



Original Article

Behavioral estimates of mating success corroborate genetic evidence for pre-copulatory selection

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In promiscuous species, fitness estimates obtained from genetic parentage may often reflect both pre- and post-copulatory components of sexual selection. Directly observing copulations can help isolate the role of pre-copulatory selection, but such behavioral data are difficult to obtain in the wild and may also overlook post-copulatory factors that alter the relationship between mating success and reproductive success. To overcome these limitations, we combined genetic parentage analysis with behavioral estimates of size-specific mating in a wild population of brown anole lizards (*Anolis sagrei*). Males of this species are twice as large as females and multiple mating among females is common, suggesting the scope for both pre- and post-copulatory processes to shape sexual selection on male body size. Our genetic estimates of reproductive success revealed strong positive directional selection for male size, which was also strongly associated with the number of mates inferred from parentage. In contrast, a male's size was not associated with the fecundity of his mates or his competitive fertilization success. By simultaneously tracking copulations in the wild via the transfer of colored powder to females by males from different size quartiles, we independently confirmed that large males were more likely to mate than small males. We conclude that body size is primarily under pre-copulatory sexual selection in brown anoles, and that post-copulatory processes do not substantially alter the strength of this selection. Our study also illustrates the utility of combining both behavioral and genetic methods to estimate mating success to disentangle pre- and post-copulatory processes in promiscuous species.

Key words: *Anolis sagrei*, body size, GTseq, parentage analysis, post-copulatory selection.

INTRODUCTION

In species where females mate with multiple partners, sexual selection on male traits can continue to occur after copulation, through sperm competition and cryptic female choice. These post-copulatory processes can alter the siring success of males and thereby modify the strength of sexual selection on traits linked to mating success (Parker 1970; Kvarnemo and Simmons 2013; Simmons et al. 2017; Glaudas et al. 2020). For example, larger male water striders are more likely to mate with females, but this does not translate into strong sexual selection on body size because they have poor fertilization success in competition with smaller males (Danielsson 2001). Theory predicts that similar tradeoffs between pre- and post-copulatory components of selection may occur in many species with male–male contest competition, owing to energetic constraints

(Hayward and Gillooly 2011; Parker et al. 2013; Simmons et al. 2017). In addition to pre- and post-copulatory sexual selection, the net reproductive fitness of a male is also influenced by the fecundity of his mating partners (Wong and Candolin 2005; Venner et al. 2010; Pincheira-Donoso and Hunt 2017). Thus, total selection on any given trait due to variance in reproductive success can be partitioned into selection acting through variance in pre-copulatory mating success, post-copulatory fertilization success, and female fecundity (Arnold and Wade 1984; Koenig et al. 1991; Collet et al. 2012; Péliissié et al. 2014). Furthermore, selection mediated through any one of these components of fitness may be reinforced or weakened by selection acting through the other components (Arnold and Wade 1984; Shuster et al. 2013). Therefore, a complete understanding of selection on a given trait requires estimating phenotypic selection as a function of total reproductive success as well as its underlying components (Arnold and Wade 1984). However, our ability to partition sexual selection in wild populations is hindered by both the cryptic nature of post-copulatory processes and the difficulty of

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independently measuring mating success, mate fecundity, and fertilization success (Droge-Young et al. 2012; O Neal and Knowles 2015; Marie-Orleach et al. 2016).

Studies of sexual selection in wild populations have typically measured fitness using either genetic estimates of parentage or behavioral observations of mating success. However, either of these approaches can provide an incomplete picture of sexual selection when considered alone (Thompson et al. 2011; Marie-Orleach et al. 2016; Olsson et al. 2019), making it difficult to disentangle pre- and post-copulatory selection (Danielsson 2001; Mobley and Jones 2013; Kamath and Losos 2018; Cramer et al. 2020). Genetic parentage analysis can identify mating pairs from shared parentage, thereby providing minimum estimates of the number of mating partners for both males and females (Flanagan and Jones 2019). Such data can be used to estimate both pre-copulatory mating success (minimum number of known mates per male) and post-copulatory fertilization success (proportion of offspring sired with females who have multiple known mates) (Rose et al. 2013; Evans and Garcia-Gonzalez 2016). However, many copulations may go undetected in genetic estimates of mating success if females mate with multiple partners but those copulations do not result in offspring production, potentially leading to misestimation of selection via mating success (Pemberton et al. 1992; Flanagan and Jones 2019; Olsson et al. 2019; Baird and York 2021). Direct observations of copulations avoid this problem, but it is usually difficult to comprehensively track all copulations in wild populations (*but see* Pemberton et al. 1992; Preston et al. 2003; Rodríguez-Muñoz et al. 2010). For example, animals may copulate in obscure or sheltered locations, the duration of mating may be short, the population density may be too low, or the population size may be too high for comprehensive observations (Candolin 1998; Dunn et al. 2012; Johnson et al. 2014; Cramer et al. 2020). Therefore, reliance on either genetic or behavioral methods alone to measure fitness may lead to misestimation of the strength of pre- and post-copulatory sexual selection (Pischedda and Rice 2012; Evans and Garcia-Gonzalez 2016; Baird and York 2021). Consequently, studies of taxa ranging from birds and fish to flies and flatworms have placed increasing emphasis on approaches that measure fitness and its components using a combination of both behavioral observations and genetic parentage analyses to help partition pre- and post-copulatory dimensions of sexual selection (Collet et al. 2012; Pischedda and Rice 2012; Péliissié et al. 2014; Devigili et al. 2015; Marie-Orleach et al. 2016). Nonetheless, studies that definitively partition pre- and post-copulatory selection on phenotypes for free-living animals in the wild are still relatively rare (Cramer 2021; *but see* Preston et al. 2003; Turnell and Shaw 2015).

To address this issue, we studied the sexually dimorphic brown anole lizard, *Anolis sagrei*, to determine which components of total male reproductive success (i.e., mating success, average mate fecundity, or competitive fertilization success) generate selection for larger body size. Adult male brown anoles are, on average, two to three times larger than adult females in body mass (Cox and Calsbeek 2010a). Larger males are more likely to succeed in competitive interactions that lead to female encounters and to sire more offspring (Tokarz 1985; Kamath and Losos 2018). However, female brown anoles produce offspring with multiple sires during the breeding season and can store sperm for several months (Calsbeek et al. 2007; Calsbeek and Bonneaud 2008; Duryea et al. 2016; Kamath and Losos 2018; Kahl et al. 2021). Females may also bias their offspring sex ratio based on the body size or condition of the males with which they mate, suggesting that post-copulatory processes can also shape selection on male body size (Calsbeek

and Bonneaud 2008; Cox and Calsbeek 2010b; Cox et al. 2011). Although several studies have detected selection for larger body size in male brown anoles (Cox and Calsbeek 2010a; Duryea et al. 2016; Kamath and Losos 2018), no study to date has assessed the extent to which the higher reproductive success of larger male anoles is due to higher mating success, higher average mate fecundity, higher fertilization success, or a combination of these components of reproductive success (Friesen et al. 2020).

Given the scope for both pre- and post-copulatory selection to act on male body size in brown anoles (Calsbeek et al. 2007; Kahl et al. 2016), we combined genetic parentage and behavioral observations of mating to estimate fitness components in a wild population of this species. Based on the established role of body size in mediating aggressive interactions among males (Trivers 1976; Tokarz 1985; Duryea et al. 2016; Kamath and Losos 2018), we hypothesized that body size is primarily subject to pre-copulatory sexual selection. Specifically, we predicted that body size would be positively associated with both total reproductive success (number of offspring sired) and mating success (number of mates identified via genetic parentage). Although anoles only lay one egg at a time, larger females produce more offspring compared with smaller females and tend to be more fecund (Andrews and Rand 1974; Cox and Calsbeek 2011; Warner and Lovern 2014; Duryea et al. 2016). Thus, we also explored whether large males preferentially mate with larger and more fecund females. Since post-copulatory selection could weaken or reinforce pre-copulatory selection (Danielsson 2001; Hosken et al. 2008; Kvarnemo and Simmons 2013; Parker et al. 2013; Turnell and Shaw 2015), we also tested whether competitive fertilization success (i.e., the proportion of offspring sired with females who also produced offspring with other males) differed as a function of male body size. To corroborate our inferences based on genetic parentage with behavioral estimates of mating success, we quantified size-specific mating rates in the wild by tracking the copulatory transfer of fluorescent powders from males to females, with different colors of powder corresponding to different quartiles for male body size. We then tested whether larger males obtained a greater number of copulations, whether larger males mated with larger females, and whether female body size and fecundity were positively correlated to examine if males preferentially mated with larger and more fecund females. Our study design thus allowed us to separate the contributions of pre-copulatory mating success, female fecundity, and post-copulatory fertilization success in shaping selection on male body size.

