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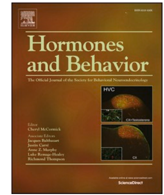

Male social niche conformance? Effects of manipulated opportunity for extra-pair mating on behavior and hormones of male zebra finches

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ABSTRACT

Success in sperm competition is an important determinant of male fitness in mating systems with female multiple mating. Thus, sperm competition risk represents a key dimension of the male social environment to which individual males are expected to adaptively adjust their reproductive phenotype. Such adaptive phenotypic adjustment we here refer to as male social niche conformance. In this pre-registered study, we investigated how male zebra finches, *Taeniopygia guttata*, adjust their behavior to sperm competition risk. We experimentally manipulated the opportunity for extra-pair mating to create two levels of sperm competition risk: 1) Single-pair, no sperm competition risk; 2) Double-pair, sperm competition risk. We compared male courtship, mate guarding, copulation rates, and aggression between the treatment groups. To identify hormonal correlates of male behavioral adjustment, we measured plasma testosterone and corticosterone levels before and after the social treatment started. Contrary to our pre-registered predictions, males from the Double-pair treatment group decreased courtship rates compared to those from the Single-pair group, and Double-pair males responded less aggressively towards intruders than Single-pair males. Testosterone levels decreased over the breeding cycle, but social treatment had no effect on either testosterone or corticosterone levels. Our results indicate that male zebra finches do not intensify courtship or competitive reproductive behaviors, or upregulate key hormones when another breeding pair is present. Although we found no evidence for the predicted adaptive behavioral responses to sperm competition risk, we show that male zebra finches plastically adjust their behavior to their social environment.

1. Introduction

Natural environments vary in space and time. Individuals are expected to adjust their phenotypes to their current environment through adaptive phenotypic plasticity, thereby maximizing their fitness (Ghalambor et al., 2007; Nussey et al., 2007; Piersma and Drent, 2003; Pigliucci, 2001; Via, 1993; for an illustrative empirical example, see e.g., Charmantier et al., 2008). Individuals can alter morphological, physiological, or behavioral phenotypes to conform (i.e., adaptively adjust) to their environment (Trappes et al., 2022; see also Müller et al., 2020). We here refer to such adaptive phenotypic adjustment as 'niche conformance' (Trappes et al., 2022). Conspecifics form an integral part of an individual's environment, and thus social niche conformance may be an important contributor to intraspecific phenotypic variation—especially in behavior (e.g., Bergmüller and Taborsky, 2010; Trappes et al., 2022). Social conflict in particular is thought to be an important driver of variation in individualized social niches and associated behavioral phenotypes (Bergmüller and Taborsky, 2010; for a somewhat different perspective, see Fokkema et al., 2021), but the role of specifically sexual competition in generating (continuous) intra-specific behavioral

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variation is less clear (but see e.g., Fraser et al., 2014; Montiglio et al., 2017; Schradin, 2013).

In sexually reproducing species, mating partners and same-sex competitors strongly shape the social environment wherein individuals compete for access to mates. Whenever females mate with more than one male per reproductive cycle, sperm competition forms an important dimension of the male social niche (Saltz and Foley, 2011). Sperm competition risk—i.e., the probability that at least one rival ejaculate is present (Parker, 1970)—should then select for competitive male behaviors that increase fertilization success, in particular courtship, mate guarding, increased copulation rate and duration, and aggression (Birkhead, 1988; Birkhead and Møller, 1992; Bretman et al., 2010; Tuni et al., 2017). Such competitive behaviors are often costly in terms of time and energy as well as potential injury (Clutton-Brock and Langley, 1997; Feder et al., 2019; Parker and Birkhead, 2013). Therefore, males are expected to adaptively adjust their behavior in response to the social environment, thereby avoiding the costly expression of competitive behaviors when the risk of sperm competition is low (Jungwirth et al., 2016; Wilson and Swaddle, 2013).

Notably, also in socially monogamous species with biparental care females occasionally copulate with multiple males, leading to sperm competition (Birkhead et al., 1989) and resulting in extra-pair offspring (Birkhead et al., 1990; Brouwer and Griffith, 2019). Several recent studies have focused on male behavioral adjustment to experimental variation in sperm competition risk (e.g., Giannakara and Ramm, 2017; Jarrige et al., 2015; Schütz et al., 2017), but such research on socially monogamous species with biparental care is rare (but see e.g., Wilson and Swaddle, 2013). In such species, males face a potential trade-off between investing in competitive reproductive traits (thereby increasing the number of fertilizations) versus parental care (thereby increasing investment per fertilization) (Magrath and Komdeur, 2003), which in turn may shape their reproductive phenotype.

Hormones play a major role in shaping behavioral variation within and among individuals (Hau and Goymann, 2015; Müller et al., 2020). Plasma testosterone (T) and glucocorticoid levels are known to be highly sensitive to changes in the social environment and are important mediators of behavioral responses (Brown and Spencer, 2013; Goymann et al., 2007; Hau et al., 2000; Wingfield et al., 1990). T is a key hormone related to sexual behavior and sperm production (de la Peña et al., 2021; Fusani, 2008; Hau, 2007; Oliveira, 2004; Wingfield et al., 2001; Wingfield et al., 1998). Glucocorticoids, such as corticosterone (CORT; e.g., Ketterson and Nolan, 1992), are major regulators of energy metabolism (Bauch et al., 2016; Jimeno et al., 2018) and social stress responses (Goymann et al., 2001). Typically, experimental studies on hormonal regulation of male competitive behavior manipulate hormone levels, for example using T implants (Hau et al., 2000; Hunt et al., 2019). However, few studies have investigated the hormonal and behavioral responses of individuals to experimental variation in the level of sexual competition in their environment (but see Maruska and Fernald, 2013; Mutwill et al., 2020).

In this study, we manipulated the opportunity for extra-pair mating to investigate if experimental variation in sperm competition risk affects male competitive behavior and plasma T and CORT levels in breeding zebra finches, *Taeniopygia guttata*. The zebra finch is an established model organism in research on sexual selection and sperm competition (Birkhead and Montgomerie, 2020) as well as on (neuro-)endocrinology (Griffith and Buchanan, 2010). It is a colony-breeding songbird, which forms socially monogamous pair bonds and shows biparental brood care (Zann, 1996). Zebra finches show low rates of extra-pair paternity in the wild (Birkhead et al., 1990; Griffith et al., 2010), but extra-pair offspring are commonly observed in captive populations (Burley et al., 1996; Ihle et al., 2015; Wang et al., 2020). The social environment generally has a strong influence on their behavior and hormone levels (e.g., Bölting and von Engelhardt, 2017; Brandl et al., 2019; Perez et al., 2012).

We specifically test the hypothesis that male zebra finches respond to variation in the risk of sperm competition by adjusting their behavioral and hormonal phenotype—i.e., conform to their social niche. To this end, we exposed breeding pairs to two social treatment levels differing in the opportunity for extra-pair mating. In the Single-pair treatment group, a zebra finch pair was breeding alone in a cage; thus, there was no opportunity for extra-pair mating and males experienced no sperm competition risk. In the Double-pair treatment group, two pairs bred in a shared cage creating an opportunity for extra-pair mating and leading to sperm competition risk. We predicted that the presence of another breeding pair (Double-pair treatment group, sperm competition) promotes increases in male courtship and competitive behavioral traits—such as singing, allopreening, mate guarding, copulations, and aggression—at an early stage of the breeding cycle when the female is fertile (Birkhead et al., 1989). Additionally, we expected that these experimental effects on male behavior are associated with higher T levels in males of the Double-pair treatment group compared to Single-pair males. We further investigated the effect of the social treatment on CORT levels. Our detailed predictions are specified in Table S1 in the supplement. To increase research transparency and the confirmatory power of our statistical hypothesis tests, we pre-registered our specific predictions and (statistical) methodology prior to data collection through the Open Science Framework (OSF; see Lilie et al., 2019 for the pre-registration). We consider all pre-registered data analyses with a priori and pre-registered predictions as confirmatory, and analyses without explicit predictions or those testing a posteriori hypotheses as exploratory.

2. Methods

2.1. Summary of experimental approach

We closely adhered to our pre-registered methods (Lilie et al., 2019). All birds used in the experiment were derived from the domesticated zebra finch stock population of the Department of Animal Behaviour at Bielefeld University, Germany, and were specifically bred for this study. On attaining sexual maturity, birds were housed in single-sex flocks until the experiment started to avoid pair formation prior to the experiment. For the experiment we used young adult birds that were at least 100 days of age (mean ± SD = 190 ± 45 days; range = 125, 305 at the start of pair formation). For further details on the breeding of experimental birds, see Text S1 in the supplement.

