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Adult sex ratio influences courtship behaviour, contest behaviour and breeding success in *Onthophagus taurus*

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Social interactions (e.g. aggression, mating and parenting) often depend on the social environment, and recent studies suggest that the adult sex ratio (ASR, the proportion of males in the adult population) modulates these social interactions. While observational studies and phylogenetic analyses provide strong support for the proposition that the relative frequencies of adult males and females impact social decisions, the experimental evidence for the impacts of ASR on social interactions is moot. In this study, we manipulated ASR in dung beetles, *Onthophagus taurus*, to test the responses of males and females in contest behaviour, courtship behaviour and breeding success. We found that ASR influenced the intensity of both contest and courtship, with both contests and courtships being more frequent in male-biased ASRs than in female-biased and unbiased treatments. The weight of brood balls did not differ significantly between ASR treatments, suggesting that ASR does not affect the breeding success of dung beetles by changing the parental investment of females and males. Moreover, the mean number of brood balls per male was fewer in male-biased treatments than in female-biased and unbiased treatments, while there was no significant difference in the mean number of brood balls per female between treatments, supporting the hypothesis that a male-skewed ASR can impair the breeding success of organisms by influencing the reproductive capacity of males but not females. Our results extend the understanding of the influence of ASR on the reproductive strategy decisions of males and females in breeding success. Future analyses are needed to understand the physiological and genetic implications of mate choice and contest behaviour in the context of a variable social environment, as represented by ASR.

Adult sex ratio (ASR, the proportion of males in the adult population, Schacht & Smith, 2017; Schacht et al., 2022; Székely et al., 2014) is a fundamental concept in evolutionary biology (Hardy, 2002; Schacht et al., 2022; West, 2009), having far-reaching consequences for sexually selected traits in behaviour, ecology and evolution (Komdeur et al., 2017; Székely et al., 2014). Via frequency-dependent interactions between males and females, ASR also impacts mortalities, emigration and immigration, and thus has knock-on effects on population densities, population viabilities and resilience (Ancona et al., 2017; Le Galliard et al., 2005).

As a predictor for intensity of competition for mates, ASR has been widely considered to impact interactions among individuals through direct intrasexual interactions such as contest competition and intersexual interactions such as courtship, which are both common patterns of mating competition during the reproductive season (Andersson, 1994; Kekäläinen & Evans, 2018; Weir et al., 2011). A skewed ASR alters mating opportunities and enhances intrasexual competition and sexual selection of the abundant sex (Kokko & Jennions, 2008; Queller, 1997). This phenomenon, characterized by intensified intrasexual competition resulting from a substantial presence of individuals of the same sex, is not limited to males but is also commonly observed in females (Clutton-Brock, 2007, 2009). In male-biased populations, males may increase their aggressive behaviour and reduce courtship behaviour towards females (Clark & Grant, 2010; Forsgren et al., 2004) and an increase in harassment of females may result in higher female mortality (Hailey

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& Willemsen, 2000; Le Galliard et al., 2005). Extreme ASR was noted in Hermann's tortoise, *Testudo hermanni*, where a dearth of females led to increased male–male interactions and coercive copulations, often resulting in female harm (Bonnet et al., 2016). Conversely, female-biased ASR results in less male aggression and increased courtship (Grant et al., 2000). In the dung beetle *Lethrus apterus*, an increased ASR significantly intensifies male–male competition (Rosa et al., 2017). Similar observations can be found in the other dung beetles (Pomfret & Knell, 2008) and the flour beetle *Tribolium castaneum* (Michalczyk et al., 2011). A meta-analysis shows that when the OSR (operational sex ratio, the proportion of sexually active males in the mating pool) leans towards males, competitors' aggression escalates with the rising OSR, but it decreases again after reaching a critical point (≥1.99), presumably due to the increased number of rivals (Weir et al., 2011). By contrast, the frequency of courtship tends to decrease as the OSR becomes increasingly male biased, while the incidence of mate guarding and the length of copulation tend to rise (Weir et al., 2011). This opposite trend between these two behaviours clearly indicates that ASR may have different effects on contest and courtship behaviours.

Breeding success is critical for species survival and reproductive success, often achieved through strategies such as increasing fecundity (reproductive capacity of an organism, Bradshaw & McMahon, 2008) or offering parental care (care to offspring from parents, Smiseth et al., 2014). For instance, species like Atlantic cod, *Gadus morhua*, produce many offspring to boost survival rates (Rowe & Hutchings, 2003; also see mealworm beetle, *Tenebrio molitor*; Rho & Lee, 2016; and the dung beetle *Onthophagus*, Hunt et al., 2002; Simmons & Emlen, 2008), while others including birds and insects invest in building nests, procuring food and protecting their young (Cockburn, 2006; Eggert et al., 1998; Smiseth et al., 2014; Wong et al., 2013). However, there is often a trade-off, because devoting too much to parental care can restrict the number of offspring produced in a season (Badyaev & Ghalambor, 2001; Furness et al., 2022; Ratikainen et al., 2018), and vice versa. For example, as the altitude rises, a strong negative relationship between number of offspring and duration of parental care occurs (Badyaev & Ghalambor, 2001). Meanwhile, the role of ASR in influencing parental care and fecundity for breeding success remains less studied. Some research suggests that the dominant sex in a biased population provides more care to maximize fitness (Kokko & Jennions, 2012; McNamara et al., 2000; Remeš et al., 2015). For example, male burying beetles, *Nicrophorus vespilloides*, exhibit an extension, rather than a reduction, in the duration of parental care in the male-biased condition (Hopwood et al., 2015). Meanwhile, others argue that males in a male-dominant population might decrease parental investment to, for example, enhance paternity (Alonzo, 2010; Fromhage & Jennions, 2016; Garcia-Navas et al., 2013; Houston & McNamara, 2002; Neff, 2003). Furthermore, a skewed ASR can limit mate availability, thereby impacting fecundity and, consequently, breeding success (Bouchard et al., 2006; Gou et al., 2019). Nevertheless, some species like the flea beetle, *Agasicles hygrophila* (Coleoptera: Chrysomelidae), show higher fecundity in male-biased situations (Guo et al., 2014). Here, using dung beetles *Onthophagus taurus*, we investigated the influence of ASR on the parental care, fecundity and the trade-off between parental care and fecundity of individuals for breeding success.