METHODS

Field site and sampling

We studied an island population of brown anole lizards (*A. sagrei*) in the Guano Tolomato Matanzas Natural Estuarine Research Reserve (GTM NERR) in northern Florida (29°37'53"N, 81°12'46"W) using procedures approved by the University of Virginia Animal Care and Use Committee (protocol 3896) and under permits granted by GTM NERR. Adults begin mating around March (Lee et al. 1989) and females typically lay one egg every 7–14 days from April through October. Juveniles emerge between late May and November, and most do not enter the breeding population as adults until the subsequent year. To assay the reproductive success of males, we sampled all adults and juveniles of the population at four different times during the breeding season (March, May, July, and October) in 2019. We marked each new individual with a unique toe clip and preserved a small (1–2 cm) tail clip in 100% ethanol at –20 °C for genotyping. This population has been the focus of a

long-term mark-recapture study since 2015, such that most adults in the 2019 breeding season were first captured, marked, and genotyped as juveniles in 2017 or 2018. We measured snout-vent length (SVL, nearest 1 mm) and body mass (nearest 0.01g) of all individuals prior to releasing them at their exact site of capture the following day. Although we did not measure male home ranges or behavioral dominance either prior to or after removal, we simultaneously removed the majority of the male population first, followed by the removal of adult females after releasing males. Thus, removal of animals from the site for 1 day is unlikely to have dramatically altered home ranges or mating dynamics. At two points in the middle of the breeding season (May and July), we tracked copulations in the wild by dusting the venters of adult males with fluorescent powder as we released them (see *Assessing size-specific mating success with fluorescent powders*). In 2019, we captured and measured a total of 920 adults (hatched prior to 2019) and 905 juveniles (hatched in 2019) on the island. All individuals who were first captured as juveniles could be assigned with certainty to the 2019 cohort based on body size. All individuals that were first captured as adults in 2019 were assigned to the 2018 cohort based on their mass and SVL, and because it is highly unlikely that we would have failed to capture them across four successive censuses in 2018 if they had been present as adults at that time. Recapture rates were estimated at 85–95% for any given census of the adult population across previous years (Reedy 2018; Wittman 2022).

Genotyping and parentage assignment

We extracted DNA by adding 3–5 mg of tail tissue to 150 μL of 10% Chelex resin (Bio-Rad, Inc.) with 1.4 μL of Proteinase K (20 mg/mL, Qiagen, Chatsworth, CA), incubating at 55 $^{\circ}\text{C}$ for 180 min, and denaturing at 99 $^{\circ}\text{C}$ for 10 min. If the DNA concentration was not within the desired range of 10–25 ng/ μL , we repeated extractions and modified the above protocol by incubating new tail samples in 40 μL of 10% Chelex resin with 1.5 μL of Proteinase K. After centrifugation at 2250 $\times g$ at 4 $^{\circ}\text{C}$ for 15 min, we collected 3 μL of supernatant from these extractions to genotype individuals using the Genotyping-in-Thousands by sequencing (GT-seq) protocol (Campbell et al. 2015) with a custom panel of primers for 215 biallelic SNP loci that were previously identified from RAD-seq data (HA Seears, unpublished). For all extractions with an average DNA concentration of <10 ng/ μL ($n = 282$ of 1319 samples), we carried out an additional purification step on the supernatant using 1.8 \times volume of AMPure XP beads (Beckman Coulter, Brea, CA, USA) and eluted samples in 20 μL 1 \times TE (Fisher Bioreagents, Fair Lawn, NJ, USA) to concentrate the DNA to >10 ng/ μL . After extraction, we shipped DNA samples to GTseek LLC (Twin Falls, ID, USA) for library preparation, sequencing, and data processing to obtain genotypes. Briefly, all 215 loci were simultaneously amplified and tagged with Illumina priming sequences in a multiplexed polymerase chain reaction (PCR). Each sample was then tagged with well- and plate-specific indices in a second PCR. The PCR products were then standardized to similar concentrations, pooled, cleaned, and then sequenced on an Illumina NextSeq 550 with 1 \times 75 bp reads. The raw Illumina reads were checked for quality using FastQC and then de-multiplexed and assigned genotypes following Campbell et al. (2015).

We used SNPPIT 2.0 (Anderson 2012) to assign genetic parentage. We included all offspring known to have hatched in 2019 that were successfully genotyped at a minimum of 128 loci (<40% missing loci; $n = 885$ successfully genotyped of 905 total offspring). We included adults as potential parents if they were successfully

genotyped at a minimum of 165 loci (<23% missing loci). Since individuals that were present but were not captured in 2019 may have also produced offspring in that year, we included all successfully genotyped individuals captured on the island between 2015 and 2018 as potential parents ($n = 7042$ individuals genotyped in previous studies using the GT-Seq protocol). Of these potential parents, 870 individuals were captured as adults in 2019 ($n = 489$ females, 381 males). Only males captured at the start of the breeding season in March 2019 were considered in analyses of selection on body size (see below). Any parentage calls with a false discovery rate >0.05, as implemented by SNPPIT, were excluded from further analysis (Anderson 2012). We successfully assigned 736 offspring (83.2% of 885 genotyped offspring) to a total of 610 parents ($n = 357$ females, 253 males). Of these 610 parents, 479 (78.5%) were among the 870 successfully genotyped adults that we captured in 2019 ($n = 276$ females, 203 males) and 131 (21.4% of 610) were only captured in previous sampling years ($n = 81$ females, 50 males). Because we did not measure body size for this subset of 50 adult males in 2019, we excluded them from our calculations of relative fitness and our analyses of sexual selection. However, the subset of 81 females, their offspring, and their mates were considered when calculating fitness components for each male included in the selection analyses (see below). Among the 870 successfully genotyped adults that we captured in 2019, a total of 391 individuals ($n = 213$ females, 178 males, 44.9%) were found to have zero reproductive success, since they were included in the SNPPIT analysis but were not assigned offspring.

Partitioning reproductive success into fitness components

Prior to selection analyses (see *Statistical analyses of selection differentials*), we partitioned our genetic estimates of reproductive success (i.e., the total number of offspring produced in 2019) components (i.e., mating success, average mate fecundity, and competitive fertilization success), into three components: mating success, average mate fecundity, and competitive fertilization success. We measured mating success as the total number of unique females with which a male sired offspring. We measured average mate fecundity as the mean number of offspring produced across all female partners of a male, including offspring sired by other males. We measured competitive fertilization success by calculating the mean proportion of offspring sired by a male with each of his partners. To detect competing males from parentage data, a female must produce at least two offspring that are assigned to at least two mates. Thus, our measure of competitive fertilization success excluded all situations in which females produced either a single offspring or multiple offspring sired by a single male, following Devigili et al. (2015). To account for the fact that the null expectation for proportional fertilization success decreases with the number of additional males with which a female has mated, we used the following formula (Devigili et al. 2015):

$$\text{Competitive fertilization success} = \sum_{i=1}^{i=k} \left(\frac{\text{PS}_i \cdot (n_i - 1)}{\text{PS}_i \cdot (n_i - 2) + 1} \right) / k,$$

where PS_i is the proportion of offspring sired for each i th female with which that male mated, k is the total number of females with which that male mated that had more than two mates, and n_i is the total number of mates of the i th female. Thus, a male that sired 33.3% of the offspring from a female that had three total mates would have a competitive fertilization success score of 0.5, which

would be the same as a male that sired 50% of the offspring from a female that had only two mates.