Minimally 8 weeks before the start of the experiment, individual male and female birds were randomly assigned to pairs. At the start of the experiment, these pairs were allocated to one of the two social treatment levels, i.e., Single-pair or Double-pair (see Fig. 1). The subsequent provision of nest boxes and nesting material stimulated the birds to start breeding. To give males sufficient time to respond to the social treatment, we let the breeding pairs produce two consecutive clutches. For both clutches, eggs were replaced by plastic dummy eggs on the day they were laid. Zebra finches usually lay one egg per day until the clutch is complete with a typical clutch size of 5 with a range from 2 to 7 eggs (Zann, 1996). To induce the laying of a second clutch (i.e., a replacement clutch), we removed the first clutch 15 days after laying of its first egg. The collected eggs from the Double-pair treatment group were artificially incubated to enable collection of embryonal tissue for microsatellite-based parentage analysis (see Section 2.7 below). To investigate the males' behavioral and hormonal responses to the social treatment we recorded their behavior and collected blood samples for measuring plasma hormones (see Sections 2.5. and 2.6 below). We standardized most of the data and sample collection with regard to the focal pairs' breeding cycle, i.e., the start of egg-laying of the replacement clutch (= day 0; for a detailed time schedule, see Fig. 1), ensuring that females were receptive when male courtship and competitive behaviors were recorded.

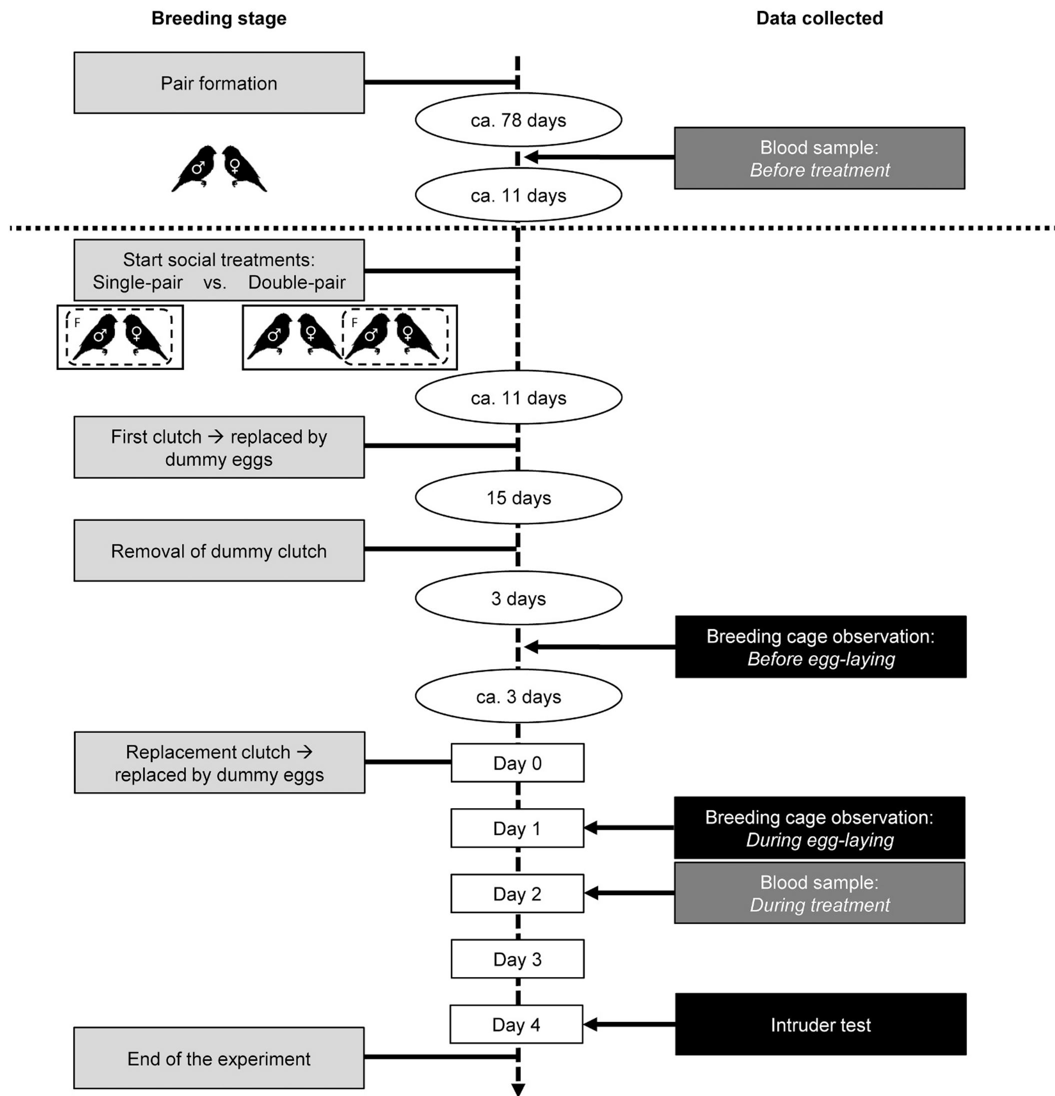

Fig. 1. Experimental approach and sampling scheme. Light grey boxes on the left mark the breeding stage of the birds. The vertical timeline in the middle shows the fixed (in boxes) or variable time (in ellipses) intervals. Variable time intervals in ellipses are indicated with 'ca.' and the observed overall mean value. Boxes show the time schedule set for data collection *during egg-laying* of the replacement clutch. Times are summarized in Table S2. Dark boxes on the right indicate the time of data/ sample collection (black = behavioral recording, dark grey = blood sample for plasma hormones). A total of 120 pairs (i.e., 40 replicates for each treatment level) were assigned to the two social treatment groups: Single-pair (no sperm competition risk) versus Double-pair (sperm competition risk); from the latter one pair per replicate was assigned as the focal pair. For an overview of sample sizes for each dataset collected, see Table S8.

2.2. Housing conditions during the experiment

We conducted the experiment in two animal rooms at the Department of Animal Behaviour at Bielefeld University holding approximately the same number of experimental replicates. Both animal rooms had a combination of natural daylight and artificial light (daylight lamps). The birds were kept under a 14:10 h light:dark cycle (light from 7:00 to 21:00 h), using time-controlled window blinds in addition to the artificial lighting. Cages for the Single-pair treatment groups measured 102.5 × 50 × 52 cm; for the Double-pair treatment group, two cages were combined resulting in a total size of 205 × 50 × 52 cm (see Fig. S1 in the supplement). The bottom of each cage was covered with sand, and four perches of two different diameters were installed at two different heights. Birds were provided with ad libitum water and seed food mix (Elles Exotenfutter, L. Stroetmann Saat GmbH & Co. KG, Münster, Germany). In addition, they received germinating seeds and egg food (Premium Eggfood, CéDé N.V., Evergem, Belgium) three times a week, and fresh chickweed (*Stellaria media*) once per week. Korvimin® (ZVT + Reptil, Wirtschaftsgenossenschaft deutscher Tierärzte eG, Garbsen, Germany) was mixed with the germinating seeds and egg food once a week. A water bath was provided once per week.

2.3. Experimental setup and assignment of birds to social treatment levels

We assigned birds to either the Single-pair or the Double-pair treatment level using random numbers in a stepwise procedure: 1) We allocated 6 birds (3 males and 3 females) to a 'triplet' group (= 3 pairs), while avoiding allocation of related or familiar birds to the same triplet. 2) Within one triplet group, we randomly assigned male and female breeding partners forming three breeding pairs. 3) We then randomly assigned one pair to the Single-pair treatment group and the other two pairs to the Double-pair treatment group. 4) In case of the Double-pair treatment group, we randomly assigned one of the two pairs to be the focal pair to avoid pseudoreplication (Fig. 1). All pairs from the Single-pair treatment group were considered as focal pairs. After allocation to the treatment levels, each of the assigned pairs were transferred to a standard (single) experimental breeding cage (102.5 × 50 × 52 cm) allowing pair formation for a period of minimally 8 weeks (mean ± SD

= 83 ± 9 days; range = 65, 91) before the experiment started. During this time, the birds had no possibility to breed, as we did not provide nest boxes and nesting material.

We aimed for a sample size of 40 experimental replicates per treatment group, totaling 120 breeding pairs, as indicated in our preregistration (see Lilie et al., 2019). Ten out of 120 pairs had to be excluded during the pair formation phase due to sickness (1 male) or death (2 males, 7 females; cause of death unknown) of birds. As a result, we started the experimental treatment with slightly lower sample sizes than planned (Single-pair: N = 38 replicates; Double-pair: N = 36 replicates). We ran the experiment in seven batches of experimental replicates starting at weekly intervals from July to August 2019 (batch size: mean ± SD = 10 ± 3.9 replicates; range = 7, 16).

At the start of the social treatment, we transferred Single-pair males to a new, but identical breeding cage together with their social mate. Double-pair males were transferred to a double-sized breeding cage together with their social mate and an additional unrelated and unfamiliar social pair from the same triplet group. Birds from both social treatment groups were each fitted with three additional plastic leg rings of the same color (either black, white, green, yellow, red, or blue) to enable individual identification during the behavioral recordings. Ring colors are unlikely to be important in mate choice and sexual selection in zebra finches (Wang et al., 2018), but were nevertheless evenly distributed between the sexes and social treatment levels. We provided one wooden nest box per pair and coconut fibers as nesting material to stimulate breeding.

