate choice cannot explain why the females with which males copulated were of bigger size than those with which they did not copulate. Third, the sperm production or the sperm maturity may limit the male's copulation probability and thus intrinsic factors may affect male behaviour (Olsson, 1993; Olsson & Madsen, 1996). In this study neither the copulation duration of the first copulation ($F_{1,110} = 0.91$, $P = 0.342$) predicted whether a male copulated a second time, nor did the inter-copulation interval (time between the first and the second copulation) affect the probability of copulating with the first presented female during the second copulation ($F_{1,49} = 0.87$, $P = 0.356$) and the interactions between inter-copulation interval and the male quality were not significant (all interactions: $F_{1,44} \leq 2.12$, $P \geq 0.153$). It is therefore, unlikely that sperm maturation and/or sperm limitation may have

caused the observed patterns. Forth, male sexual harassment (Fitze *et al.*, 2005; Le Galliard *et al.*, 2005a) may lead to the observed positive relationship between male quality and access to reproduction, since better quality males might be better in harassing. If male coercion is the reason why males in better condition get more copulations, one would expect that male condition or size relative to female condition or size would be important. Thus, only males, which are large, relative to females, would be successful. This would manifest itself as a significant male condition times female condition interaction. However, in our study the probability of copulating with the first encountered female was not significantly affected by an interaction between the male's and the female's trait (interaction body mass: $F_{1,187} = 0.03$, $P = 0.872$; interaction body size: $F_{1,188} = 0.03$, $P = 0.863$), also male body mass significantly predicted the probability of copulating with the first encountered female ($F_{1,193} = 12.84$, $P = 0.0004$, estimate: 1.027 ± 0.287). This indicates that male sexual harassment is unlikely to be an important mechanism behind inter-sexual selection in this study.

All together our results support the existence of inter-sexual selection by male mate choice and female mate choice, while intrinsic factors and sexual harassment are unlikely the cause of the observed patterns. Most importantly, neither inter-sexual selection imposed by males nor inter-sexual selection imposed by females can explain all results, suggesting that multi-factorial inter-sexual selection may act on male reproductive success. However, the evidence for the different mechanisms of inter-sexual selection is of purely observational nature and only experimental studies may explain which mechanisms of inter-sexual selection led to the observed patterns. Consequently, the presented evidence for the different mechanisms of inter-sexual selection should be interpreted with caution.

In summary, our study contrasts the general belief that mainly intra-sexual selection determines male reproductive success. We experimentally demonstrate that inter-sexual selection significantly determines male reproductive success and that intra-sexual selection also contributes to a male's fitness. Our study further indicates that intra-sexual selection is likely to be responsible for the stronger sexual selection in both the field study and the combined mating experiment. Our findings on inter-sexual selection are consistent with female mate choice and male mate choice but not with intrinsic factors determining a male's reproductive success and sexual harassment. The study thus indicates that both intra-sexual selection imposed by male–male competition (Lecomte *et al.*, 2004; Richard *et al.*, 2005) and inter-sexual selection may exist concurrently and thus that male reproductive success is the result of multi-factorial sexual selection, suggesting that males should find the optimal balance between investing in the traits favourable for inter-sexual selection and for intra-sexual selection.

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