Dung beetles, particularly the species *O. taurus*, serve as an excellent model for studying intrasexual competition, contest dynamics and breeding success. This is attributed to their relatively prolonged reproductive period (≥6 months within a 1-year period under natural conditions) and repetitive reproduction capacity, as well as their biparental care, significant sexual size dimorphism and diverse sexual behaviours, including courtship and contest (Hanski & Cambefort, 2014; Scholtz et al., 2009; Simmons & Ridsdill-Smith, 2011). *Onthophagus taurus* can amass densities of 5000 adults per dung pad (Hunt et al., 1999), leading to widespread competition among individuals for mates and resources. Their sexual dimorphism extends to morphology and reproductive strategy, with dominant males showcasing large horns indicative of a mate-guarding strategy, while subordinate males usually lack horns and are smaller (Moczek & Emlen, 2000). Horn length plays a role in male–male competition and males with a larger horn have an advantage in securing paternity (Simmons & Ridsdill-Smith, 2011). Reproduction in this species is strictly reliant on dung availability, which is crucial for larval growth. Females prepare brood balls from dung, serving as the sole food source for larvae until they emerge (Hanski & Cambefort, 2014; Scholtz et al., 2009). The brood ball weight, primarily determined by females, directly impacts offspring fitness due to its nutritional content (Hanski & Cambefort, 2014; Hunt & Simmons, 1997, 2001; Simmons & Ridsdill-Smith, 2011). In general, larger females produce heavier brood balls and large males selectively assist females based on environmental context, while smaller males generally do not (Hunt & Simmons, 1998). Additionally, females with male companions produce fewer but larger brood masses than females on their own or with hornless males, indicating that the relative number of males and females may significantly affect both parental care and fecundity of parents (Hunt & Simmons, 1998). The impact of ASR at larger gradients, however, remains untested.

We had two objectives in this experiment. First, we investigated the influence of ASR on the intensity of courtship and contest behaviour. We expected (1) an overall higher intensity of courtship and contest behaviour in the male-biased treatment compared to female-biased and unbiased treatments, and (2) males in the male-biased treatment and females in the female-biased treatment to initiate contest events more frequently. Second, we investigated the influence of ASR on breeding success. We predicted that (1) ASR can affect breeding success by impacting dung beetles' parental care, with lighter brood balls more likely in the male-biased treatment than in female-biased and unbiased treatments and (2) ASR may alter individual reproductive capacity, leading to fewer brood balls produced in the male-biased treatment compared to female-biased and unbiased treatments.

METHODS

Maintenance

Virgin beetles used in this experiment were first-generation beetles whose parents were transported from a laboratory in Germany (Terrestrial Ecotoxicology Laboratory, Niefern-Öschelbronn, Germany), bred and housed separately at 24 °C with a photoperiod of 16:8 h light:dark until sexually mature. Adequate dung was supplied every 5 days for more than 2 months to guarantee that the beetles were in a good reproductive state during experiments. Fresh cow dung droppings were collected in the milking room at an organic dairy farm in the north of Groningen (Martinizicht, 53° 15′ 37.8″N, 6° 34′ 45.5″E), then stored and frozen in 10-litre buckets at −20 °C in our laboratory. The dung was allowed to thaw and reach room temperature for 24 h before feeding. All beetles were used only once during the experiment. We assumed that ASR equalled OSR, because all dung beetles used in the experiment were sexually mature and in good physical condition.

Experimental Design

Adult sex ratio manipulation

For the sequential behavioural observations and breeding work (Table 1), 180 adult dung beetles were housed in groups of six in three ASR treatments (female-biased treatment, unbiased

Table 1
The manipulation of adult sex ratio (ASR) in the experiment

Treatment	ASR (%)	Replication	Number of males	Number of females	Body size of males (mm)	Body size of females (mm)
Female-biased	33.33	× 10	2	4	4.925±0.075	4.901±0.055
Unbiased	50.00	× 10	3	3	4.948±0.039	4.925±0.062
Male-biased	66.66	× 10	4	2	4.908±0.026	4.823±0.101

Body size is given as mean ± SE.

treatment and male-biased treatment). Each replication and each treatment were conducted in parallel. To tell beetles apart they were individually marked with small differently coloured dots on their backs using permanent marker (Edding 751, Japan). Maximum pronotum length of beetles was measured with digital callipers (0.01 mm, Mahr, Germany; Hunt et al., 1999; Hunt et al., 2002) and used as body size. Previous research has consistently shown that measurements of the pronotum can serve as a reliable indicator of body size in the genus *Onthophagus* (Emlen, 1997). For instance, in the species *O. sagittarius*, the pronotum width was found to be highly repeatable ($F_{9,10} = 1005.6$, $P < 0.001$, repeatability estimate = 0.999; Watson & Simmons, 2010). All measurements were performed only once, and consistently by the same person to ensure measurement stability. In future studies, to increase the precision of measurements, we recommend employing noninvasive methods to assess the body morphology of dung beetles. This could involve capturing high-resolution photographs using cameras equipped with standard scales, followed by the utilization of analytical software (e.g. ImageJ, https://imagej.net/ij/) for body shape measurement (for examples, see Broeke et al., 2015; Fowler et al., 2021). There was no significant difference in body size between the sexes and between the three ASR treatments (sex: $F_1 = 0.919$, $P = 0.338$; ASR treatment: $F_2 = 0.927$, $P = 0.391$).

