Fitness costs of female competition linked to resource defense and relatedness of competitors

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ABSTRACT

Female reproductive success is often limited by access to resources and this can lead to social competition both within and between kin groups. Theory predicts that both resource availability and relatedness should influence the fitness consequences of social competition. However, testing key predictions requires differentiating the effects of these two factors. Here we achieve this experimentally by manipulating the social environment of house mice, a facultative communal breeding species with known kin discrimination ability. This allows us to investigate: 1) the reproductive costs of defending a limited resource in response to cues of social competition, and 2) whether such costs, or their potential mitigation via cooperative behavior, are influenced by the relatedness of competitors. Our results support the hypothesis that resource defense can be costly for females, potentially trading-off against maternal investment. When the availability of protected nest sites was limited, subjects: 1) were more active, 2) responded more strongly to simulated territory intrusions via competitive signaling, and 3) produced smaller weaned offspring. However, we found no evidence that the propensity for kin to cooperate was influenced by relatedness of rivals. Communal breeding between sisters occurred independently of the relatedness of competitors, and communally breeding sisters weaned fewer offspring when competing with unrelated females, despite the design of our study to prevent infanticide between kin groups. Our findings thus demonstrate that female competition has fitness costs, and that associating with kin is beneficial to avoid negative fitness consequences of competing with non-kin, in addition to more widely recognized kin-selected benefits.

Introduction

Female reproduction is often constrained by access to limited resources, such as safe nest sites or territories (Heinsohn et al. 2005; Clutton-Brock 2007; Stockley and Campbell 2013). This leads to resource competition between females, broadly defined as a form of social competition (West-Eberhard 1979; Stockley and Bro-Jorgensen 2011; Tobias et al. 2012). Female resource competition is widespread among mammals and other vertebrates, with important consequences for the evolution of social and reproductive systems (Stockley and Bro-Jorgensen 2011; Clutton-Brock and Huchard 2013; Stockley and Campbell 2013), and for population dynamics (Charnov and Finerty 1980; Lambin and Krebs 1993). However, despite this widespread significance, the fitness consequences of female resource competition are still poorly understood. Competition between females is typically less overtly aggressive than that between males but may still be costly (Stockley and Campbell 2013). For example, where females require key resources to reproduce successfully, they may invest in competitive signaling or other energetically costly behaviors to defend such resources (LeBas 2006; Cain and Ketterson 2013). Although it has been hypothesized that investment in competing for or defending key resources should be constrained by trade-offs with producing and rearing young, empirical tests of this theory are lacking (Fitzpatrick et al. 1995; Cain and Ketterson 2013).

Where females live in social groups, dispersal patterns lead to competition among group members of varying levels of relatedness (Greenwood 1980; Perrin and Lehmann 2001; Silk 2007). This is an important consideration, because kin selection theory predicts that the relatedness of competing females could influence the expression and fitness consequences of their behavior in multiple complementary ways (Hamilton 1964a; Hamilton 1964b; Gardner 2020; Kay et al. 2020). Firstly, directing more helpful and less harmful behaviors towards relatives may promote increased inclusive fitness benefits (Hamilton 1964a; Hamilton 1964b; Kay et al. 2020). However, where competition for limited resources is concentrated among relatives, this can negate the benefits of cooperation (Taylor 1992; West et al. 2001; West et al. 2002; Platt and Bever 2009). These opposing

influences are difficult to tease apart in natural populations, because conditions that influence within-group relatedness, such as reduced dispersal, often also lead to higher levels of competition. However, assuming that subjects are able to discriminate kin and adjust their behavior adaptively according to local conditions, an experimental approach can be used to test responses to resource competition and relatedness of competitors independently (Giron et al. 2004; Kapranas et al. 2016).

Following the theory outlined above, the benefits of cooperating with kin should be negated when local competition occurs exclusively between closely related females. Hence resource limitation within such groups could result in reduced cooperation and heightened competitive behaviors between close kin. Secondly, following similar logic, local competition that occurs both within and between kin groups is predicted to favor increased levels of cooperation within kin groups (Frank 1998; Lehmann et al. 2007). In this case, the benefits of cooperating could lead to greater inclusive fitness benefits for related females, although competing with unrelated females might also result in greater fitness costs. Examples of harmful behaviors directed to conspecifics by female mammals include inhibition of competitors' reproduction via low level aggression or harassment, even when resources do not appear to be immediately limiting to reproductive success (e.g. Dunbar 1980; Wasser and Starling 1988). Such behavior is thought to be beneficial since inhibiting reproduction of competitors will reduce future resource competition, either directly for the female inhibiting others, or indirectly for her offspring and other relatives (reviewed in Stockley and Bro-Jorgensen 2011).