Assessing size-specific mating success with fluorescent powders

We assessed the relationship between body size and number of copulations at two points in the middle of the breeding season: 12–16 May and 26 July–3 August 2019. In the first 2 days of each sampling period, we captured as many adult males on the island as possible and distributed them into size quartiles based on their body mass (May: $n = 153$; July: $n = 128$). We used body mass as an estimate of size because its measurement at 0.01-g resolution yielded a more continuous distribution and a balanced assignment of males across size quartiles than did SVL, which was only measured at only 1-mm resolution. The use of body mass also precluded any confounds between size and age because the few males that were 2 years old (5 of 240 total males in May and July) were distributed across the full range of size quartiles. Immediately prior to releasing each male at its exact site of capture, we powdered its venter with one of four colors of fluorescent powder corresponding to its size quartiles (A/AX Series, DayGlo Color Corp., Ohio). The four colors (orange, yellow, pink, and green) were selected after our pilot studies confirmed that different colors of powder transferred during successive copulations could be clearly distinguished in the event of multiple mating across different size quartiles. These powders are nontoxic, easily differentiated under ultraviolet (UV) light, and wear off after a few days without negatively affecting the fitness of animals (Holbrook et al. 1970; Rojas-Araya et al. 2020).

We switched the colors assigned to each size quartile between May and July to ensure that any observed mating patterns were not due to underlying differences in our ability to detect each color. We were not blind to the size quartile associated with the colors during the study. Subsequent studies in the same population using a double-blind study design have not uncovered significant biases in estimation of copulation rates (RS Bhave, unpublished data). Two days after males were powdered and released, we captured as many adult females on the island as possible in a single day in May ($n = 132$) and across 5 days in July ($n = 312$; 50% of these captures occurred on the first day). We noted the color of any powder on or near the cloaca under UV light. If two colors were detected on the same female, we recorded this as two separate copulations by males from different size quartiles. The presence of color found on any other part of the body was uncommon and treated as non-copulation contact.

Statistical analyses

All statistical analyses were performed in R v4.2.1 (R Core Team 2015) using the RStudio interface (Posit team 2023). All data visualizations were created using *ggplot2* (Wickham 2016) and *ggpubr* (Kassambara 2023) packages in R. A complete list of R packages used to format and analyze data can be found in files provided by Bhave et al. (2023).

Statistical analyses of selection differentials

We estimated selection differentials for body mass following Lande and Arnold (1983). We standardized body mass to a mean of 0 and a standard deviation of 1. We calculated relative fitness by dividing total reproductive success and each of its components (i.e., mating success, average mate fecundity, competitive fertilization success, see *Partitioning reproductive success into fitness components*) by the mean value of that fitness component across all males in the population that were included in the analysis (i.e., males that were measured for body mass in March

2019 and successfully genotyped for inclusion in parentage analysis). Males with zero reproductive success were included when analyzing selection via reproductive success and mating success but excluded when analyzing selection via average mate fecundity and competitive fertilization success. This approach assumes that failure to reproduce is due to failure to mate when it could, in principle, also reflect low mate fecundity and/or poor competitive fertilization success.

To estimate univariate linear (s) and nonlinear (c) selection differentials for body mass, we used ordinary least-squares regressions of each measure of relative fitness on standardized body mass with separate regressions for each fitness component. We included only the linear term for body mass to estimate s , and included both the linear and quadratic terms (i.e., $0.5 \times \text{body mass}^2$) to estimate c (Lande and Arnold 1983; Stinchcombe et al. 2008). We used the *glm.nb* function from the *MASS* package in R (Venables and Ripley 2002) to test the significance of selection differentials ($P < 0.05$) while using generalized linear models to account for non-Gaussian distributions of fitness components. We computed effect sizes and significance of predictors using a type II sum of squares and specified a likelihood ratio or F -test statistic in the *Anova* function from the *car* package (Fox and Weisberg 2019). We used a negative binomial distribution for all components of fitness except competitive fertilization success, which had a Gaussian distribution. To confirm that including individuals with zero reproductive success and zero mating success did not bias our inferences about the relative partitioning of selection among components of reproductive success, we repeated all estimates of selection acting through reproductive success and mating success using only the subset of males that had at least one offspring (Supplementary Figure S1), making these estimates directly comparable to those using average mate fecundity and competitive fertilization success as fitness components. Nonlinear selection differentials were not significant for any fitness component, so we only present visualizations of linear selection differentials.

Statistical analysis of powdering experiment

We tested whether observed copulations within each size quartile (as determined by the color of transferred powder) differed from the null expectation of equal number of copulations across size quartiles, using a chi-square test with 3 degrees of freedom. The expected number of copulations for each size quartile was a product of the proportion of powdered males that were assigned to that quartile and the total number of copulations detected across all females captured in May and in July. To understand how data from each month contributed to overall patterns observed, we repeated the above analyses to compare observed and expected copulations separately in May and in July.

To test whether large males mated more frequently with large females, we conducted an ordinary least-square regression of female body mass (continuous dependent variable) on male size quartile (ordinal independent variable) and included size quartile \times month interaction to assess month-specific relationships. We estimated the significance of each variable with a type II sum of squares (or type III sum of squares if the interaction between size quartile and month was significant) using the *car* package (Fox and Weisberg 2019) in R. To test the underlying assumption that female fecundity is positively correlated with body size, we regressed the total number of offspring assigned to a female on their body mass using generalized linear models with a negative binomial error distribution and a log link function. Because female body mass can vary depending on the presence or absence of oviductal eggs, we also repeated the above analyses by considering SVL as an alternate

measure of female body size. For all analyses, we combined data from the May and June iterations of the powdering experiment, then conducted separate analyses within each month as needed to further explore any differences between months.

A small subset of females for whom we detected copulations in both May and July ($n = 8$) were included twice in our combined analysis comparing female size across male size quartiles. Given that these females constituted only 5% of all individuals ($n = 180$ unique females) that were known to have copulated, our models did not converge within the iteration limit when specifying individual ID as a random effect. Similarly, 49 females were included twice in our combined analysis comparing female fecundity with female size, which was only 13% of all individuals ($n = 391$ unique females) captured in May and July, resulting in non-convergence of model specifying ID as a random effect. Thus, we opted to report results from a generalized linear regression while retaining repeated measures for individuals without specifying a random effect. We also re-analyzed the data by randomly assigning the 8 or 49 individuals to either the May or July dataset and found that our interpretation of results did not change for either analysis (Supplementary Table S1). Coefficients (β) from linear regressions with an ordinal predictor variable (size quartile) denote the magnitude and direction of the linear (L), quadratic (Q), or cubic (C) effect of size on the response variable, each corresponding to the slope, curvature, or shape of the modeled polynomial regression. For these regressions, we report higher-order coefficients only when illustrating differences between months or if they were significantly different from zero at $P < 0.05$. We report coefficients from generalized linear regressions (log-link) on the original scale by exponentiating them wherever relevant, when using continuous measures of body size (body mass or SVL) as the predictor. Also known as incident rate ratios (IRR), these values represent the fold-change in fitness for a unit change in body size (per g of body mass or per mm of SVL) relative to when body size is at the hypothetical value of zero.

Statistical comparison of behavioral and genetic approaches

To compare behavioral and genetic approaches, we assessed whether males belonging to larger size quartiles in our powdering experiment (behavioral) also differed in their fitness components as measured by parentage (genetic). We conducted separate generalized linear regressions with reproductive success (negative binomial), mating success (negative binomial), average mate fecundity (negative binomial), and competitive fertilization success (Gaussian) as response variables. We treated the size quartile that males belonged to in each month as an ordinal predictor variable. In each analysis, we only considered males that were powdering in that month and successfully genotyped. To test whether associations between size quartile and fitness components varied across months, we conducted the above analyses on data combined across May and July and included a size quartile \times month interaction. A subset of successfully genotyped males that were captured and powdering in May and in July ($n = 37$ of 225 males), so these individuals were included twice in our combined analysis. Given that these males constituted only 15% of all individuals that were powdering, our models did not converge within the iteration limit when specifying individual ID as a random effect. We also repeated all the above analyses by randomly assigning the 37 individuals to either May or July and found that our interpretation did not change (Supplementary Tables S2 and S3). Thus, we opted to report results from a generalized linear regression while retaining repeated

measures for individuals without specifying a random effect. We then conducted separate analyses within each month as needed to further explore patterns between months. We obtained effect sizes of all main effects in these models from a type II sum of squares unless the interaction of size quartile \times month was significant, in which case we conducted a type III sum of squares using the *Anova* function within the *car* package in *R* (Fox and Weisberg 2019). We report coefficients from generalized linear regressions on the original scale by exponentiating them (i.e., IRR) wherever relevant. Since the predictor in each regression was an ordinal variable (size quartile), the reported IRR values denote the estimated linear (L), quadratic (Q), or cubic (C) effect of body size on fitness which respectively represent the slope, curvature, or shape of the modeled regression.