Behavioural observations

After grouping, the beetles from each treatment were placed in observation boxes for behavioural observations. For the experiment we tried to raise/obtain as many beetles as possible within our capacity. Owing to the size constraints of the experimental population, six individuals were selected as a single sample size in each observation box to ensure a balance between the number of dung beetles in each observation box and the total sample size. This experimental design guaranteed the possibility of intrasexual competition of the minor sex (e.g. two females in the male-biased treatment). Similarly, owing to the limited population size, we did not segregate males into major and minor groups. Instead, all males and females were randomly distributed into various boxes, ensuring an even distribution of body size across the groups. Plastic boxes (16 × 9 cm and 10 cm high), with a little soil covering the inner surface of the box but not affecting our observations, were used as the behaviour observation boxes. Three plastic boxes, containing one ASR treatment each, were placed in a triangle, 50 cm apart on a horizontal desk in uniform dim light under 24 ± 2 °C constant temperature. The density in all boxes was controlled as six beetles in one box (24 cm^2/individual). A video camera (GZ-R405BEU, JVC, Japan) was positioned above each box to observe and trace the movements of each beetle. At the onset of each experiment, when beetles were put together in the box, the camera was started and all beetles were simultaneously recorded for 90 min for behavioural analyses of contest and courtship behaviour (Fig. 1a). Two persons scored behaviour independently in half of the observational videos after achieving consensus on the differentiation and identification of various behavioural categories, and then the data were combined for analyses.

Courtship

Courtship behaviour was defined as a male rapidly drumming its foreleg tarsi on the pronotum or elytra of a female beetle (Beckers et al., 2017; Fig. 1a). To investigate the influence of ASR on the intensity of courtship behaviour, the frequency of initiating courtship events per box and the frequency of initiating courtship events of each specific male were recorded.

Contest

Contest behaviour was expressed as fighting, including primarily head butting and pushing (Beckers et al., 2017; Fig. 1a), either among males (main type), among females or between the sexes. We calculated the frequency of contest events in each box and the attacking frequency of each individual to investigate the influence of ASR on intensity of contest behaviour.

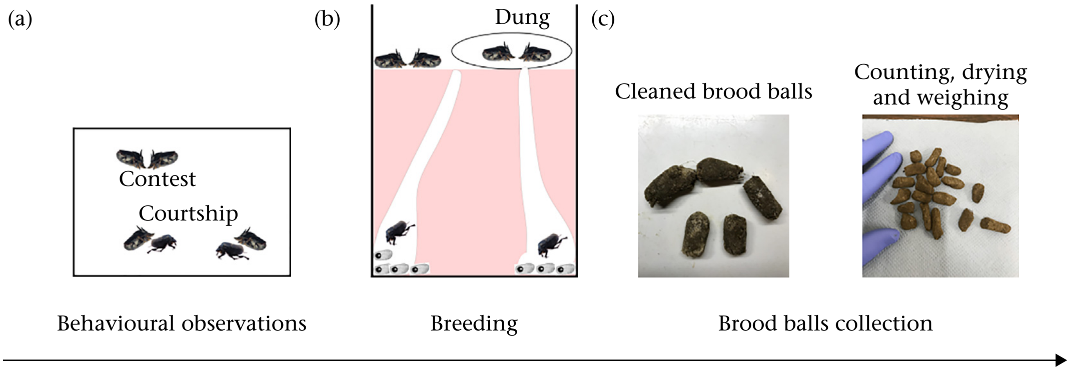

Figure 1. Experimental flowchart (taking male-biased treatment as a representative case). (a) Behavioural observations. We recorded the courtship frequency of males and the contest frequency of individuals at both the population and individual levels. (b) Breeding. The construction of brood balls by two females in the presence of four males. (c) Brood balls collection. Brood balls obtained from the male-biased treatment were subjected to surface soil removal, counting, drying and weighing procedures. The arrow indicates the experimental procedures in a sequential manner.

Breeding and Brood Balls Collection

Breeding success in this study refers to the combined strategies individuals employ in a single breeding cycle to maximize reproductive success, including increased parental investment and offspring quantity (Badyaev & Ghalambor, 2001). After the behavioural recording, all beetles (both males and females) from the observation box were immediately transferred to a breeding chamber (Fig. 1b). Breeding chambers (15 cm diameter and 20 cm high) were three-quarters filled with moist sand and soil (mixed in a ratio of 1:1) and provided with 150 g of dung on the top. Breeding duration was set to 6 days.