The extent to which the costs of competition between kin groups might be mitigated by cooperation within kin groups is largely unexplored, but potential examples include shared resource defense, communal breeding or shared efforts to prevent infanticide (Wrangham 1980; Packer et al. 1990; Dobson et al. 2000). Each of the predictions outlined have very broad evolutionary significance, but empirical tests of the way in which resource competition and the relatedness of competitors interact to influence behavior and reproductive success are lacking, particularly for vertebrate animals such as mammals.

House mice (*Mus musculus domesticus*) are an ideal model species to explore fitness consequences of social competition. They are social, often living at high population density in natural populations. At high density, social units typically consist of several closely related reproductive females, their offspring and a dominant male (Bronson 1979). Local competition can occur both within and between female kin groups, including within the same dominant male's territory, and competition to reproduce is particularly intense at high density (Crowcroft and Rowe 1963; Lidicker 1976; Hurst 1987; König and Lindholm 2012). Importantly, food is not usually limiting for house mice exploiting human environments. Rather, females compete for safe nest sites needed for successful reproduction within shared territories (Hurst 1987). As in many other mammals, competitive interactions between female house mice are largely mediated by scent communication (Hurst 1990; Stockley et al. 2013; Coombes et al. 2018). Females scent mark under competitive conditions, with major urinary proteins (MUPs) in their scents acting as specialized components that are important for both competitive signaling and kin-recognition (Garratt et al. 2011; Thonhauser et al. 2013; Green et al. 2015). The social and breeding systems of house mice also make them particularly suitable to explore the effects of social competition on cooperative behavior and inclusive fitness. Mechanisms of kin discrimination are well established (Green et al. 2015), and cooperative behaviors can occur in the context of facultative communal breeding, whereby typically two or more sisters share a nest and combine their respective offspring into a single large litter (König and Lindholm 2012; Ferrari et al. 2019).

To test how resource competition and relatedness of competitors affect reproductive success, we manipulated the social environment of female house mice living together under carefully controlled naturalistic conditions. To manipulate levels of resource competition, subjects were offered contrasting numbers of protected nest sites for breeding in the presence of potential competitors. Competing females were physically separated to prevent infanticide when offspring were present in the nest, while refreshing social odor cues of competitors within the territory to stimulate the requirement for resource defense. To test the influence of kin structure on

cooperative and competitive behaviors, we also manipulated the relatedness of potential competitors within each social group. This allowed us to test: 1) if limited nest site availability leads to increased activity levels and competitive signaling, indicative of greater investment in resource defense, 2) if reproductive success is constrained by costs of social competition when protected nest sites are limited, 3) if relatedness of competitors affects the propensity of kin to cooperate or compete, with greater propensity to form communal nests when competitors are unrelated, combined with increased productivity or lower reproductive skew within communal nests, and 4) if competition between non-kin is ultimately more costly than between kin. Our results support the hypothesis that social competition has direct fitness costs for females, but we find no evidence that such costs are mitigated by increased cooperation between kin when competitors are unrelated. Rather, reproductive success of cooperating sisters was lower under social competition with unrelated females, revealing that competition with non-kin is more costly overall.

Methods

Subjects

Subjects were from a captive outbred colony of house mice (*Mus musculus domesticus*), derived from wild ancestors originating from several populations in the northwest of England, UK, with regular addition of new wild-caught animals. The colony is maintained under controlled environmental conditions, with temperature 20–21 °C, relative humidity 45–65%, and a reversed 12:12 h light cycle (lights off at 08:00). All animals are provided with *ad libitum* access to water and food (Lab Diet 5LF2 Certified Rodent Diet, Purina Mills, St Louis, MO, USA), and housed on Corn Cob Absorb 10/14 substrate with paper wool nest material. Subjects were bred in standard laboratory cages (MB1, North Kent Plastics, UK; 45 × 28 × 13 cm) with behavioral enrichment. Radio frequency identification (RFID) tags were used for individual identification and animals were handled with handling tunnels to minimize stress (Hurst and West 2010). Breeding males used in experimental

treatments were derived from the captive colony and were always unrelated to breeding females. Unrelated animals were classed as those with no shared full sibling grandparents ($r < 0.032$).