We carried out a chi-square test with 3 degrees of freedom to test whether the number of copulations in each size quartile, as determined by powdering (observed), corresponded to the number of copulations predicted from genetic parentage (expected). To calculate the expected proportion of copulations in each size quartile, we first estimated the number of unique dam-sire pairs from genetic parentage corresponding to each male powdering in May or in July. We assumed that each parental pair indicates the occurrence of at least one copulation of a female with a male belonging to a particular size quartile. We then divided the total copulations estimated for each size quartile by the total number of copulations attributable to all males that were measured and powdering in either May or in July. The expected number of copulations was calculated by multiplying this proportion by the total number of copulations observed from the transfer of fluorescent powder in the respective months. We first assessed the overall relationship between behavioral and genetic estimates of copulations across both months and then repeated the analyses for estimates attributable to each month separately.

RESULTS

Multiple paternity and multiple mating

Among 160 females that produced at least 2 genetically assigned offspring (i.e., females for which multiple paternity could potentially be detected), we found that 108 (67.5%) produced offspring with more than one male (mean = 2.4, range 2–4 male mates), suggesting that multiple paternity was common. This estimate of multiple paternity is likely conservative because the number of offspring produced by these females was relatively low (mean = 3.4, range = 2–9). In our powdering experiment, 189 of 444 total number of captured females (42.5%) displayed evidence of copulation within several days following the release of powdering males, and 11 (6.6% of mated females, 2.5% of all females) displayed evidence of multiple mating with males from different size quartiles in the span of 1–5 days. This is also a conservative estimate because it does not include copulations from different males in the same size quartile.

Partitioning pre- and post-copulatory selection on body size

We found significant directional selection favoring large male body mass when using total reproductive success as a measure of fitness ($s = 0.40 \pm 0.08$; $\chi^2 = 22.43$, $P < 0.001$; Figure 1a), and we found similarly strong selection when using only its pre-copulatory component (i.e., mating success, $s = 0.33 \pm 0.07$; $\chi^2 = 19.36$,

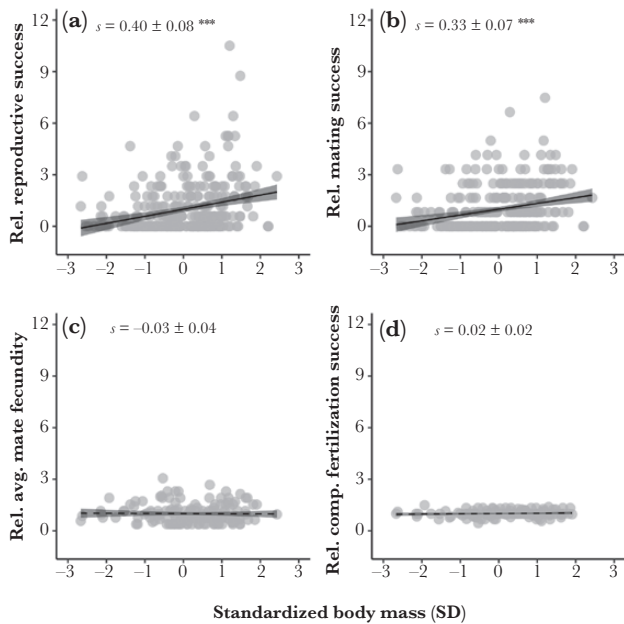


Figure 1

Linear selection on adult body mass as a function of different components of male fitness, including relative measures of (a) reproductive success (total number of offspring), (b) mating success (total number of mates), (c) average mate fecundity (mean fecundity across all mates), and (d) competitive fertilization success (mean proportion of offspring sired across all mates, adjusted for the number of competing males). For each fitness component estimated from genetic parentage, we divided individual fitness by the population mean to obtain relative measures. Adult body mass was measured at the start of the breeding season in March and standardized to a mean of 0 and standard deviation of 1. Trendlines with 95% confidence intervals (CIs) from linear regressions are used to visualize linear selection. Solid lines and asterisks in panels (a) and (b) indicate significant selection differentials while dotted lines in panels c and d indicate nonsignificant selection differentials (** $P < 0.001$).

$P < 0.001$; Figure 1b). Directional selection favoring large size persisted when we excluded males who did not sire any progeny from our analyses using reproductive success and mating success (Supplementary Figure S1). However, neither average mate fecundity ($s = -0.03 \pm 0.04$; $\chi^2 = 0.15$, $P = 0.70$; Figure 1c) nor competitive fertilization success (adjusted for number of competing males) generated significant selection on male body mass ($s = 0.02 \pm 0.02$; $F_{1,115} = 2.18$, $P = 0.17$; Figure 1d). There was no significant quadratic (nonlinear) selection on male body mass with respect to total reproductive success ($c = 0.30 \pm 0.12$; $\chi^2 = 2.51$, $P = 0.11$), mating success ($c = 0.30 \pm 0.12$; $\chi^2 = 2.33$, $P = 0.13$), average mate fecundity ($c = 0.19 \pm 0.09$; $\chi^2 = 1.21$, $P = 0.27$), or competitive fertilization success ($c = -0.0009 \pm 0.02$; $F_{1,115} = 0.001$, $P = 0.97$).

Behavioral estimates of size-specific mating success

We powdered a total of 281 males ($n = 240$ unique males) across May and July to test whether actual copulation rates differed across male size quartiles (Figure 2a-c). Based on detection of transferred powder (Figure 2d), we found that 38 of 132 (28.8%) females in May and 151 of 312 (48.4%) females in July mated within the 5-day collection period, with most of these copulations detected within 3 days of the release of powdered males. We omitted seven

copulations in May and July from our analysis, since we could not accurately resolve the color of fluorescent powder. Omitting these instances from the analyses did not bias the number of copulations for any size quartile. Across both months, the observed number of copulations differed significantly from the expectation of equal mating across size quartiles ($\chi^2 = 11.64$, $df = 3$, $P = 0.009$, $n = 193$ copulations). This result is primarily attributable to the low number of copulations in the smallest size quartile and is consistent across both iterations of the experiment (May: $\chi^2 = 8.03$, $df = 3$, $P = 0.045$, $n = 37$ copulations; July: $\chi^2 = 8.33$, $df = 3$, $P = 0.039$, $n = 156$ copulations; Figure 2e, f).

When considering data combined across both months, we found a weak but significant positive linear correlation between female body mass and the size quartiles of males with which they mated ($\beta_{(L)} = 0.22$; Size Quartile: $F_{3,185} = 3.24$, $P = 0.02$; Month: $F_{1,185} = 3.71$, $P = 0.06$; Size Quartile \times Month: $F_{3,185} = 0.75$, $P = 0.52$). Although there was a weak trend toward positive size-assortative mating, when considering each month separately, female body mass did not differ significantly across male size quartiles in either May ($\beta_{(L)} = 0.22$; Figure 3a) or in July ($\beta_{(L)} = 0.06$; Figure 3b). Female SVL also did not differ significantly across male size quartiles (Size Quartile: $F_{3,185} = 1.48$, $P = 0.22$; Month: $F_{1,185} = 1.23$, $P = 0.27$; Size Quartile \times Month: $F_{3,185} = 1.43$, $P = 0.24$, Figure 3c, d). The total number of offspring produced by a female in a year and body mass was strongly positive, with a 2.49-fold increase in number of offspring per g increase in body mass (IRR = 2.49; Mass: $\chi^2 = 5.42$, $P = 0.020$; Month: $\chi^2 = 5.10$, $P = 0.024$; Mass \times Month: $\chi^2 = 1.16$, $P = 0.28$). However, this relationship was not significant when considering the data individually in May (IRR = 2.46; $\chi^2 = 3.50$, $P = 0.06$) or in July (IRR = 1.43; $\chi^2 = 2.54$, $P = 0.11$). When considering SVL as a measure of female size, total number of offspring had a strong positive association with female body size, on average increasing by 1.24-fold per mm increase in SVL (SVL: $\chi^2 = 10.96$, $P < 0.001$; Month: $\chi^2 = 7.89$, $P < 0.005$) and this relationship differed between months (IRR = 0.82; SVL \times Month: $\chi^2 = 7.36$, $P < 0.005$). Total number of offspring produced by a female increased with each unit change in SVL measured in May (IRR = 1.28; $\chi^2 = 9.91$, $P = 0.002$), though not in July (IRR = 1.01; $\chi^2 = 0.11$, $P = 0.74$).