After breeding finished, all brood balls were gently removed and sifted out using a sieve with a 0.5 cm aperture. All brood balls were adhered to the bottom vicinity of the breeding chambers (ca. 15–17 cm deep; Fig. 1b). Soil and other impurities from the surface of the brood balls were removed using small tweezers. Impurities stuck on the inner dung were cleaned away with a tiny dissecting needle. Brood balls were then dried at 60 °C for 48 h to constant weight, excess sand was removed with a dissecting probe, and they were weighed using an analytical balance (AG204, Mettler Toledo, Switzerland; Hunt & Simmons, 2002; Fig. 1c). We weighed each individual brood ball within every box, rather than assessing the total weight of brood balls in a box. Weight of brood balls, number of brood balls per breeding box and mean number of brood balls per female and per male (i.e. number of brood balls per breeding box/number of females or males in breeding box) were set as indicators for breeding success.

Ethical Note

The dung beetles used in this study were sourced from a laboratory in Germany and transported to our laboratory in spring under suitable temperature conditions. The laboratory population was cultivated, sustained and expanded in a climate-controlled house with constant temperature and humidity, and fed a suitable, sufficient diet. No anthropogenic or other injuries were inflicted on the dung beetles during the experiment. The vast majority of dung beetles did not exhibit any significant physical injuries following the experiment. Upon completion of the experiment, all dung beetles were returned to their original boxes, allowed to recover, and then used to produce the next generation of the laboratory population.

Statistical Analyses

Data analyses of contest and courtship behaviour were performed in R version 4.0.3 (R Development Core Team, 2020) using generalized linear mixed models (GLMMs) or linear mixed models (LMMs) from package lme4 (Bates et al., 2015) according to response variable types.

We conducted homogeneity level, data dispersion and normality tests on all the data we analysed. The results of these tests demonstrated that the homogeneity level and data dispersion of all the data conformed to the basic characteristics inherent to their respective data types. However, some of the data exhibited poor normality, and we applied a logarithmic transformation to increase the normality of these specific data sets (see below). In each model, ASR treatment, body size and/or sex were added as explanatory variables. In all models, according to our research targets, we added the interaction effect between ASR treatment and other explanatory variables (body size or sex), when they were of special interest (e.g. the interaction between sex and body size in the models of contest behaviour). Body size of individuals was regarded as a covariate, but the focus of the study was on the effects of the experimental ASR treatments on the outcome variables. Box and ID of beetles, when appropriate, were used as random variables. All models we selected showed acceptable dispersion level and Akaike information criterion. The glht function from the package multcomp (Hothorn et al., 2008) and the emmeans and emtrends functions from the emmeans package (Searle et al., 1980) were used to do post hoc tests if necessary. We used cor and cor.test functions from the package Matrices (Bates et al., 2017) to test the Pearson correlation coefficient and significance of the correlation between body size and horn size of male beetles. There was strong collinearity between body size and horn size in male beetles in our experiment (PCCs: $r = 0.742$, $P < 0.001$) and the results of models were qualitatively the same when using body size or horn size as explanatory variable. Therefore, we only show results of models with body size, not horn size, as the explanatory variable.

Courtship behaviour

For courtship behaviour, we first ran a GLMM with a Poisson error distribution on frequency of courtship events in each box. Subsequently, we applied a logarithmic transformation to the courtship frequency of male individuals in each box and ran an LMM to investigate which males exhibited higher courting frequency.

Contest behaviour

To test the effect of ASR on the intensity of contest, we first ran a GLMM with a Poisson error distribution on frequency of contest events in each box. Subsequently, we performed a logarithmic transformation on the frequency of contest events initiated by each individual in each box and ran an LMM to examine which males were more likely to initiate courtship events.

Breeding success

We ran an LMM to compare the weight of brood balls among different treatments. We used a linear model to compare the number of brood balls per box, the mean number of brood balls per female and the mean number of brood balls per male in each box among different treatments.

RESULTS

Courtship and Contest Behaviour

ASR significantly impacted courtship behaviour. The male-biased treatment resulted in the highest courtship frequency, surpassing both female-biased ($z = 6.860$, $P < 0.001$) and unbiased ($z = 9.766$, $P < 0.001$) treatments, with the latter also being lower than the female-biased treatment ($z = 2.841$, $P = 0.012$; Table 2,

Table 2
The influence of experimental manipulation of adult sex ratio (ASR) on courtship behaviour of male dung beetles among ASR treatments during a 90 min trial

Explanatory variable	χ^2/*F*[a]	*df*	*P*
Total no. of courtship events in boxes (*N*=29)			
ASR treatment	109.860	2	<0.001
Frequency of courtships of individual males (*N*=88)			
ASR treatment	4.452	2	**0.019**
Male body size	0.009	1	0.921
ASR treatment * male body size	0.553	2	0.577

The ID of each box was added as a random effect in the GLMM model when testing total number of courtship events in boxes and the LMM model when testing frequency of courtships exhibited by individual males. Bold text indicates a significant difference.

[a] The chi-square value is given for total number of courtships and *F* values for frequency of courtships.