Experimental design

Our study was designed to test the effects of resource competition and relatedness of competitors on female behavior and reproductive success in a 2x2 factorial experimental design, where subjects had the opportunity to share rearing of their offspring in communal nests. Since female house mice strongly prefer protected nest sites for breeding (Rich and Hurst 1998), we manipulated resource competition by providing contrasting numbers of protected nest sites within semi-natural enclosures. Each enclosure housed a social group consisting of two adult sister pairs of different age (median age difference 82 days, Table S1), thus creating a naturalistic social grouping where older females have a competitive advantage and younger females may be reproductively suppressed (Ferrari et al. 2019). Sister pairs within each social group are hereafter described as competitors in the context of the experiment, and females given the opportunity to breed will be referred to as subjects. To manipulate relatedness of competitors, sister pairs were either derived from the same parents (related) or from different parents (unrelated), and were always unfamiliar on introduction. The four treatments were: 1) single protected nest site, related competitors; 2) multiple protected nest sites, related competitors; 3) single protected nest site, unrelated competitors; 4) multiple protected nest sites, unrelated competitors (Fig. 1A).

As outlined in Fig. 1B, the study was divided into four phases. During the pre-reproductive phase, all females within each group were interacting, as represented by a double-sided arrow connecting older and younger sister pairs in Fig. 1B. During this phase, all females had access to protected and unprotected nest boxes, according to their treatment group (Fig. 1A). A sexually mature male was introduced for the mating phase and removed prior to the reproductive phase, when litters were raised in communal or single nests, as represented by the presence of offspring in

Fig. 1B. Weaned offspring were removed prior to the post-reproductive phase, when behavioral assays were performed to test scent-marking behavior in response to simulated intrusions, as represented by the presence of tiles to record scent marking behavior in Fig. 1B. Movements of younger sister pairs were restricted during the mating phase to prevent mating (except in block 1 – see below), and direct interaction between older and younger sister pairs was prevented during the reproductive phase to prevent infanticide. Restricted interactions between older and younger sister pairs are represented in Fig. 1B as a cross over the double-sided arrow connecting them. Further details of each phase and the behavioral assays are provided below.

Treatment groups were approximately balanced across five blocks (Table S2), with sister pairs randomly allocated to treatment groups within this restriction. In the first block, all females (N= 16) in four social groups were given the opportunity to breed, and all produced litters. Given this initial high breeding success, we restricted breeding opportunities to older females in subsequent blocks (N=32 subjects in 16 social groups). This adjustment was necessary for both ethical and logistical reasons, because the same high level of breeding throughout the experiment would have required culling and genotyping very large numbers of offspring in order to achieve sufficient replicates within each treatment group. Younger females (N=32) still functioned as competitors to older subjects but were prevented from breeding by restricting their mating opportunities (see below). Subjects therefore consisted of 16 older and younger females in block 1, and 32 older females in blocks 2-5 (N=48 females in 20 social groups in total). We quantified the reproductive success of subjects as the number of offspring weaned, which was assessed by genotyping in cases where females formed a communal nest (Supplementary methods S1).

Experimental set-up

Social groups were each housed in one of eight melamine enclosures (1.2 × 1.2 m), containing four inter-linked compartments (Fig. 1A, Supplementary methods S2). Previously unfamiliar animals were

introduced within the enclosures following an ethical protocol to prevent escalated aggression (Supplementary methods S3), and females were left to establish social relationships for between 17 and 22 days, during the pre-reproductive phase (Fig. 1B). In half of the trials (N=10), only a single protected (preferred) nest box was provided, with unprotected (non-preferred) nest boxes available in the other three compartments. For the other half (N=10), four protected nest boxes were provided. An MB1 cage, placed within each enclosure, was used to separate sister pairs when necessary to prevent mating, or escalated aggression, while maintaining protected social contact (Table S1). Older and younger sister pairs were always separated to prevent infanticide, while maintaining protected social contact. In block 1, where all females were allowed to breed, we prevented infanticide by restricting sister pairs to separate halves of the enclosure while litters were reared, with protected contact via wire mesh barriers. For those enclosures with a single safe nest site, an additional protected nest box was provided during the separation period so that all breeding females had access to at least one equivalent protected nest. In blocks 2-5, where only older sisters were allowed to breed, we prevented infanticide by restricting younger sisters to MB1 cages within the enclosure while older females reared their litters (see also Supplementary methods S3).

Offspring were counted and sexed at postnatal day (PND) 14 and at weaning (PND 28-30). No offspring mortality was recorded between these observation points. Body masses of offspring and dams were also recorded at weaning. In cases where sisters formed a communal nest, we were not able to match offspring to their respective mothers prior to genotyping, and hence to assign average offspring weaning mass per dam. We therefore calculated the average weaning mass by sex of offspring for each communal or single litter.