Comparing behavioral and genetic approaches

Of the 381 males that we successfully genotyped, measured, and included in genetic parentage analysis in 2019, 225 were also powdered in either May, July, or in both months. The overall relationship between male size quartile and reproductive success was not significant (IRR_(L) = 1.55; Size Quartile: $\chi^2 = 6.93$, $P = 0.07$), although there was a significant difference between months in the quadratic relationship between size and reproductive success (IRR_{(Size (Q) \times Month)} = 2.20; Size Quartile \times Month: $\chi^2 = 14.16$, $P = 0.002$; Table 1) and a large effect of month on reproductive success (IRR = 0.54; Month: $\chi^2 = 19.30$, $P < 0.001$; Table 1). When analyzing these data separately in each month, we found that reproductive success estimated from genetic data increased linearly with male size quartile in May (IRR_(L) = 2.56; Figure 4a), although this positive relationship was weaker and not significant in July (IRR_(L) = 1.55; Figure 4b). Likewise, we found that the relationship between size and mating success was not significant overall (IRR_(L) = 1.43; Size Quartile: $\chi^2 = 5.31$, $P = 0.15$), although it differed significantly across months (IRR = 0.67, Month: $\chi^2 = 14.12$, $P < 0.001$; IRR_{(Q) \times Month} = 1.63, Size Quartile \times Month:

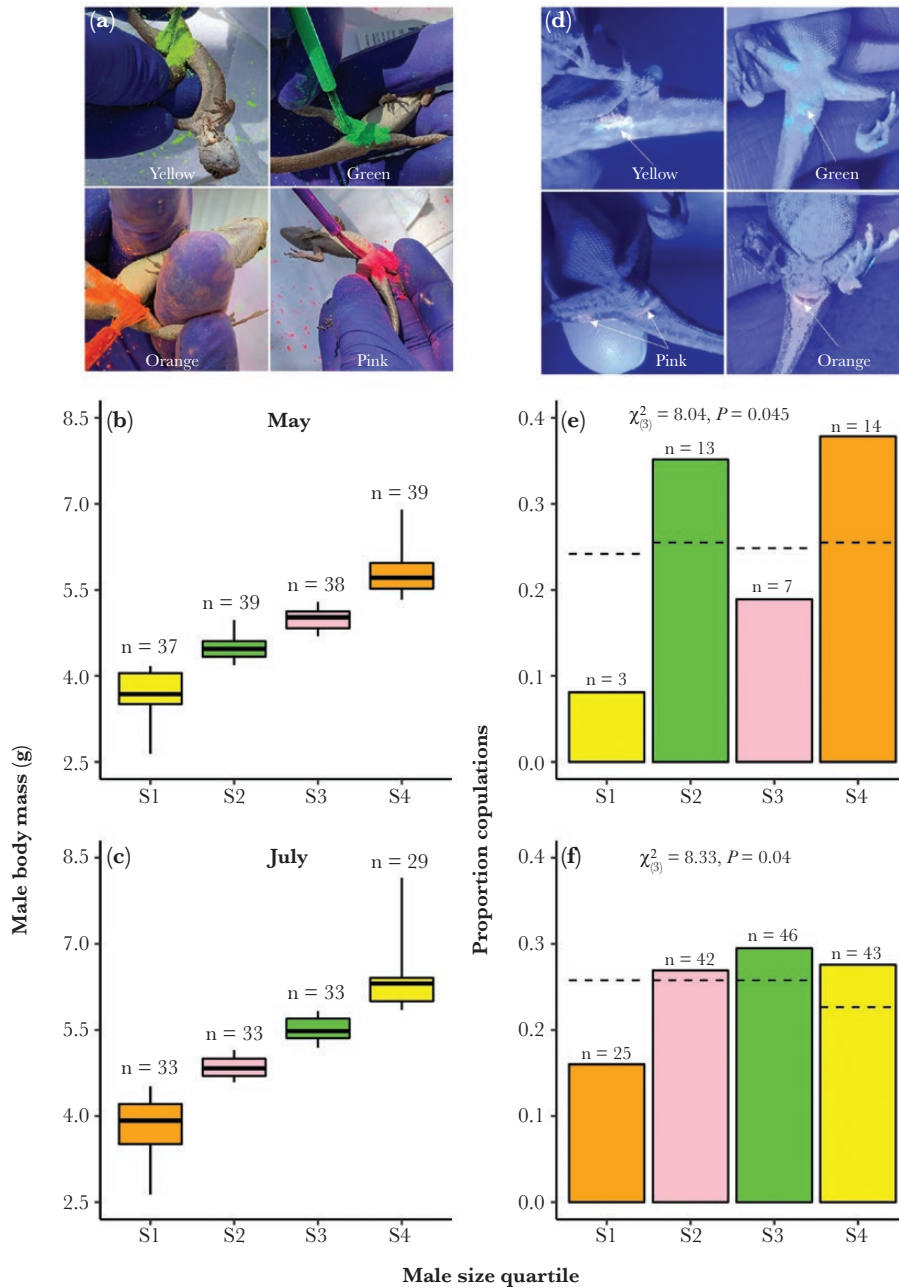


Figure 2

Procedure for detecting copulations in the wild. (a) Males were dusted with one of four colors of fluorescent powder based on size quartiles for body mass in (b) May and (c) July, with colors alternated among size classes between months. Boxplots in b and c depict medians (lines), interquartile ranges (boxes), and minimum and maximum values (whiskers), with the number of males in each quartile shown above each boxplot. After males were released and allowed to interact freely with females for 2 days, females were captured and (d) inspected under UV light for the presence and color of any powder transferred near their cloaca. The proportions of total copulations detected among females that we correctly attributed to males from each size category are shown separately for (e) May and (f) July. The number of females with each color of powder is indicated within each bar. The dotted lines give the expected proportion of copulations in each size quartile if mating is random with respect to male size. Colors of bars and box plots indicate the color of powder used for that size quartile.

$\chi^2 = 10.52$, $P = 0.014$, Table 1). Specifically, male mating success increased linearly with size quartile in May ($IRR_{(L)} = 2.09$, $IRR_{(Q)} = 1.46$; Figure 4c), but this relationship was weaker and not significant in July ($IRR_{(L)} = 1.43$, $IRR_{(Q)} = 0.88$; Figure 4d). We found a weak but significant tendency for average mate fecundity to decrease with male size quartile ($IRR_{(L)} = 0.81$; Size

Quartile: $\chi^2 = 9.39$, $P = 0.025$, Month: $\chi^2 = 0.19$, $P = 0.66$, Size Quartile \times Month: $\chi^2 = 1.10$, $P = 0.78$; Table 1). However, when analyzing data separately in each month, average mate fecundity was unrelated to male size quartile in May ($IRR_{(L)} = 0.88$; Figure 4e) and in July ($IRR_{(L)} = 0.81$; Figure 4f). Competitive fertilization success was unrelated to male size when pooling data across months