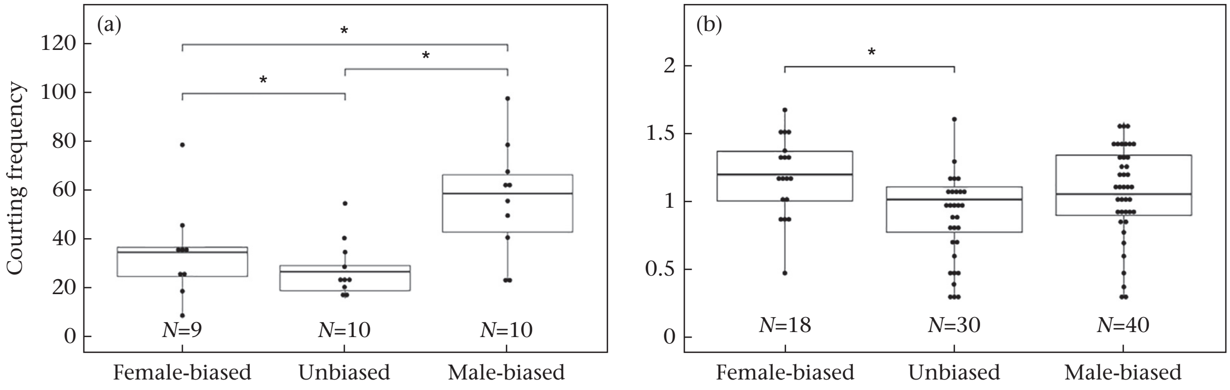

Figure 2. Intensity of courtship behaviour. (a) Frequency of courtship events in each box in the three treatments. Points represent the frequency of all courting events occurring within a given box in each treatment. (b) Frequency of initiating courtship events of males in the three treatments. Points represent the frequency of courting initiation by each male within each treatment. (a, b) Box plots illustrate the median, as well as the 25th and 75th percentiles. Whiskers extend to the furthest data point within 1.5 times the interquartile range following the Tukey method (R package, ggplot2). Sample size is indicated above the *X* axis. *$P < 0.05$.

Fig. 2a). Courtship initiations by males were lower in the unbiased compared to the female-biased treatment ($z = 2.862$, $P = 0.012$), but comparable to the male-biased treatment ($z = 2.078$, $P = 0.093$). No significant variation was found in male courting frequency between female-biased and male-biased treatments ($z = 1.234$, $P = 0.431$; Table 2, Fig. 2b).

ASR significantly impacted contest behaviour, with contests more frequent in the male-biased treatment compared to the female-biased ($z = 3.633$, $P < 0.001$) and unbiased ($z = 3.006$, $P = 0.007$) treatments, and no difference was observed between the latter two ($z = 0.771$, $P = 0.719$; Table 3, Fig. 3a). A similar trend was found for individual initiations of contests, with the male-biased treatment leading to more events than female-biased ($z = 3.054$, $P = 0.006$) and unbiased treatments ($z = 3.121$, $P = 0.005$), but no difference between female-biased and unbiased treatments ($z = 0.093$, $P = 0.995$; Table 3, Fig. 3b). Both sexes initiated contests at a similar frequency (Table 3, Fig. 3c), and larger individuals were more prone to initiate attacks (Table 3).

Breeding Success

ASR did not significantly alter parental care. There was no significant difference in weight of brood balls between treatments (Table 4, Fig. 4a). Male-biased treatments produced fewer brood balls than unbiased treatments ($t = 2.704$, $P = 0.036$), but not fewer than female-biased treatments ($t = 2.536$, $P = 0.052$), with no difference between female-biased and unbiased treatments ($t = 0.256$, $P = 0.96$; Table 4, Fig. 4b). The mean number of brood balls per female was similar across all treatments (Table 4, Fig. 4c). However, the mean number of brood balls per male in the male-biased treatment was fewer than those in female-biased ($t = 5.567$, $P < 0.001$) and unbiased ($t = 3.053$, $P = 0.017$) treatments, with no significant difference between female-biased and unbiased treatments ($t = 2.414$, $P = 0.064$; Table 4, Fig. 4d).

Table 3
The influence of experimental manipulation of adult sex ratio (ASR) on contest behaviour of females and males among ASR treatments during a 90 min trial

Explanatory variable	χ^2/F[a]	*df*	*P*
Total no. of contest events in boxes (*N*=29)			
ASR treatment	16.172	2	<0.001
Attacking frequency of individuals (*N*=174)			
ASR treatment	6.341	2	**0.005**
Sex	0.020	1	0.887
Body size	6.138	1	**0.014**
ASR treatment * sex	2.109	2	0.125
ASR treatment * body size	2.262	2	0.107
Sex * body size	1.229	1	0.269

The ID of each box was added as a random effect in the GLMM model when testing total number of contest events in boxes and the LMM model when testing attacking frequency of individuals. Bold text indicates significant differences.

[a] The chi-square value is given for total number of contest events and *F* values for attacking frequency.

DISCUSSION

Our study underscores ASR's significant impact on contest and courtship behaviours' intensity and on population breeding success. More contest and courtship events were observed in male-biased environments. Despite varying courtship intensity in female-biased and unbiased treatments, contest intensity remained similar. A similar pattern was observed when examining individual behaviours. ASR appeared not to influence parental care directly, with consistent brood ball weight across treatments. Male reproductive success was lower in male-biased settings, with no impact on female fecundity, indicating ASR's intricate effects on dung beetle breeding success, predominantly via fecundity rather than parental care.