2.4. Monitoring of breeding and egg collection

During the experiment, we carried out daily nest checks in the afternoon between 13.00 and 18.00 h. Most pairs bred in the nest box provided; a few built a nest on the bottom of the cage. As described in brief in Section 2.1 above, all breeding pairs were stimulated to produce two clutches. We collected all eggs from both clutches (in case of the Double-pair treatment group these were used for parentage analysis), and replaced them with plastic dummy eggs on the day they were laid. Double-pair eggs were incubated in an artificial incubator until embryonal development was detectable (but for max. 7 days), after which they were stored at −18 °C until DNA extraction for molecular parentage analysis (see Section 2.7 below). Single-pair eggs were not incubated, but collected for another study. The replacement of laid eggs with dummy eggs ensured that females continued their normal egg-laying sequence. For the first clutch, we removed the dummy eggs 15 days after laying of the first egg—which closely matches the regular incubation period in zebra finches (Zann, 1996)—to stimulate the females to produce a replacement clutch. The date of laying of the replacement clutch set the standardized schedule for collection of behavioral data and blood samples (Fig. 1; for a detailed breeding summary, see Table S2). In a few cases we deviated from our standard breeding scheme: 1) When a focal pair took a longer time to start breeding, we let the non-focal pair lay an additional clutch to give the focal pair more time (Double-pair: $N = 1$). 2) When two of the non-focal pairs changed the nesting place during egg-laying, we considered their clutch at the second nest to be their first clutch (Double-pair: $N = 2$). 3) When females started laying a new clutch before the dummy clutch was removed, we removed both the dummy clutch and the new clutch together allowing them to produce a third clutch (Single-pair: $N = 4$; Double-pair: $N = 1$). For more details, see Table S3.

2.5. Measuring behavior

2.5.1. Video equipment and behavioral analysis software

For the video recordings, we used LAMAX X8.1 Sirius action cameras fitted with additional batteries. The cameras were attached to the front of the cages with custom-made aluminum bars (see Fig. S1). For the breeding cage observations, we attached cameras to the cages one day prior to the actual recording to allow for habituation. For the intruder tests, we attached cameras to the cages at least 1 h before the test started (due to logistical reasons); at this point, the birds had been exposed to the cameras already several times before during the breeding cage observations. The recordings were processed and quantified in the event-logging software BORIS (version 7.9.8; Friard and Gamba, 2016).

2.5.2. Breeding cage observations

2.5.2.1. Recording. We conducted video recordings at two specific breeding stages. The first recording was conducted three days after removal of the first dummy clutch, shortly *before* the anticipated start of laying of the replacement clutch, when we assumed the focal female's fertility reached its peak. Most females laid the first egg of the replacement clutch 5 days after removal of the dummy eggs (mean ± SD = 5.6 ± 2.4 days; range = 1, 25), so we mostly hit the target time window of female peak fertility (87.3 % of females started laying the replacement clutch within one week (89.5 % Single-pair; 86.2 % Double-pair)). The second recording, *during egg-laying*, was made on day 1 after the initiation of the replacement clutch, so the day after the first egg was laid (typically two eggs were present in the nest at this point). This allowed us to obtain behavioral recordings of males at standard time points during their female's breeding cycle. Both video recordings were conducted immediately after 'artificial sunrise', i.e., after the blinds of the windows went up and the light turned on in the animal rooms (between 6.55 and 7.05 h). Each recording lasted for 2 h, during which no person entered the animal rooms.

2.5.2.2. Data processing. Video recordings were aimed to run for 2 h (mean ± SD = 119.4 ± 2.0 min; range = 105.3, 120.0, $N = 135$). For the 35 recordings that did not reach the maximum duration, we corrected the duration of the state event variables (i.e., singing and allopreening) by extrapolation. From those 35 recordings, 17 were at least 119 min long, 16 were between 115 and 119 min long, and only two recordings were <110 min long. Full blinding of the two treatment groups was not possible, because we could not conceal the treatment group of the focal male. The videos were analyzed by two observers (for details on inter-rater reliability, see Text S2 and Table S4). They were unaware of our hypotheses and did not assist in data collection (e.g., nest checks, video recording, blood sampling).

We included male singing, allopreening, and within-pair copulation attempts as pre-registered response variables (Table S1). Male singing included behavior that was either directed to the social mate (or the non-focal female in case of the Double-pair treatment group), or undirected (for a detailed ethogram, see Table S5). Additionally, we included the time spent in close proximity to the social mate as a proxy for mate guarding (not pre-registered; see Tables S1 and S5). For this, we used instantaneous sampling with 1-minute intervals (Bateson and Martin, 2021). Every minute of the recording, we assessed whether the focal pair was in close proximity (i.e., in body contact, within one body-width (<5 cm gap between their bodies), or together in the nest) or further apart. From these data points (max. 120 per recording session), we estimated the proportion of the total time the focal pair spent in close proximity. For the Double-pair treatment group, we additionally collected data on interactions with the other pair. For this, we recorded extra-pair copulations with the non-focal female as well as aggressive interactions between the focal and the non-focal male.

2.5.3. Intruder tests

2.5.3.1. Recording. To quantify male aggressiveness, we conducted a standardized intruder test on day 4 of the replacement clutch (Fig. 1). For this, we released a pre-selected stimulus male (from a pool of nine males) into the focal pair's breeding cage and video recorded their behavioral response focusing on agonistic interactions (for details on the

selection of stimulus males, see Text S3). The stimulus male was brought into the animal room in a standard bird bag in which it was left undisturbed for 5 min before starting the trial. For the Double-pair treatment group, we separated the focal pair from the non-focal pair just before the test started by sliding an opaque partition between the two merged cages, making sure that the focal pair stayed in the part of the cage with their own nest. We introduced the stimulus male into the focal pair's cage and video recorded the males' behavioral responses for 10 min, during which no person entered the animal room. After the test, we removed the stimulus male as well as the experimental male from the breeding cage, and the social treatment experiment was terminated.

2.5.3.2. Data processing. Since we separated the focal pair from the non-focal pair, and the camera only filmed the focal pair's part of the cage, video recordings from both social treatment groups looked alike. Therefore, blinding of the social treatment levels was possible in this case. The videos were analyzed by one observer who was unaware of the treatment level of the birds. Following our pre-registration, we quantified aggression during the 10-minute test by using 1) the total time a focal male spent chasing the stimulus male (i.e., escalated aggression), and 2) the total time a focal male spent in any aggressive interactions with the stimulus male (see Tables S1 and S6). Behaviors included here were (from the focal/resident male's perspective): time spent chasing (given and received), pecking rate (given and received), mounting rate (given and received), time spent bill fighting, and other uncategorized aggressive interactions. Pecking and mounting were included as point events with a duration of 1 s per event.

2.6. Measuring hormones

2.6.1. Blood sampling and processing

To investigate the effect of the social treatment on plasma levels of T and CORT, we aimed to collect two blood samples from each focal male. The first—*before-treatment*—sample was taken a few days (mean ± SD = 10.9 ± 3.0 days; range = 4, 17; Table S2) before the birds entered the experimental treatment. The second sample was taken *during treatment*, on day 2 of the replacement clutch (Fig. 1, Table S2). We took all samples between 10.30 and 12.30 h to minimize bias by circadian fluctuation in hormone levels (Breuner et al., 1999; Kraus et al., 2020; Remage-Healey and Romero, 2000). To avoid any short-term influence of human disturbance, no person entered the animal room for at least 30 min prior to blood sampling, and birds were caught immediately after entering the animal room. Blood samples (ca. 100 μl) were collected into two heparinized capillaries via brachial venipuncture with a sterile disposable 27-gauge hypodermic needle. Since hormone levels, especially of CORT, are highly reactive to external stimuli (Romero and Romero, 2002), we recorded the time elapsed between entering the animal room and collection of a blood sample (separately for each of the two capillaries). When the sampling times of both capillaries per bird were shorter than 3 min, both samples were pooled for CORT and T analyses. However, when the sampling took longer than 3 min, we used the first one for CORT analysis and the second one for T, the latter of which is less sensitive to sampling durations. On collection, blood samples (capillaries) were immediately placed on ice until they were processed. Processing began mostly within an hour of sampling (mean ± SD = 31 ± 14 min; range = 9, 74). We extracted blood plasma by centrifugation at 13,000 rpm for 5 min and removed any residues and the blood pellet. We froze the clean plasma sample at −20 °C until further processing. The remaining blood cells from the pellet were used for molecular parentage analysis (see Section 2.7 below).

2.6.2. ELISA

T concentrations from 187 plasma samples were determined in duplicate for each of the samples using an enzyme immunoassay kit (RE52151, IBL/Tecan, Hamburg, Germany). The used antiserum cross-reacted with relevant steroids as follows: testosterone 100 %, 11β-OH-testosterone 8.67 %, 11α-OH-testosterone 3.24 %, dihydrotestosterone 1.92 %, androstenedione 0.83 %, and all other tested steroids <0.1 %. The intra-assay coefficient of variation (CV) was 5.4 % and the inter-assay CV was 7.4 %.

CORT concentrations from 106 plasma samples were determined in duplicate for each of the samples using a corticosterone enzyme immunoassay kit (501320, Cayman Chemical, Michigan, USA; obtained from Biomol, Hamburg). The anti-serum cross-reacted with relevant steroids as follows: corticosterone 100 %, 11-deoxycorticosterone 15.8 %, prednisolone 3.4 %, 11-dehydrocorticosterone 2.9 %, cortisol 2.5 %, progesterone 1.4 %, aldosterone 0.47 %, 17α-hydroxyprogesterone 0.21 %, 11-deoxycortisol 0.14 %, androstenedione 0.11 % and all other tested steroids <0.1 %. The intra-assay as well as inter-assay CVs were <10 %.