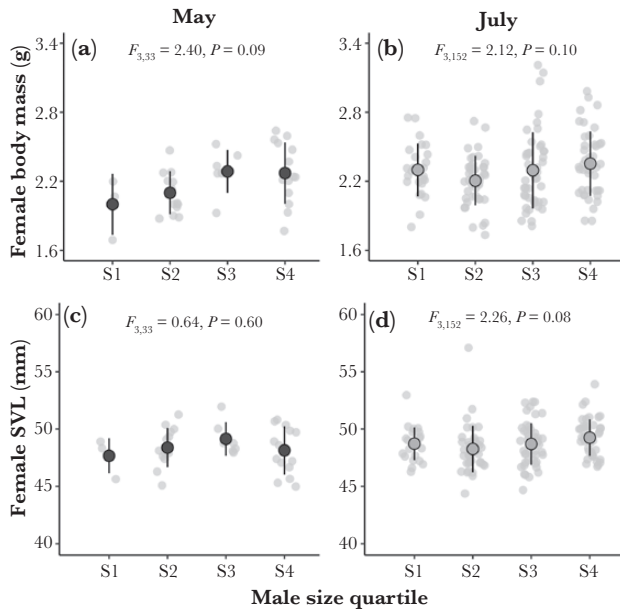


Figure 3

Tests for size-assortative mating with respect to (a, b) body mass or (c, d) SVL of females that mated with males from each size quartile in May (left panels) and in July (right panels), based on the color of fluorescent powder detected on the female. Small filled circles (light gray) are individual values and larger overlaid symbols are mean \pm SD values for each quartile. Mating was not strongly size assortative in either May (left) or in July (right), as shown by F statistics from a type II ANOVA.

(Size Quartile: $F_{3,127} = 1.73$, $P = 0.17$, Month: $F_{1,127} = 1.87$, $P = 0.35$, Size Quartile \times Month: $F_{3,127} = 1.82$, $P = 0.15$, Table 1). This relationship was also consistent when considering data from each month separately (Figure 4g, h).

Overall, the size distribution of copulations inferred from the transfer of fluorescent powder was not significantly different from the size distribution of copulations estimated from genetic parentage ($\chi^2 = 4.52$, $df = 3$, $P = 0.21$). However, when considering each month separately, observed copulations were significantly different from those estimated from genetic parentage in May ($\chi^2 = 8.35$, $df = 3$, $P = 0.039$, Figure 5a). In particular, males in the second size quartile had more observed copulations than expected from genetic parentage, whereas males in the smallest and largest size quartiles had fewer copulations than expected (Figure 5a). However, our analyses for May are based on substantially fewer observed copulations ($n = 37$) than our analyses for July ($n = 156$), in which size-specific copulations observed in the wild were very similar to the distribution estimated from parentage ($\chi^2 = 1.41$, $df = 3$, $P = 0.70$, Figure 5b).

DISCUSSION

Pre- and post-copulatory components of sexual selection can be difficult to disentangle in wild populations, particularly when females mate frequently with multiple males. In brown anoles, our genetic parentage data revealed that 67.5% of females that produced two or more offspring (i.e., females for which multiple paternity could be detected) did so with more than one mate, and our behavioral data revealed that 6.6% of females that mated within a brief 5-day period did so with more than one male. Both of these

estimates are also likely conservative, suggesting the strong potential for post-copulatory processes to modulate pre-copulatory sexual selection in this system. We detected strong positive directional selection on male body size using estimates of total reproductive success from genetic parentage. Partitioning male reproductive success into its components revealed that the higher reproductive success of larger males was primarily mediated by an increase in their mating success, the pre-copulatory component of fitness. This result was corroborated by our behavioral assay involving the transfer of fluorescent powder from males to females during copulation, which allowed us to track copulations in the wild and revealed that individuals in the smallest size quartile mated much less frequently than larger individuals. In contrast, neither of the remaining fitness components (i.e., average mate fecundity and male competitive fertilization success) covaried positively with male body size, suggesting that pre-copulatory sexual selection is largely responsible for the strong association between reproductive success and body size in male brown anoles. This was further confirmed by our finding that both behavioral and genetic parentage estimates of mating success were similarly distributed across different male size quartiles. Thus, despite multiple mating by females, post-copulatory processes did not significantly modify pre-copulatory sexual selection for large male body size in this population.

Body size and mating success

We found that larger body mass is directly associated with greater mating success in the wild, a pattern corroborated by both behavioral and genetic estimates of mating success. Consequently, larger males sired a greater number of offspring than average throughout the breeding season. Our findings are in line with the general consensus that there is strong pre-copulatory sexual selection on male body size across a wide range of taxa with extreme male-biased size dimorphism (reviewed in Stamps et al. 1997; Kingsolver and Pfennig 2004; Fairbairn et al. 2007; Kingsolver and Diamond 2011). In brown anoles, as in many other taxa, the observed pattern of pre-copulatory sexual selection for large body size is likely due to success in male–male competition (Andersson and Iwasa 1996; Eberhard 1996; Cox et al. 2003; Roff and Fairbairn 2007; Janicke and Fromonteil 2021). Previous studies have shown that larger male anoles are more active (Tokarz 1985; Jenssen et al. 2005), move across larger areas (Trivers 1976; Stamps et al. 1997; Kamath and Losos 2018), and are more likely to win in aggressive interactions with other males, resulting in more frequent encounters with females (Steffen and Guyer 2014). This is also the case in many other vertebrate and invertebrate species that are characterized by male-biased size dimorphism or contest competition (Cox et al. 2003; Fairbairn et al. 2007; Emlen 2008; Janicke et al. 2016; Horne et al. 2020). Although examples of sexual selection via female choice are relatively rare in reptiles (Olsson and Madsen 1995; Tokarz 1995; Cox and Kahl 2014; Ord et al. 2015; Rosenthal 2017), our study cannot eliminate the role of female choice for large males (Wong and Candolin 2005; Fitze et al. 2008; Karsten et al. 2009; Debelle et al. 2016). Selection due to female choice in lizards may occur directly for body size or indirectly through correlated traits such as territory quality, display behaviors, activity levels, and ornaments (Cooper and Vitt 1993; Censky 1997; Hamilton and Sullivan 2005; Swierk and Langkilde 2013; Flanagan and Bevier 2014; Ord et al. 2015). Regardless of whether larger body size is advantageous in male competition or female choice, our study provides comprehensive evidence that

Table 1

Summary of coefficient estimates^{a,b} from linear regressions with corresponding error distributions, carried out separately for each measure of fitness as the response variable when pooling data from May and July: reproductive success (total number of offspring), mating success (total number of mates), average mate fecundity (mean fecundity across all mates), and competitive fertilization success (mean proportion of offspring sired across all mates, adjusted for the number of competing males), with size quartile (ordinal), month (categorical) and their interaction included as predictors.

Coefficient	Reproductive success	Mating success	Average mate fecundity	Competitive fertilization success
	IRR ^c (95% CI)	IRR (95% CI)	IRR (95% CI)	β (95% CI)
Intercept	2.87*** (2.38 to 3.47)	1.92*** (1.63 to 2.25)	2.76 (2.51 to 3.03)	0.51*** (0.48 to 0.54)
Size quartile (Linear—L)	1.56* (1.06 to 2.30)	1.44* (1.04 to 2.00)	0.81* (0.67 to 0.98)	0.04 (-0.02 to 0.09)
Size quartile (Quadratic—Q)	0.75 (0.52 to 1.10)	0.90 (0.65 to 1.23)	1.12 (0.93 to 1.35)	-0.06* (-0.11 to -0.005)
Size quartile (Cubic—C)	1.02 (0.71 to 1.47)	0.96 (0.70 to 1.30)	1.00 (0.83 to 1.21)	-0.01 (-0.06 to 0.05)
Month [May]	0.54*** (0.41 to 0.71)	0.63*** (0.50 to 0.80)	0.95 (0.82 to 1.10)	-0.02 (-0.06 to 0.02)
Size (L) × Month	1.64 (0.95 to 2.85)	1.45 (0.91 to 2.34)	1.09 (0.83 to 1.44)	-0.00 (-0.08 to 0.08)
Size (Q) × Month	2.20*** (1.27 to 3.82)	1.64* (1.01 to 2.65)	1.12 (0.83 to 1.50)	0.09* (0.01 to 0.17)
Size (C) × Month	1.47 (0.85 to 2.56)	1.46 (0.90 to 2.37)	0.98 (0.72 to 1.34)	0.02 (-0.06 to 0.10)
<i>N</i>	262	262	176	135
<i>R</i> ²	0.279	0.225	0.090	0.081/0.030

^aSince the predictor in each regression was an ordinal variable (size quartile), the reported Incident Rate ratios (IRR) derived from a generalized linear regression or β coefficient values from an ordinary least-squares regression denote the estimated linear (L), quadratic (Q), or cubic (C) effect of body size on fitness. These values correspond to the respective slope, curvature, or shape of the modeled regression in the month of July which was treated as the baseline in this analysis. Values corresponding to Size × Month, denotes how these aspects of the modeled regression differ in May.