Influence of ASR on Intensity of Contest and Courtship Behaviours

Agonistic contests play a crucial role in sexual selection across various animal taxa, involving both intrasexual and intersexual competition (Briffa & Sneddon, 2007; Clutton-Brock, 2007; Rosvall, 2011). Studies show that the overrepresented sex in a population usually experiences intensified sexual competition for mates (Emlen & Oring, 1977; Janicke & Morrow, 2018; Weir et al., 2011). This concept is broadly corroborated by extensive, multispecies empirical data in both contest (e.g. mammals, Clutton-Brock, 2007, 2009; birds, Székely et al., 2014; fishes like gobies (Gobiidae), Forsgren et al., 2004; Kvarnemo et al., 1995; pipefishes (Syngnathidae), Silva et al., 2010; guppies, *Poecilia reticulata*, Chuard et al., 2016; insects like dung beetles, Pomfret & Knell, 2008; flour beetle, *Gnatocerus cornutus*, House et al., 2019; dung beetle *L. apterus*, Rosa et al., 2017; flour beetle *T. castaneum*, Michalczyk et al., 2011; fruit fly *Drosophila melanogaster*, Bath et al., 2021) and courtship behaviour (e.g. fish, *Syngnathus typhle*, Aronsen et al., 2013; fruit fly *Ceratitis capitata*, Leftwich et al., 2012; fruit fly *Drosophila*, Vahl et al., 2013; Colorado potato beetle, *Leptinotarsa decemlineata*, Vahl et al., 2013). Meta-analysis found that increasing OSR corresponds to increased male aggression, which declines when OSR becomes more male-biased, probably due to competition costs

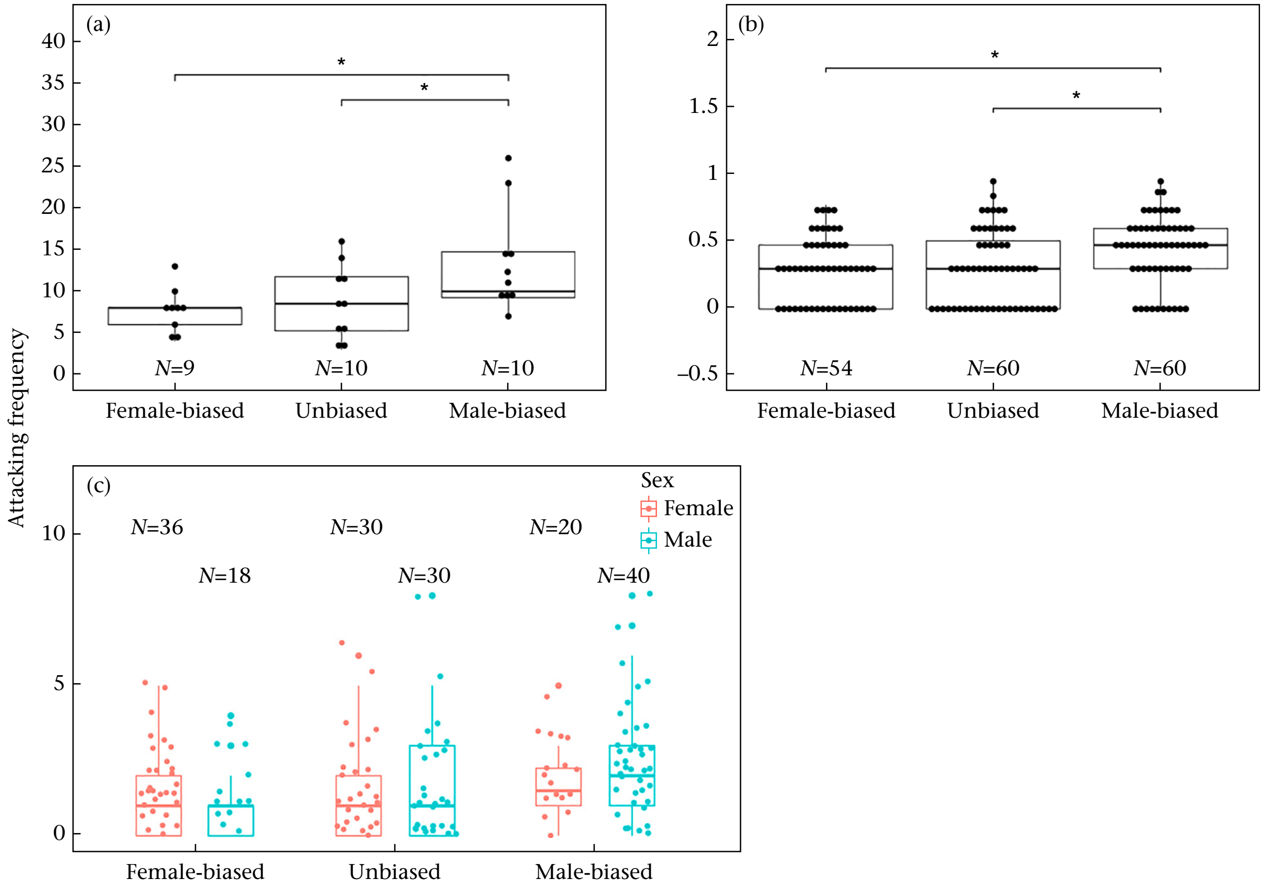

Figure 3. Intensity of contest behaviour. (a) Frequency of contest events in each box in the three treatments. Points represent the frequency of all attacking events occurring within a given box in each treatment. Sample size is indicated above the *X*-axis. (b) Frequency of initiating contest events of individuals in the three treatments. Points represent the frequency of attacking initiation by individuals within each treatment. Sample size is indicated above the *X* axis. (c) Frequency of initiating contest events between males and females in the three treatments. Points represent the attacking frequency of males or females in each treatment. Sample size is indicated above the box plots. (a, b, c) Box plots illustrate the median, as well as the 25th and 75th percentiles. Whiskers extend to the furthest data point within 1.5 times the interquartile range following the Tukey method (R package, ggplot2). $*P < 0.05$.