2.7. Molecular parentage analysis

To assign paternity in the Double-pair treatment group, we collected all eggs from both clutches of focal and non-focal pairs (see Section 2.4 above). Altogether, we collected 671 eggs from 136 clutches (clutch size: mean ± SD = 4.6 ± 0.95 eggs; range = 1, 6), from which 490 eggs could eventually be included for parentage assignment (for details, see Table S7). Additionally, we took blood samples from all potential parents (i.e., all adult breeders in a cage) for DNA extraction. Parentage assignment based on 16 polymorphic microsatellite markers followed an established protocol (Caspers et al., 2013; for details, see Text S4).

2.8. Statistical analysis

2.8.1. Statistical approach and software

The statistical analyses adhered to our pre-registration (Lilie et al., 2019) and were carried out in R (version 4.1.2; R Core Team, 2022). The data and code used for the analyses can be found at https://osf.io/46wgr/. We fitted generalized linear mixed-effects models (GLMMs) using the 'lme4' package (version 1.1-29; Bates et al., 2015). We constructed full models providing estimates of all fixed and random effects when fitted simultaneously. All estimates of variance components and other parameters presented are based on models fit with the restricted maximum likelihood method. To obtain *P* values for fixed effects, we used the 'lmerTest' package (version 3.1-3; Kuznetsova et al., 2017). To test for significance of the random effects, we dropped the respective random effect from the full model and compared model fits of the reduced and the full models using the function *anova*. To infer *P* values, this function uses a log-likelihood ratio test comparing nested models refitted with the maximum likelihood method. Significance testing was based on *P* values, with alpha set to 0.05. All statistical tests were two-tailed. Figures were created with the packages 'ggplot2' (version 3.3.6; Wickham, 2016), 'dplyr' (version 1.0.9; Wickham et al., 2022), 'ggpubr' (version 0.4.0; Kassambara, 2020), 'forcats' (version 0.5.1; Wickham, 2021), 'ggpp' (version 0.4.4; Aphalo, 2022), and 'ggbreak' (version 0.1.0; Xu et al., 2021). For sample sizes for all behavioral and hormonal data included in the analyses, see Table S8.

In cases where response variables deviated from a Gaussian distribution, we either fitted GLMMs with appropriate non-Gaussian error distributions (e.g., binomial, Poisson) or we fitted models with a Gaussian error distribution after applying appropriate data transformations (following recommendations from Knief and Forstmeier, 2021). When fitting non-Gaussian error distributions, we checked for potential overdispersion and, in case the data were overdispersed, we included observation level random effects (OLRE) in the model to account for this (following Harrison, 2014). When fitting Gaussian models, we applied a set of different data transformations—specifically, $\sqrt{(x)}$, log(x), log(x + 0.01), log(x + 0.1), and log(x + 1)—after which we assessed the distribution of the models' residuals by inspecting plots of the residual versus fitted values and Q-Q plots. For each of the response variables, we selected the data transformations that yielded the most

Gaussian-like distributions of model residuals before testing for statistical significance in our final analyses (i.e., we selected the most appropriate data transformations based on assessment of the models' residuals, while being blind to the statistical outcomes of the models). In case behavioral data were zero-inflated, we applied a two-step approach (following Rossi et al., 2017). Briefly, in the first step we analyzed whether the likelihood of occurrence of a behavior was dependent on social treatment (and other relevant predictor variables) using a GLMM with a binomial error distribution. In the second step, we excluded the cases in which the behavior did not occur (i.e., with a zero expression) and analyzed the *extent* to which the behavior was expressed for the remaining cases (i.e., with non-zero expression). Whenever necessary, in the second step we also applied data transformations before final analysis as described above.

2.8.2. *Statistical analysis of breeding cage observation data*

The two behavioral recording sessions at different breeding stages (see Section 2.5 above) essentially represented repeated measures. We investigated three pre-registered response variables: time spent singing, time spent allopreening, and the number of within-pair copulation attempts. Additionally, we investigated the proportion of sampling points within a recording at which the focal male was in close proximity to its social mate as a proxy for mate guarding in an exploratory analysis. We transformed the data (see Section 2.8.1 above) for time spent singing ($\log(x + 1)$) and fitted the model with a Gaussian error distribution. The data for time spent allopreening were zero-inflated, and therefore we followed a two-step approach of analysis (see Section 2.8.1 above). In the first step, we fitted a model with a binomial error distribution, analyzing whether males showed any allopreening or not (1 or 0) depending on the predictor variables. In the second step, after excluding all zero values, we fitted a model with a Gaussian error distribution on the $\log(x)$-transformed data. For the number of within-pair copulation attempts we applied a model with a Poisson error distribution. This model was appropriate given the data showed no evidence for overdispersion (dispersion ratio = 0.986, $P = 0.53$, as tested with the 'performance' package (version 0.9.1, Lüdecke et al., 2021)), with similar values for the mean and variance for the number of within-pair copulations (mean = 0.53; variance = 0.61 per 2-hour recording session). For the proximity data we applied a model with a binomial error distribution. This model showed overdispersion (dispersion ratio = 4.554, $P < 0.001$, as tested with the 'performance' package (version 0.9.1, Lüdecke et al., 2021)). In this case, we included an OLRE in the model to account for extra variance due to overdispersion.

In all models, we included social treatment level (Double-pair or Single-pair) as a fixed effect, which was our primary interest. Additionally, we controlled for breeding stage (i.e., whether the recording session took place *before* or *during egg-laying* of the replacement clutch) by including it as another fixed effect. As we were primarily interested in the main effect of treatment, we deviated from our pre-registered statistical models in this case by not including the two-way interaction of the fixed effects. To account for non-independence due to the replicates belonging to the same batch, genetic relatedness and a shared brood environment, as well as multiple observations on the same individual, we included experimental batch ID, mother ID, and individual ID as random effects in the models (Table S1). As mentioned above, observation ID was included as additional OLRE in the binomial model of the proximity data to account for overdispersion. As an exploratory analysis, we calculated detailed time budgets for male behavioral categories split by social treatment group and breeding stage (for details, see Text S5).

2.8.3. *Statistical analysis of intruder test data*

As the two main pre-registered response variables for the intruder tests, we analyzed the total time of chasing performed by the focal male directed to the stimulus male, and the total time the focal male spent in aggressive interactions with the stimulus male. Both response variables were zero-inflated, and therefore we followed a two-step approach of analysis (see Section 2.8.1 above). In the first step, we fitted a model with a binomial error distribution, analyzing whether males showed any chasing or aggressive responses or not (1 or 0) depending on the predictor variables. In the second step, after excluding all zero values, we fitted Gaussian models. Data transformations as mentioned above did not improve the models' fit, so we proceeded with the untransformed data. We included treatment as the only fixed effect, and experimental batch ID, mother ID, and stimulus male ID as random effects (Table S1).

2.8.4. *Statistical analysis of hormone data*

We included 139 plasma T measurements (Single-pair: *before treatment:* $N = 36$, *during treatment:* $N = 36$; Double-pair: *before treatment:* $N = 34$, *during treatment* $N = 33$) and 71 plasma CORT measurements (Single-pair: *before treatment:* $N = 13$, *during treatment:* $N = 24$; Double-pair: *before treatment:* $N = 12$, *during treatment:* $N = 22$) into the statistical analyses. Measurements from other samples were excluded before statistical analyses for one of the following reasons: 1) They were from non-focal males (T: *before treatment:* $N = 35$, *during treatment:* $N = 8$; CORT: *before treatment:* $N = 18$, *during treatment:* $N = 5$). 2) They were from individuals that were excluded from the experiment due to sickness or death (T: $N = 4$, CORT: $N = 2$). 3) They were mistakenly not run on the same assay plate (CORT: $N = 10$). 4) Because we analyzed two samples of the same individual (T: $N = 1$).

T concentration level was pre-registered as a response variable where we had a clear directional prediction, i.e., higher T levels for the Double-pair treatment level. However, we considered the analysis of CORT to be exploratory in our pre-registration because we did not have a clear a priori expectation on how the social treatment might influence plasma CORT levels (see Table S1). We transformed the data ($\log(x + 0.1)$) and fitted models with a Gaussian error distribution for both T and CORT. In all models, we included social treatment group (Double-pair or Single-pair) as the fixed effect of primary interest. Additionally, we controlled for the breeding stage, i.e., whether the measurement (= the blood sample) was taken *before* or *during treatment*, by including it as another fixed effect. Furthermore, we included the two-way interaction between treatment level and breeding stage, and blood sampling duration as fixed effects. Again, we included the random effects of experimental batch ID, mother ID, and individual ID (see Table S1) to account for the non-independence of the data. We stated in our pre-registration that we would also estimate random slopes in the final model, but this was not possible with our dataset that contained only two data points (plasma hormone samples) per individual (Dingemanse and Dochtermann, 2013).