^bEstimates indicate the relative increase (IRR >1 or β > 0) or decrease (IRR <1 or β < 0) with an increase in size quartile for each predictor variable.

^cTerms are significant if the 95% confidence intervals indicated in brackets do not overlap IRR at 1 or β at 0 (***) $P < 0.001$; * $P < 0.05$.

body size is primarily subjected to pre-copulatory selection despite extensive promiscuous mating in the population.

Body size and average mate fecundity

Male body mass was mostly unrelated to, or sometimes even negatively correlated with, the average fecundity of female partners (Figures 1c and 4e, f; Table 1). This may reflect the fact that the relationship between female body mass and male size quartile was weak and nonsignificant within each month (Figure 3a, b), and that female mass itself was unrelated to fecundity in each month. Although an alternative measure of female size (SVL) was significantly related to fecundity, consistent with previous work showing that larger female anoles may achieve a higher reproductive output (Warner and Lovern 2014; Duryea et al. 2016) by laying eggs more frequently (Cox and Calsbeek 2011), we did not find any association between male size quartile and female SVL (Figure 3c, d). Thus, neither body mass nor SVL of females provided a strong intermediate, linking male size to female fecundity via size-assortative mating. These findings are consistent with the general observation that size-assortative mating is rare, particularly in species with male-biased sexual size dimorphism, such as anoles (Shine et al. 2001; Hofmann and Henle 2006; Harrison 2013; Rios Moura et al. 2021). When mate choice has been detected in anoles, males appear to prefer novel females rather than larger females (Tokarz 1992; Orrell and Janssen 2002). Male preference for novel females would be expected if males are primarily under selection to mate with a greater number of females, rather than mating with more fecund females. This pattern may also be a general characteristic of

species with male-biased sexual size dimorphism, given that in contrast, it is more common for larger males to mate with large and/or more fecund females in species with female-biased size dimorphism (Verrell 1989; Olsson 1993; Whiting and Bateman 1999; Cox et al. 2005; John-Alder et al. 2009; Jiang et al. 2013).

Body size and competitive fertilization success

Consistent with previous findings in brown anoles, we found that majority of females with at least two genotyped offspring produced these offspring with more than one mate (Calsbeek et al. 2007; Duryea et al. 2016; Kahlr et al. 2021). Moreover, some females in our powdering experiment mated with multiple males in the span of several days. Although multiple mating by females was common in our population, male size was unrelated to fertilization success even in situations where females produced offspring with multiple males. Theory predicts that post-copulatory processes can oppose pre-copulatory selection on a given trait if investment in corresponding fitness components is drawn from the same limited resources, or if the genetic covariance among fitness components is negative (Roff and Fairbairn 2007; Parker et al. 2013). Accordingly, inter- and intraspecific comparisons across several lineages, including reptiles, have shown that traits typically subjected to pre-copulatory selection trade off with those under post-copulatory selection (Moczek and Nijhout 2004; Fitzpatrick et al. 2012; Dines et al. 2015; Kahlr et al. 2016; Somjee et al. 2018). On the other hand, when there is high variance in resource acquisition, as can occur in the wild, individuals with high acquisition are predicted to invest in and achieve high fitness through both pre- and

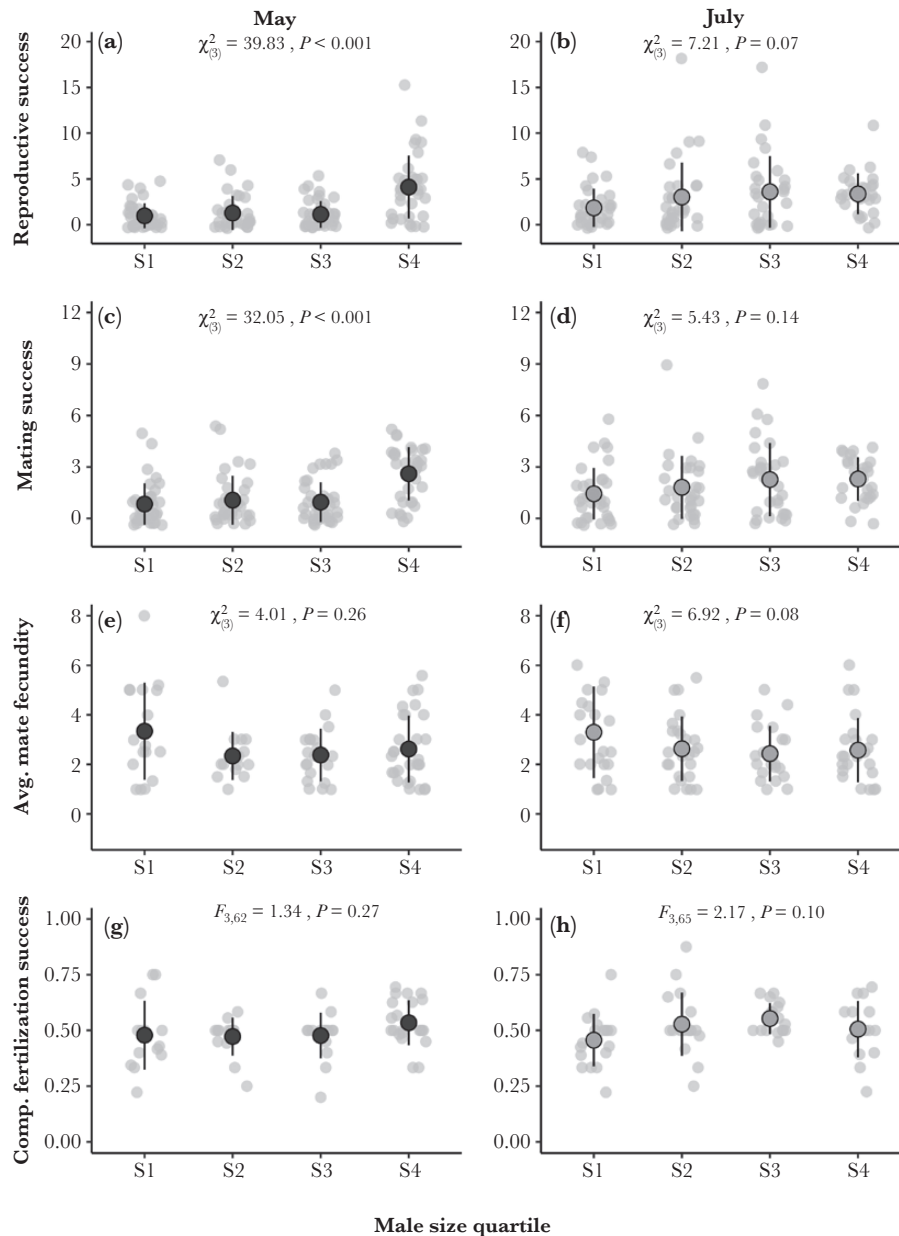


Figure 4

Distribution of (a, b) reproductive success (total number of offspring), (c, d) mating success (total number of mates), (e, f) average mate fecundity (mean fecundity across all mates), and (g, h) competitive fertilization success (mean proportion of offspring sired across all mates, adjusted for the number of competing males), for males powdered in May (left panels) and July (right panels) as a function of their corresponding size quartile. Fitness components were determined using genetic parentage analysis. Small symbols are individual values and larger overlaid symbols are mean \pm SD values for each quartile. Large males had significantly higher reproductive success and mating success than small males in May, but not in July. Average mate fecundity and competitive fertilization success did not differ as a function of size quartile in May or in July.