Table 4
The influence of experimental manipulation of adult sex ratio (ASR) on breeding success of dung beetles *Onthophagus taurus*

Explanatory variable	Mean±SE per box	*F*	*df*	*P*
Weight (g) of brood balls in boxes (*N*=301)				
ASR treatment		0.029	2	0.972
FB (*N*=124)	1.071±0.033			
UB (*N*=113)	1.055±0.027			
MB (*N*=64)	1.090±0.045			
No. of brood balls in boxes (*N*=22)				
ASR treatment		4.541	2	**0.024**
FB (*N*=8)	15.500±1.581			
UB (*N*=7)	16.142±2.642			
MB (*N*=7)	10.142±0.553			
Mean no. of brood balls of each female (*N*=22)				
ASR treatment		1.807	2	0.191
FB (*N*=8)	3.875±0.395			
UB (*N*=7)	5.380±0.880			
UB (*N*=7)	4.571±0.277			
Mean no. of brood balls of each male (*N*=22)				
ASR treatment		15.517	2	**< 0.001**
FB (*N*=8)	7.750±0.790			
UB (*N*=7)	5.380±0.880			
MB (*N*=7)	2.290±0.138			

The mean ± SE of response variables in each treatment was calculated separately to show the difference of a specific response variable between ASR treatments. FB: female-biased treatment; UB: unbiased treatment; MB: male-biased treatment. Bold text indicates significant differences. The ID of each box was added as a random effect in the LMM model when testing weight of brood balls.

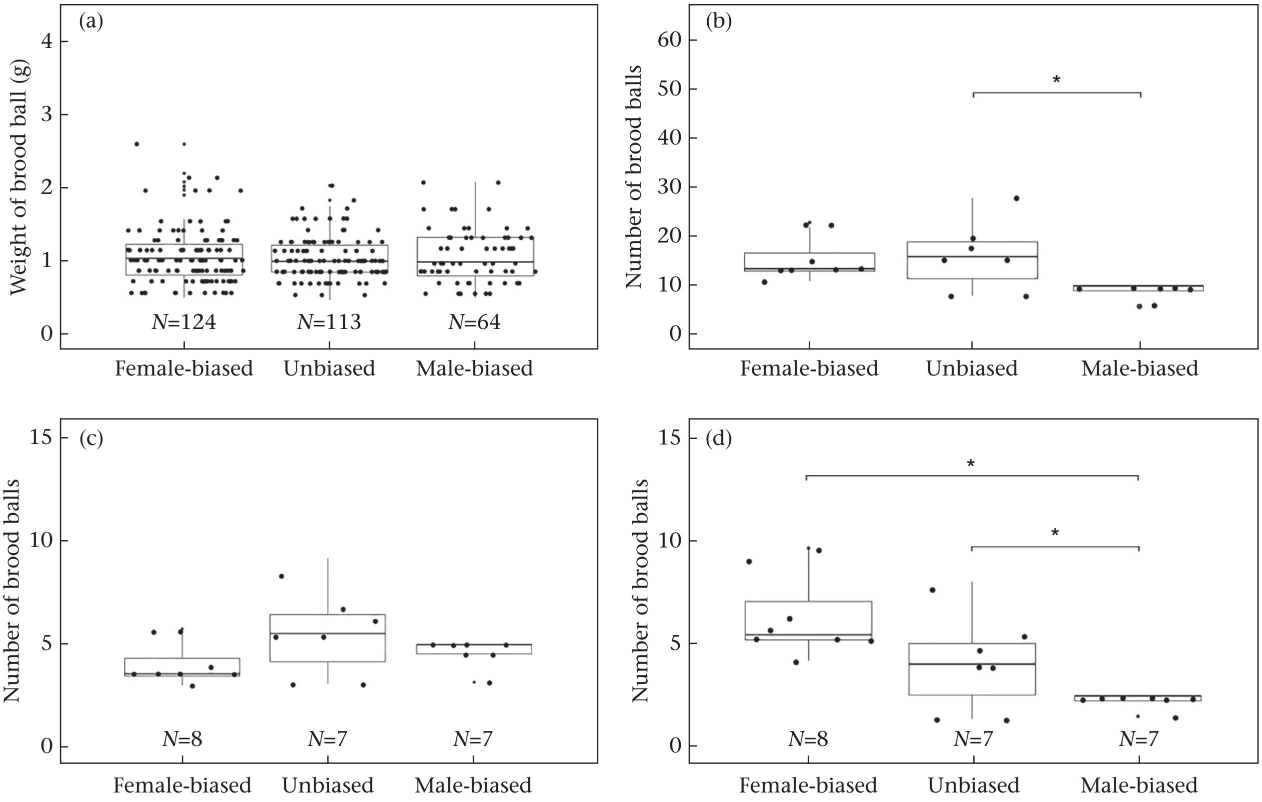

Figure 4. Implications of ASR for the weight of brood balls, number of brood balls and mean number of brood balls per female and per male in the three treatments. (a) Weight of brood balls. Points represent the weight of each brood ball in the three treatments. (b) Number of brood balls in boxes in the three treatments. Points represent the number of brood balls in a box in each treatment. (c) Mean number of brood balls of each female in each box in the three treatments. Points represent the average number of brood balls produced by each female in a box in each treatment. (d) Mean number of brood balls of each male in each box in different treatments. Points represent the average number of brood balls fertilized by each male in a box in each treatment. (a, b, c, d) Box plots illustrate the median, as well as the 25th and 75th percentiles. Whiskers extend to the furthest data point within 1.5 times the interquartile range following the Tukey method (R package, ggplot2). Sample size is indicated above the *X* axis. $*P < 0.05$.