2.8.5. *Correlations among behavioral and hormonal variables*

We explored the correlations between the main behavioral and hormonal variables in order to reveal patterns of covariation that may facilitate the interpretation of our results. We ran partial correlations with regard to treatment group membership using the 'TripleR' package (version 1.5.4; Schönbrodt et al., 2012). We included behaviors from the breeding cage observations (*before* and *during egg-laying*) each: total singing duration, total time spent allopreening of the social mate, number of within-pair copulation attempts, proportion of time of the focal pair spent in close proximity, total time spent in the nest, total time spent in aggression with the non-focal male (Double-pair only). We further included time spent chasing and total time spent in aggression from the intruder test. We also included both hormone measurements (*before* and *during treatment*) for both T and CORT.

We used the same data transformations as for the models in the main text, except for allopreening, for which we used $\log(x + 1)$ instead of $\log(x)$ to include the full dataset (containing zeros). For time spent in nest and aggression, no data transformation would have improved the models' fit. We visualized correlations in a correlation matrix by using the 'ggplot2' package (version 3.3.6; Wickham, 2016) in R (version 4.2.1; R Core Team, 2022).

3. Results

3.1. Breeding summary

From 74 focal pairs that started in the experiment (Single-pair: N = 38 pairs; Double-pair: N = 36 pairs), 71 focal pairs started laying a first clutch (Single-pair: N = 37 pairs; Double-pair: N = 34 pairs) after ca. 10.6 days (Single-pair: N = 10.7 days; Double-pair: N = 10.4 days) with a mean clutch size of 4.5 (Single-pair: N = 4.8 eggs; Double-pair: N = 4.3 eggs). After dummy clutch removal, 71 focal pairs started laying a replacement clutch (Single-pair: N = 37 pairs; Double-pair: N = 34 pairs) after ca. 5.6 days (Single-pair: N = 6.0 days; Double-pair: N = 5.2 days) with a mean clutch size of 5.0 (Single-pair: N = 5.1 eggs; Double-pair: N = 4.9 eggs). For details, also on the breeding of non-focal pairs, see Tables S2 and S3.

3.2. Behavior

3.2.1. Breeding cage observations: Time budgets

Male time budgets for the two treatment groups were similar overall (Fig. 2). Differences between social treatment groups in key behavioral traits are described in detail below. In brief, for both treatment groups, the breeding stage (i.e., recording session) had a strong impact on time budgets: copulations occurred more frequently and more time was spent on allopreening *before egg-laying*, while more time was spent on singing *during egg-laying* (for statistical details, see Section 3.2.2 below). Males spent more time in their nest *during egg-laying* (30.2 % more, $P < 0.001$; see Table S9). For the Double-pair treatment group, we recorded a few additional behaviors, which cannot be compared between treatment groups. Specifically, focal males from the Double-pair treatment group spent time in aggressive interactions with the non-focal male. They further spent some time inside the nest of the non-focal pair.

3.2.2. Breeding cage observations: treatment effects

3.2.2.1. Pre-registered analyses. Males from the Double-pair treatment group sang less (directed and undirected song taken together) than males from the Single-pair treatment group (mean = 1.50 versus 2.13 min per 2-hour recording session, $P = 0.020$; Fig. 3a, Table S10a). We also found a strong effect of recording session, with males singing more *during* the *egg-laying* phase (of the replacement clutch) than *before egg-laying* (mean = 2.61 versus 1.04 min per 2-hour recording session, $P < 0.001$; Fig. 3a, Table S10a). While individual ID explained substantial variation (33 % of variance, $P = 0.013$), batch ID, and mother ID explained negligible variation (Table S10a). After splitting the total time spent singing into directed and undirected song, we found that the observed treatment effect was mainly driven by the time spent on undirected song (Tables S10b and S10c, Fig. S2).

Data on allopreening behavior were zero-inflated, so we used a two-step analysis (see also Methods, Section 2.8.1). The likelihood of occurrence of allopreening was similar between social treatment groups ($P = 0.83$), but was higher *before egg-laying* than *during egg-laying* ($P < 0.001$; Table S11a). None of the random effects, batch ID, mother ID, or individual ID, explained significant variation. In case allopreening occurred, the time spent allopreening was similar between the social treatment groups ($P = 0.94$; Fig. 3b) as well as the recording sessions ($P = 0.95$; Fig. 3b, Table S11b). Mother ID explained substantial variation in time spent allopreening (68 % of variance, $P = 0.022$), while batch ID and individual ID explained negligible variation (Table S11b).

Frequencies of within-pair copulation attempts were similar between social treatment groups ($P = 0.82$; Fig. 3c, Table S12). Copulation attempts occurred more frequently *before egg-laying* (mean = 0.92 versus 0.13 attempts per 2-hour recording session, $P < 0.001$; Fig. 3c, Table S12). None of the random effects of batch ID, mother ID, individual ID explained significant variation (Table S12).

3.2.2.2. Exploratory analyses. Males from the Double-pair treatment group spent less time in close proximity of their social mates compared to males from the Single-pair treatment group (mean = 31.9 % versus 40.4 % of the time $P = 0.001$; Fig. 3d, Table S13). Pairs spent somewhat more time in close proximity *during egg-laying* (mean = 39 % versus 33.7 %, $P = 0.02$; Fig. 3d, Table S13). Observation ID explained significant variation in time spent in close proximity ($P < 0.001$), while batch ID, mother ID, and individual ID were not significant (Table S13).

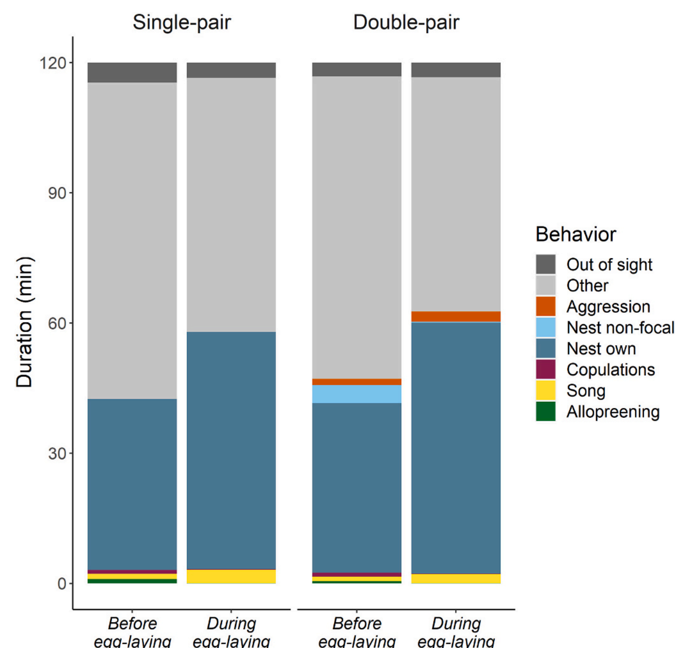

Fig. 2. Time budgets for the different social treatment groups (Single-pair versus Double-pair) from breeding cage observations in relation to recording sessions (*before egg-laying* versus *during egg-laying* of the replacement clutch). Within-pair and extra-pair copulation attempts are summed up for visualization purposes and scaled to 1 min per copulation event.

3.2.3. Intruder tests

3.2.3.1. Pre-registered analyses. Data from the intruder tests were zero-inflated, so we used a two-step analysis (see also Methods, Section 2.8.1). The likelihood of occurrence of chasing and other aggressive behaviors was similar between the social treatment groups ($P = 0.86$ and $P = 0.78$, respectively; Tables S14a and S15a). For the cases where the birds responded aggressively, we found that males from the Double-pair treatment group spent less time chasing the stimulus male compared to males from the Single-pair treatment group (mean = 1.63 versus 2.59 min per 10-minute test, $P = 0.023$; Fig. 4a, Table S14b). They also spent less time in aggressive interactions overall (mean = 1.78 versus 2.72 min per 10-minute test, $P = 0.045$; Fig. 4b, Table S15b). Note that these two measures of aggressiveness were highly correlated (see Section 3.4 below), and that the total time spent in aggressive interactions was mainly a reflection of the time spent chasing. The random effects from both models were mostly not significant, except for mother ID (43 %, $P = 0.06$) and stimulus male ID (20 %, $P = 0.06$) which explained variation in the total time spent in aggressive interactions (Tables S14 and S15).