post-copulatory competition. Conversely, individuals with low resources may invest in neither, resulting in a positive correlation between pre- and post-copulatory components in a population (Saeki et al. 2014; Simmons et al. 2017). Consistent with this idea, several intraspecific studies have also reported a positive correlation between targets of pre-copulatory sexual selection and ejaculate traits (reviewed in Mautz et al. 2013; Supriya et al. 2019). Although some studies report positive associations between standardized fertilization success and traits such as body size, singing effort, and/or weapon size (Preston et al. 2001; Hosken et al. 2008; Turnell

and Shaw 2015; House et al. 2016), others report negative associations (Danielsson 2001; Evans et al. 2003; Kelly and Jennions 2011). Our findings are however, consistent with those studies in which male fertilization success is unrelated to body size or ornament size (Keogh et al. 2013; Rose et al. 2013; Flanagan et al. 2014; McDonald et al. 2017). This may indicate that investment in mate acquisition in anoles does not trade off or positively correlate with investment in fertilization success, possibly due to the predicted low cost of producing ejaculates when these are distributed across several copulations during the breeding season (Uller and

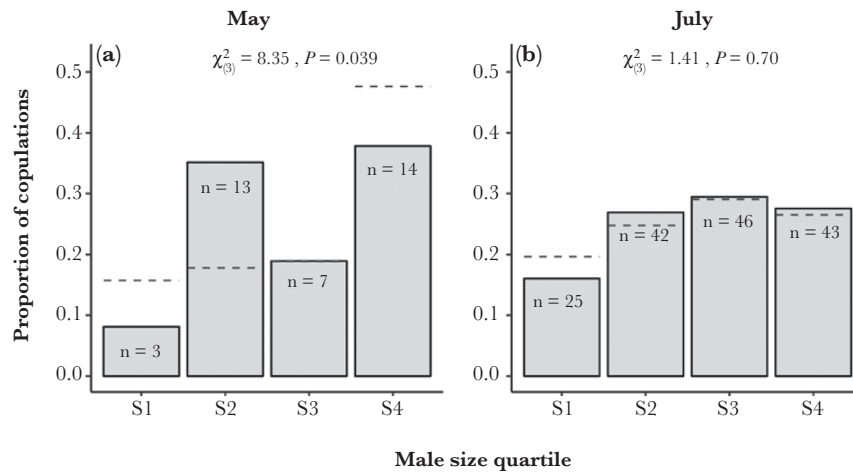


Figure 5

Comparison between behavioral and genetic estimates of male mating success in (a) May and (b) July. Bars represent the observed proportion of copulations for males in each size quartile based on transfer of powder, and dotted black lines indicate the expected proportion of copulations in each size quartile based on the number of mates inferred from genetic parentage data for the same males. Numbers (*n*) indicate the total number of copulations observed in each size quartile. The observed number of copulations based on powder transfer differed significantly from the expected number based on genetic parentage in May, but not in July.

Olsson 2008; Hayward and Gillooly 2011; Parker 2016; Kahl et al. 2021; *but see* Kahl and Cox 2015).

One caveat is that our measure of competitive fertilization success required us to exclude all instances in which a single male sired all of the offspring produced by a female, potentially excluding extremely strong or weak sperm competitors from our analysis (Supplementary Figures S2D and S3B). However, failure to account for the number of competing males in this way may result in spurious correlations because the estimated proportion of offspring sired by a male will increase, regardless of the focal male's competitive ability, if females produce offspring with fewer mates (Rose et al. 2013; Devigili et al. 2015; McCullough et al. 2018). Indeed, when we re-analyzed our data using unadjusted fertilization success in brown anoles, we found significant, albeit very weak, positive selection on male body size (Supplementary Figure S3). Thus, post-copulatory selection on body size may be weaker in natural populations, than previously reported by studies using unadjusted measures of male fertilization success (Preston et al. 2001; Hosken et al. 2008; Turnell and Shaw 2015; House et al. 2016). Our study suggests that, at least for body size, post-copulatory selection on body size is negligible when compared with pre-copulatory selection. It is more likely that post-copulatory selection acts primarily on ejaculate traits, as has been demonstrated in brown anoles (Kahl and Cox 2015), and that this selection may operate independent of male body size (Kahl et al. 2021).

Comparing behavioral and genetic measures of mating success

We found a close association between measures of size-specific mating success derived from genetic parentage and those inferred from copulations in the field, particularly in July (Figure 5b). This highlights the utility of fluorescent powder transfer as a relatively inexpensive and effective method for detecting copulations, particularly in natural populations, and for linking mating success to broad categories of phenotypic variance. Our findings are in line with other studies that have found behavioral proxies, such as the frequency of male–female associations in space and time, to be

closely predictive of the realized mating and reproductive success of males (Kamath and Losos 2018; Olsson et al. 2019; Baird and York 2021). However, our technique is much easier to execute compared with detailed observations of individual copulations or movements in the wild. Thus, this method can be used to uncover associations between mating success and categorical simplifications of continuous traits (as in this study), naturally categorical traits or groups (e.g., morphs), or experimental treatments (e.g., Wittman et al. 2022). It can also be used to uncover mating patterns of secretive or spatially dispersed species that can be difficult to observe in the wild for long hours (Gosden and Svensson 2007; Johnson et al. 2014). It is important to note that behavioral estimates of size-specific mating success based on powder transfer only corresponded closely with genetic mating success when extensive sampling of the female population was possible, and when the mating rate was high (Figure 5a, b). For example, in May, we only sampled females for 1 day and the inferred mating rate was half of that seen in July, when we sampled the population for 5 days (Figure 5a, b). Perhaps as a result, the relatively low number of observed copulations in May differed significantly from our expected distribution of size-specific mating success, which was likely more accurate because it was based on a much larger number of inferred copulations from genetic parentage (Figure 5a). Thus, behavioral observations or genetic parentage alone may not adequately capture fitness when populations are partially sampled, or if mating is infrequent within a short sampling period.

CONCLUSIONS

Overall, our study supports the widely held assumption that large body size is associated with higher reproductive success in brown anoles, and that this is primarily due to the increased mating success of large males. Although previous work has suggested that sexually antagonistic viability selection may favor large male size and promote male-biased sexual size dimorphism in this species (Cox and Calsbeek 2010a; *but see* Cox and Calsbeek 2015), our results support a parallel body of work suggesting that sexual selection

strongly favors large male size, potentially through male–male contest competition (Trivers 1976; Tokarz 1985; Jenssen et al. 2005; Duryea et al. 2016; Kamath and Losos 2018). Importantly, we extend this work by specifically resolving the importance of pre-copulatory sexual selection despite the potential for female fecundity or post-copulatory processes mediating selection on male body size. We also link pre-copulatory selection for large male size to both behavioral and genetic measures of mating success. Our results further illustrate that strong pre-copulatory sexual selection and extremely male-biased sexual size dimorphism can occur even in promiscuous mating systems in which access to females cannot be monopolized and multiple paternity is common. Finally, our findings also emphasize the importance of incorporating both behavioral and genetic methods in the same study to achieve a more robust understanding of the roles of pre- and post-copulatory processes in sexual selection.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behco.oxfordjournals.org/>.

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AUTHOR CONTRIBUTIONS

Rachana S. Bhavé (Conceptualization [lead], Data curation [equal], Formal analysis [lead], Funding acquisition [supporting], Investigation [lead], Methodology [equal], Project administration [equal], Resources [equal], Supervision [supporting], Validation [lead], Visualization [lead], Writing – original draft [lead], Writing – review & editing [equal]), Heidi A. Seears (Data curation [equal], Investigation [supporting], Methodology [equal], Writing – review & editing [supporting]), Aaron M. Reedy (Data curation [supporting], Investigation [supporting], Methodology [supporting], Writing – review & editing [supporting]), Tyler N. Wittman (Investigation [supporting], Writing – review & editing [supporting]), Christopher D. Robinson (Investigation [supporting], Writing – review & editing [supporting]), and Robert M. Cox (Conceptualization [supporting], Funding acquisition [lead], Investigation [supporting], Methodology [equal], Project administration [equal], Resources [lead], Supervision [lead], Visualization [supporting], Writing – review & editing [equal])

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY

Analyses reported in this article can be reproduced using the data provided by Bhavé et al. (2023).

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