(Weir et al., 2011). Our study found that varying ASRs produced distinct effects on the performance of these two behaviours. A male-biased ASR yielded a higher frequency of contest events compared to unbiased and female-biased treatments. Consistently, individuals in the male-biased treatment displayed a higher frequency of contest events, highly aligning with previous extensive research. Interestingly, both female-biased and male-biased treatments exhibited elevated courtship rates compared to unbiased ASR. When analysing individual courtship performance, each male in the unbiased treatment initiated fewer courtship events than in the female-biased treatment, but not in the male-biased treatment. This finding partially aligns with Weir et al. (2011), who discovered that increasing OSR led to reduced courtship rates, while mate guarding and copulation duration increased. Traditional OSR theory suggests that male courtship rates should increase with higher OSR (de Jong et al., 2012; Kvarnemo & Ahnesjö, 1996), yet empirical systems often report the opposite trend (Chuard et al., 2016; Verrell & Krenz, 1998; Weir et al., 2011). This could be attributed to limited female availability in higher OSR treatments. Consequently, heightened male competition in male-biased OSRs might reduce male investment in courtship displays (de Jong et al., 2012; Driscoll et al., 2022; Jirotkul, 1999), suggesting a trade-off between contest and courtship behaviours. Particularly, we were puzzled by the observation that courtship intensity in the female-biased treatment was higher than in the unbiased treatment, at both the population and individual levels. A plausible explanation for this phenomenon could be that all virgin males in our experiments were raised separately, devoid of any interaction with females. Upon their first encounter with females, these virgin males exhibited a strong mating desire. Consequently, in this scenario, it appears that the frequency of male courtship is primarily determined by the number of accessible females in their proximity, rather than the individual male's mating intent.

Influence of ASR on Breeding Success

The balance between parental investment and breeding success is complex. Parental care can boost offspring fitness but may reduce parents' future reproductive success (Wong et al., 2013). ASR can affect parental care strategies diversely. Males in male-biased populations might invest more in offspring care (Kokko & Jennions, 2012; Liker et al., 2013, 2014; Remeš et al., 2015) due to limited female availability (Kokko & Jennions, 2008). For instance, shorebird studies show that male care increases and female care decreases in male-biased populations (Liker et al., 2013). Male burying beetles, *N. vespilloides*, typically extend the duration of parental care under conditions of male bias (Hopwood et al., 2015). However, higher paternity uncertainty, extrapair copulations and the risk of sperm competition could drive males to prioritize, for example, paternity assurance (Fromhage et al., 2008) over caregiving (Alonzo, 2010; Fromhage & Jennions, 2016; Houston & McNamara, 2002; Neff, 2003). In male-biased populations, zebra finch males increase parental care while females remain unaffected (Burley & Calkins, 1999). Similarly, the dung beetle *L. apterus*, under male-biased conditions, shows reduced leaf collection, indicative of lower parental care levels, possibly due to intensified male–male competition (Rosa et al., 2017). In addition, a study on the dung beetle *O. taurus* indicated that horned males tend to provide parental care during brood ball formation, generally resulting in heavier brood balls (Hunt & Simmons, 1998). Excessive males, however, might disrupt females' brood ball production by obstructing tunnels during contests, consequently reducing fecundity. Yet, our study found no significant difference in brood ball weight across treatments, even though male-biased populations experienced heightened intrasexual competition, suggesting male care levels may be consistent across ASRs. Alternatively, varying degrees of male care could have been offset by female care. This contrasts with previous findings (Hunt & Simmons, 2002), which reported that brood masses produced by unaided females were significantly lighter, signifying incomplete compensation by females. Moreover, our findings indicated the mean number of brood balls per male in male-biased treatments was less than that in female-biased and unbiased treatments. In contrast, no significant variation was found in mean number of brood balls per female across all ASR treatments. This suggests ASR impacts male reproductive success, but not female fecundity, possibly due to fewer mating opportunities for males. Past research suggests ASR's dual role in fecundity. A male-biased ASR can increase fecundity by giving females a broader choice of mates (Aitken & Child, 1992; Carrillo et al., 2012; Schacht & Smith, 2017; Solberg et al., 2002; Zhan et al., 2020). However, it can also limit offspring number in species with multiple matings by females, as some males may miss mating opportunities or excessive male presence could lead to increased sexual harassment (Galicia-Mendoza et al., 2017; Le Galliard et al., 2005; Rankin & Kokko, 2007; Rossi et al., 2010). This concurs with our findings that an ASR tailored to meet all females' mating needs exhibits similar fecundity. However, male reproductive success may be hindered in male-biased ASR due to increased competition intensity or limited mating opportunities. In summary, ASR seems to impact dung beetle reproductive success by modulating fecundity, rather than parental care.

Conclusions

Our study ascertains that in a male-biased scenario, the intensity of both contest and courtship behaviours markedly increases. ASR exerts its influence on the breeding success of a dung beetle population primarily by modulating fecundity, especially impacting male reproductive capacity without significant alterations in female fecundity, rather than through variations in parental care. Our results extend the understanding of the influence of ASR on the fitness of dung beetles.

Author Contributions

Lisheng Zhang: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft., Writing – review & editing. **Dennis Sliep**: Investigation, Data curation, Formal analysis. **Maaike A. Versteegh**: Formal analysis, Writing – review & editing. **Tamás Székely**: Methodology. **Jan Komdeur**: Methodology.

Data Availability

The data set used for this study is available as Supplementary material.

Declaration of Interest

The authors declare that they have no competing interests.

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Supplementary Material

Supplementary material associated with this article is available, in the online version, at https://doi.org/10.1016/j.anbehav.2024.03.004.

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