3.3. Hormones

3.3.1. Pre-registered analyses

Male plasma T levels were similar between the social treatment groups ($P = 0.51$; Fig. 5a, Table S16). However, T levels were

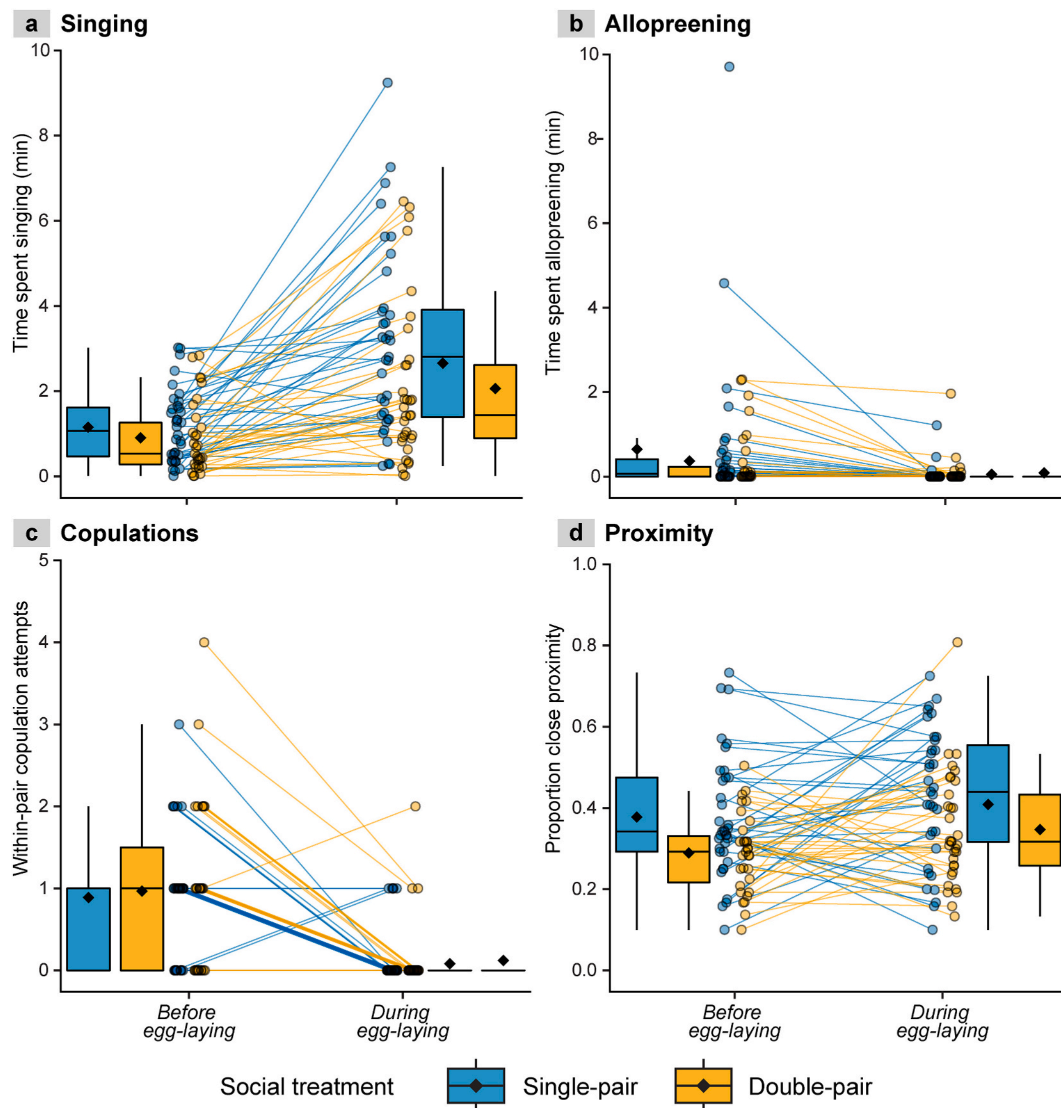

Fig. 3. Treatment effects on behavior based on breeding cage observations. Plots show behavioral responses (raw values) by social treatment and recording session (Single-pair: *before egg-laying*: $N = 36$, *during egg-laying*: $N = 35$; Double-pair: *before egg-laying*: $N = 31$, *during egg-laying*: $N = 33$). a) Total time spent singing of the focal male (min). b) Total time spent allopreening the social mate. c) Within-pair copulation attempts including both the ones that have been accepted or rejected by the social mate. d) Proportion of time in close proximity to the social mate. The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). Whiskers extend from the hinge to the largest and smallest value no further than 1.5 * IQR. Diamonds indicate mean values. For test statistics, see Tables S10 to S13.

substantially lower *during treatment* than *before treatment* (mean = 0.66 versus 1.66 ng/ml, $P < 0.001$; Fig. 5a, Table S16). Blood sampling duration had no effect on T levels (Table S16). Random effects of batch ID, mother ID, and individual ID explained negligible variation in T levels (Table S16).

3.3.2. Exploratory analyses

Plasma CORT levels were similar between social treatment groups ($P = 0.95$; Fig. 5b, Table S17). Plasma CORT levels measured *before* and *during treatment* were also similar ($P = 0.95$; Fig. 5b, Table S17). Sampling duration had no effect on CORT levels (Table S17). Random effects of batch ID, mother ID, and individual ID explained negligible variation in CORT levels (Table S17).

3.4. Correlations among behavioral and hormonal variables

3.4.1. Exploratory analyses

In partial correlation analyses controlling for treatment group membership, we found positive correlations between recording sessions (*before* and *during egg-laying*) for some behavioral variables (e.g., time spent singing: $r = 0.353$, $P = 0.004$; allopreening: $r = 0.434$, $P < 0.001$), as was also reflected by the variation explained by individual ID in some of the models (see above). Plasma T levels *before treatment* and *during treatment* were positively correlated ($r = 0.256$, $P = 0.041$). Furthermore, in the breeding cage observations we found different behavioral variables to be correlated. For example, time spent in the nest *during egg-laying* was negatively correlated with within-pair copulation attempts *during egg-laying* ($r = -0.299$, $P = 0.014$), and also with aggression *during egg-laying* in the Double-pair males ($r = -0.365$, $P = 0.037$). Time spent in the nest *before egg-laying* was negatively correlated with T level *during treatment* ($r = -0.255$, $P = 0.040$). Finally, the time spent chasing and total time spent in aggressive interactions during the intruder test was highly correlated ($r = 0.966$, $P < 0.001$). For further details, see Fig. 6 and Table S18.

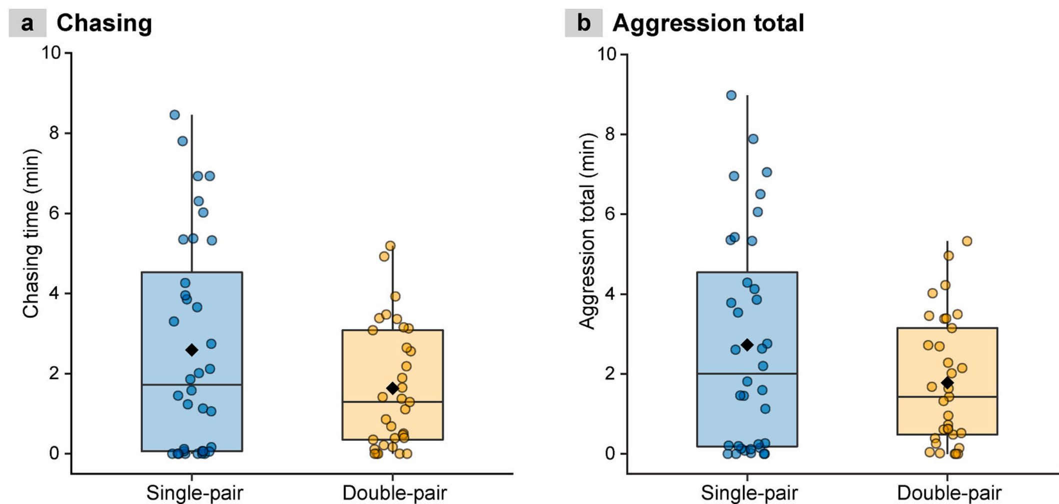

Fig. 4. Treatment effects on behavior based on the intruder test (Single-pair: $N = 36$; Double-pair: $N = 33$). Plots show behavioral responses (raw values) by social treatment. a) Total time the focal male spent chasing the stimulus male (min). b) Total time the focal and the stimulus male spent in any aggressive interactions (min). The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). Whiskers extend from the hinge to the largest and smallest value no further than 1.5 * IQR. Diamonds indicate mean values. For test statistics, see Tables S14 and S15.

3.5. *Extra-pair paternity and egg dumping*

3.5.1. *Exploratory analyses*

We found at least one extra-pair egg in 3 clutches out of a total of 136 clutches (2.2 %) from the 72 breeding pairs in the Double-pair treatment group (clutches of focal and non-focal breeding pairs), amounting to a total of 4 extra-pair eggs out of 490 eggs (0.8 %). Overall, 3 of 72 females laid extra-pair eggs, and only in their first clutches (3 out of 69) (for details, see Table S19). In one further case, which we excluded from the calculations above, the male turned out to have an injury on one of its legs, and the complete replacement clutch ($N = 4$ eggs) laid by its initial mate was sired by the other male in the breeding cage. Finally, we detected 5 eggs ($N = 4$ clutches) that were laid in the other female's nest (egg dumping in the strict sense), and an additional 15 fertilized eggs laid on the bottom of the cage (egg dumping in the wide sense) (Forstmeier et al., 2021).

4. Discussion

4.1. *Summary of main findings*

In this study, we aimed to test whether males conform to their social niche by increasing courtship and competitive behavioral traits in the presence of sperm competition risk. To this end, we manipulated the opportunity for extra-pair mating in zebra finches by either letting single pairs breed alone in a cage or two pairs together. Double-pair males sang less and spent less time in close proximity of their social mates than Single-pair males, while we found no evidence for differences in the time spent allopreening or in copulation rates. When confronted with an unfamiliar intruder, Double-pair males responded less aggressively than Single-pair males. We found that social treatment had no effect on plasma T or CORT levels. In addition to these results, we found that male behavior and hormone levels were strongly dependent on the breeding cycle of the birds. Males showed higher copulation rates and spent more time allopreening but sang less and spent less time in close proximity to their social mates *before egg-laying* than *during egg-laying* (of the second, replacement clutch). T levels during pair formation measured *before treatment* were higher than T levels *during treatment* after the start of egg-laying. We found individual consistency and/or correlations between repeated measurements for behavioral traits (i.e., singing, allopreening) as well as T levels. Extra-pair paternity in the Double-pair treatment group occurred at low levels. Finally, exploratory analyses suggested a possible trade-off between male competitive traits and paternal care (i.e., copulation attempts and aggression versus time in nest). We discuss these findings and their implications in more detail below.

4.2. *Social treatment effects on male behavior and hormones*

In socially monogamous species with biparental care, mate guarding and increased rates of within-pair courtship and copulations are thought to be strategies via which males reduce the probability of being cuckolded (Møller and Birkhead, 1991). In contrast to our pre-registered predictions (see Lilie et al., 2019), we found no evidence that an opportunity for extra-pair mating and associated elevated sperm competition risk promoted behaviors facilitating pre-copulatory access or monopolization of females, such as courtship, copulation, or mate guarding behavior. Obviously then, neither an expected need to defend within-pair paternity, nor the opportunity to gain extra-pair paternity elicited increased investment in these competitive traits in Double-pair males.

Remarkably, we found that Double-pair males sang less and spent less time in close proximity of their social mates compared to Single-pair males. Zebra finch song is likely a short-range signal, as it is relatively soft compared to other songbird species (Loning et al., 2021). It has various functions (Catchpole and Slater, 2003), and zebra finch pair members use frequent vocal exchanges to coordinate their behavior when incubating (Boucaud et al., 2017). Females prefer their mate's song over the song of others, highlighting the importance of male song in within-pair communication (Forstmeier et al., 2021; Miller, 1979; Woolley and Doupe, 2008; but see Adkins-Regan and Tomaszycki, 2006). The differences in the overall time spent singing between our treatment groups were mainly driven by differences in undirected song, which was displayed more by Single-pair males. We labelled singing behavior as undirected song when there was no apparent receiver, but song may nevertheless still have been directed at the social mate not in sight (e.g., while the female was inside the nest). Undirected song makes up the largest proportion of singing in zebra finches (Lansverk et al., 2019). It could be important for attracting a new mate (Dunn and Zann, 1996a), but it could also reinforce an already existing pair bond. Females increase their investment in egg production (Bolund et al., 2012) and incubate more when their partners produce undirected song more frequently (Dunn and Zann, 1996b). Due to the lack of additional interaction partners in their cage and no need for defending the nest, Single-pair males may have been more focused on their social mate

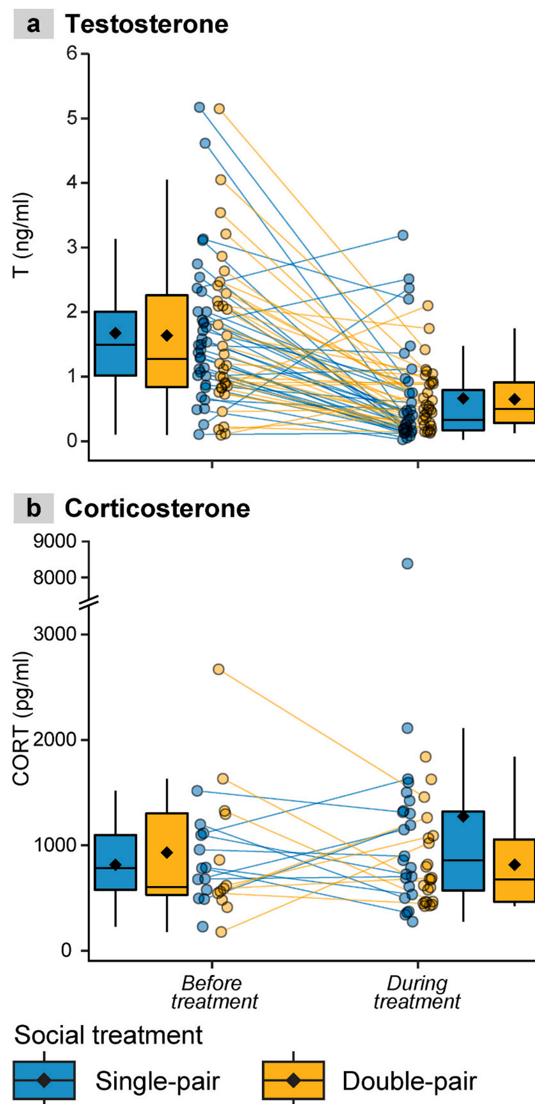

Fig. 5. Treatment effects on plasma hormones. Plots show testosterone, T, and corticosterone, CORT (raw values), by social treatment and breeding stage (measurement *before treatment* versus *during treatment*). a) T (Single-pair: *before treatment*: $N = 36$, *during treatment*: $N = 36$; Double-pair: *before treatment*: $N = 34$, *during treatment*: $N = 33$). b) CORT (Single-pair: *before treatment*: $N = 13$, *during treatment*: $N = 24$; Double-pair: *before treatment*: $N = 12$; *during treatment*: $N = 22$). The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). Whiskers extend from the hinge to the largest and smallest value no further than 1.5 * IQR. Diamonds indicate mean values. For test statistics, see Tables S16 and S17.

spending more time interacting with them through (undirected) song.

Comparative evidence suggests that T levels are related to the level of prevailing sperm competition across species (Garamszegi et al., 2005), and T is thought to regulate courtship and competitive behaviors (e.g., Adkins-Regan, 1999; but see Foerster and Kempenaers, 2005). As our results provide only weak evidence for behavioral adjustment to variation in sperm competition risk, it is perhaps not surprising that we did not find treatment effects on plasma hormone levels either. In contrast, breeding stage turned out to be an important predictor of plasma hormone levels with strongly reduced T (but not CORT) levels *during egg-laying* compared to the samples taken *before treatment* (see Section 4.3 below). Therefore, it is possible that potential differences between treatment groups in T levels that may have been present earlier in the breeding cycle diminished over time and had largely disappeared at the time of sampling (see Hill et al., 2005). We also did not find an effect of social treatment on CORT levels, despite evidence that social competition can affect CORT (Comendant et al., 2003; Raouf et al., 2006; Robertson et al., 2017). We can nonetheless conclude that despite possible differences in the level of male-male competition and the number of conspecific interaction partners present in the different treatment groups, levels of social stress (Goymann et al., 2001) and metabolic rates (Bauch et al., 2016; Jimeno et al., 2018) of the focal males remained apparently unaffected.

An increase in aggression towards potential competitors may be part of an effective mate guarding strategy (see above). To be able to directly compare aggressive responses towards male competitors between social treatment groups, we conducted a standardized intruder test. In contrast to our prediction, Double-pair males reacted overall less aggressively towards the stimulus male than Single-pair males. The likelihood of an aggressive response was not different between the two treatment groups, but in case an aggressive response occurred the Single-pair males showed more aggression than the Double-pair males. From the start of the treatment, Double-pair males were exposed to a continuously increased level of sexual competition in the weeks preceding the test. Perhaps aggressive interactions between the focal and the non-focal male mostly occurred at the start of the treatment and such behavior was later downregulated to reduce the potential costs of frequent escalated aggression. For Double-pair males the intruder test may have been a more familiar social situation (which may also be considered a habituation effect). On the other hand, for Single-pair males the first experience with a conspecific competitor in their breeding cage was at the presentation of the stimulus male. As a result, the intruder test may have presented a stronger social stimulus to the Single-pair males than to the Double-pair males, eliciting an overall stronger aggressive response in the Single-pair males. Our findings are in line with studies that have shown that individuals living in simple social environments (i.e., with few interaction partners) react more aggressively towards potential competitors compared to ones kept in more complex social environments (i.e., with many interaction partners) (zebra finch: e.g., Ruploh et al., 2013; Ruploh et al., 2014; mammals: e.g., Zimmermann et al., 2017; fish: e.g., Arnold and Taborsky, 2010; Nyman et al., 2017).

Although forced pairing can reduce reproductive success (Ihle et al., 2015), most of the assigned pairs in our study apparently formed a solid pair bond, and started egg-laying and incubation. Except for the one case in which a male of a pair had an injured leg (see also below), mate switching never occurred in the double pair treatment, also reflecting generally strong and stable pair bond formation. The long period of pair formation prior to the experiment (minimally 8 weeks), and the stable and constant social environment over the time of the experiment probably ensured strong and monogamous pair bonding (Wilson et al., 2022). Extra-pair copulation attempts in the Double-pair treatment groups occurred rarely during the breeding cage observations (2 extra-pair copulations observed in a total of 268 h of observations of Double-pair breeding cages, $N = 135$ recording sessions, versus 71 within-pair copulation attempts). Parentage analysis of the eggs from the Double-pair treatment group revealed a low rate of extra-pair paternity (in 2.2 % of all clutches, 0.8 % of all eggs, and 4.2 % of females), which is rather comparable to rates in wild zebra finches (Birkhead et al., 1990; Griffith et al., 2010) than to some previous studies on birds in captivity (Burley et al., 1996; Ihle et al., 2015; Wang et al., 2020). A possible interpretation for this discrepancy may be that zebra finches in the wild naturally form strong monogamous pair bonds with little occurrence of extra-pair behavior, while in some laboratory studies pair bonds may be weaker due to forced pairing in combination with less time and opportunity for pair bond formation. Such weaker pair bonds in captive studies may result in increased extra-pair behavior and higher extra-pair paternity rates. While the long pair formation period in our study probably ensured the establishment of strong pair bonds, our chosen experimental setup (two established pairs in one standardized cage) probably also hindered extra-pair copulations, due to the low number of interaction partners, limited space in the cages without

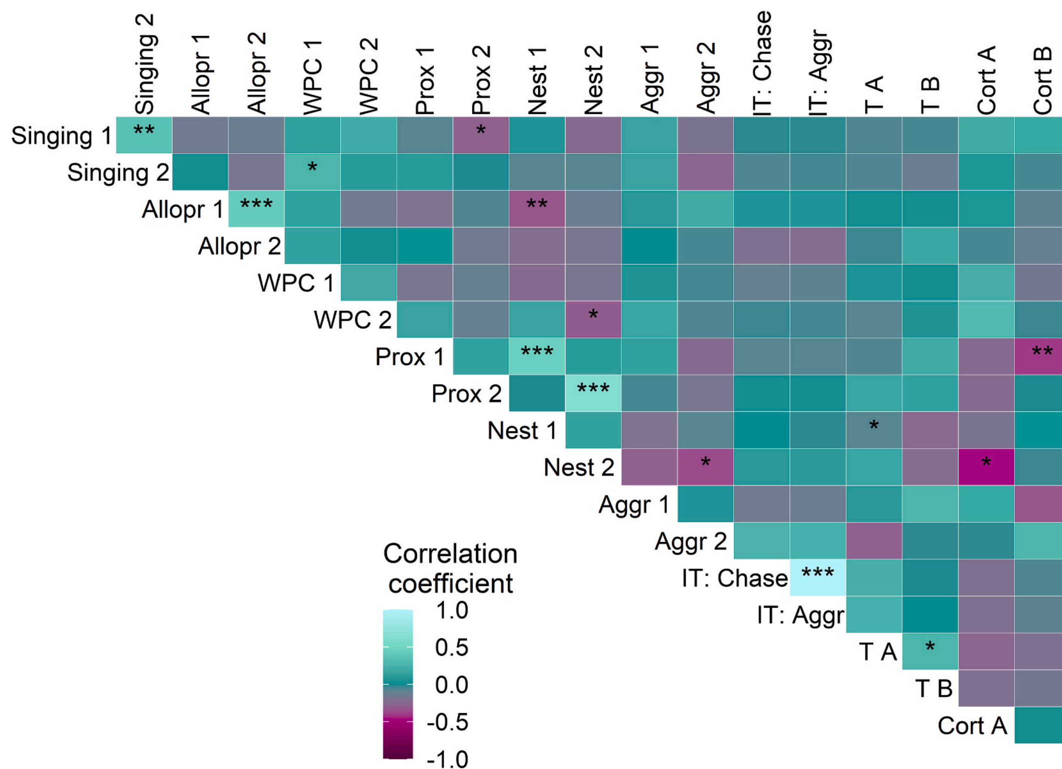

Fig. 6. Partial correlations of behavioral and hormonal variables, controlling for treatment group membership. Behaviors of interest from the breeding cage observations, the intruder test, and plasma hormones. For the breeding cage observations, behaviors of interest include singing of directed and undirected male song ('Singing'), allopreening ('Allopr'), within-pair copulation attempts ('WPC'), the proportion of time spent in close proximity to the social mate ('Prox'), and aggression with the non-focal male ('Aggr', Double-pair only, based on Pearson's correlation). The breeding stage is abbreviated with a number (*before egg-laying* of the replacement clutch = 1, or *during egg-laying* of the replacement clutch = 2). Behaviors of interest from the intruder test ('IT') include the time spent chasing the intruder ('Chase') and total time in aggression ('Aggr') between the two males. Plasma hormone values include T and CORT *before* (A) and *during treatment* (B). Data transformation was mostly applied as in the original models (see Section 2.8.5). Color of the cells represent the correlation coefficient (turquoise = 1 to magenta = −1). Asterisks represent significance values (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$).

hiding places and thus the permanent risk of attack by the other pair male. Potentially, observed extra-pair paternity rates would have been higher without successful within-pair courtship and mate guarding. In one case, a female bred with the other male when her partner was no longer available as a mate due to an injury on its leg. This suggests that female zebra finches, although generally socially monogamous, may also opportunistically seek copulations or remate to ensure fertilizations when the initial partner is no longer available or not fit to breed.

The observed behavioral responses were mostly opposite in sign from what we predicted based on adaptive phenotypic adjustment to experimental variation in sperm competition risk. Zebra finches have since long been used as a model organism in sexual selection and sperm competition research (Birkhead et al., 1989; reviewed in Birkhead and Montgomerie, 2020). However, our results provide only weak evidence for the opportunity for extra-pair mating and risk of sperm competition being important drivers of behavioral and hormonal variation. These results, in combination with the strong monogamous pair bonds with low levels of extra-pair paternity observed in the wild, suggest that although zebra finches have proven a useful model for studying *mechanisms* of sperm competition in the laboratory (Birkhead et al., 1989; Birkhead et al., 1988), they may be less suitable for testing *adaptive* hypotheses on phenotypic adjustment to the risk of sperm competition. Hence, although we manipulated the actual opportunity for extra-pair mating and therewith the level of sperm competition risk, the birds may have perceived this experimental treatment in a different way. The presence of another established pair might have been a weak stimulus to upregulate competitive male traits. Nevertheless, we found that birds adjusted their (behavioral) phenotype to our social treatment, possibly still indicating social niche conformance. Males experiencing another breeding pair in their near environment may have avoided the costs of escalated aggression by being more tolerant. However, further work would be needed to demonstrate the adaptive significance of the behavioral treatment effects observed.

4.3. Breeding stage effects on male behavior and hormones

Although for most pairs only a few days (mean ± SD = 3.5 ± 2.7 days) elapsed between the two recording sessions of the breeding cage observations, we found that within-pair social interactions, such as allopreening and within-pair copulation attempts, had decreased once the female had started laying of the replacement clutch. While zebra finch females are still fertile *during egg-laying* (Birkhead et al., 1989), they often already start parental care in the form of incubation prior to completion of the clutch (Zann, 1996), which may be accompanied by a down-regulation of mating behaviors (Magrath and Komdeur, 2003).

Zebra finch males take a large share in incubation (Zann, 1996), as also indicated by the substantial amount of time the males spent inside the nest *during* the egg-laying phase. Such paternal behavior may well trade off against male investment in sexual and competitive behaviors (Komdeur et al., 2002; Magrath and Komdeur, 2003). Consistent with this idea, we found a negative correlation between the time a male spent inside the nest versus the number of within-pair copulations and aggression *during egg-laying*. This interpretation is further consistent with the strong down-regulation of plasma T levels at this time point, as compared to levels measured *before* the start of the social *treatment*. However, we also found that males increased the time spent singing and in proximity to their social mates *during* the egg-laying phase of the replacement clutch. Song could function to attract extra-pair females or

to reinforce the pair bond and stimulate the partner's reproductive investment (Bolund et al., 2012). The time spent in proximity to the social mates was increased *during egg-laying*, because pairs spent a substantial amount of time together inside the nest, which we also recorded as time spent in close proximity. Thus, it is possible that pairs shifted affiliative behaviors, such as allopreening, to take place inside their nest, where we could not observe these.

4.4. Social niche conformance

In this study, we found evidence that birds plastically adjust their behavior to the social environment they encounter. Our aim was to investigate the importance of intrasexual competition as a dimension of the male social environment by manipulating the opportunity for extra-pair mating and thereby the risk of sperm competition. However, based on our results, the opportunity for extra-pair mating and sperm competition risk did not qualify as main drivers of courtship and competitive behavioral trait expression. Nevertheless, even though the birds were kept with many conspecifics in the same animal room where they could for example hear the vocalizations by the others, they still showed different behavior in the presence or absence of another breeding pair in the same cage. It further remains to be investigated to what extend post-copulatory competitive traits (e.g., sperm phenotype) may be sensitive to experimental variation in the opportunity for extra-pair mating and risk of sperm competition. In conclusion, our results show that zebra finches plastically adjust their behavior to variation in their social environment, which could result in individualized social niches (Bergmüller and Taborsky, 2010; Trappes et al., 2022). In this way, social niche conformance may drive among-individual phenotypic variation (Takola and Schielzeth, 2022).

Open science information and data availability

The study was pre-registered with the Open Science Framework (OSF): https://osf.io/84z5r. The raw data and code on which the manuscript is based can be accessed on OSF website: https://osf.io/46wgr/.

Ethical note

All procedures complied with the regulations covering animal experimentation within Germany (Animal Welfare Act) and the EU (European Community Council Directive 2010/63/EU), and were approved by the Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (case number 81-02.04.2018.A311).

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CRediT authorship contribution statement

PK and TS conceived of the study. NDL, PK, and TS designed and planned the experiment in consultation with SK. NDL coordinated and managed the experiment. AK, NDL, PK, SMS and SR collected data and hormone samples. SK coordinated hormone quantification. NDL analyzed the data and wrote the initial draft of the manuscript with PK. All authors provided input to the manuscript.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2022.105243.

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