Quantifying social behavior and activity levels

During the pre-reproductive phase (Fig. 1B), we quantified the frequency with which older and younger sister pairs chose to sleep together in shared nest boxes. This was recorded regularly within

each social group using a handheld transponder reader to identify the location of subjects shortly before the end of the light (inactive) phase. During the reproductive phase (Fig. 1B), following parturition we used paired transponder readers on the tunnel to the nest box currently occupied to monitor the time that breeding females spent in protected nest boxes (see also Supplementary methods S2). Recordings were made for 15 days from the birth of offspring until around the period of peak lactation at postnatal day (PND) 14. We used these recordings to also quantify the number of times that breeding females left and re-entered the nest, as an index of activity while maternal care was maximal, assessed during (a) the dark (active) phase and (b) the light (inactive) phase (see also Supplementary methods S2). These recordings reveal general activity patterns (Fig. S1), confirming higher activity during the dark (active) phase compared to the light (inactive) phase.

Scent marking assay

Behavioral assays tested the scent marking responses of older subjects within social groups in blocks 2-5 (N=32) to simulated intrusions by unfamiliar females into their territory (Supplementary methods S4). These were conducted during the post-reproductive phase (Fig. 1B). The response of each subject to urine of simulated intruders was recorded in three separate tests: (1) urine from a related but unfamiliar female versus water, (2) urine from an unrelated unfamiliar female versus water, and (3) a control test where both tiles were streaked with water. Prior to the assay, each subject was constrained to a separate compartment of the enclosure so that individual scent marking responses could be assessed. Subjects were presented simultaneously with two test tiles (15cm × 15cm × 0.5cm [W × L × H]) covered in Benchkote (GE Healthcare, UK) and streaked in the center with 2 × 4 µl streaks of female urine or double distilled water as artificial scent marks. Tiles were removed after 30 min. Scent marks were scanned under UV light using a Bio-Rad Fluor-S^{TM} MultiImager (Syngene GeneSys V 1.2.7.0: 530DF60 Filter, UV light source, Epi illumination, automatic exposure, automatic capture, high resolution mode) and the number and area covered by scent

marks was quantified using ImageJ software (https://imagej.nih.gov/ij/). Because presentation of the stimulus tile can induce higher scent marking activity on both tiles, we used the sum of scent marking activity (number or area of scent marks) on stimulus and water tiles within each test for comparison with scent marking activity in control tests, where only water was presented.

Statistical analysis

For statistical analysis we used R 4.1.1 (R Core Team 2021), with the packages lme4 (Bates et al. 2015), emmeans (Lenth 2021), and afex (Singmann et al. 2021). We used linear models (LM), generalized linear models (GLM), linear mixed effects models (LMM) and generalized linear mixed effects models (GLMM). To obtain p-values, we used either the mixed() function in the package 'afex' with a Satterthwaite's approximation for degrees of freedom, or the drop1() function to perform a type II anova. Interactions and covariates were stepwise removed if non-significant (Engqvist 2005) and reported p-values refer to the final model without non-significant interactions. An overview of all full models can be found in Table S3.

For analysis of scent marking assays, we used orthogonal contrasts to minimize Type I error when conducting pairwise comparisons of a significant interaction term. Further details of this approach are provided in Supplementary methods S5. For analysis of associations between older and younger sister pairs during the pre-reproductive phase, we focus on treatments where subjects were offered multiple safe nest sites, and hence had the option to sleep together or apart under equivalent conditions. For this analysis we calculated an index of association between older and younger sister pairs. The index was calculated by dividing the number of times at least one of the older females was sharing a nest with one of the younger females by the total number of observations. For the analysis of reproductive skew, we analyzed the absolute difference in the number of weaned offspring produced by communally breeding sisters. Further details are provided in Supplementary methods S5.

Results

The individual reproductive success of female mice within our experimental treatment groups was variable, ranging from 0 to 9 weaned offspring (4.42 ± 0.42 [mean ± SE]). Of the 48 subjects that were given the opportunity to breed, 38 produced a litter, and a total of 212 offspring were weaned. At least one litter was weaned within 19 of 20 social groups, with no significant difference across treatments in the number of females that weaned a litter, hereafter referred to as breeding females (nest site availability: χ^2=0.7, p=0.41; relatedness of competitors: χ^2=0.01, p=0.93, Table S4). Fifteen communal nests and 8 single nests were formed. The average number of offspring weaned per breeding female was greater for subjects rearing their young in communal nests (Table 1, factor: 'Communal or single nest' [$F_{1,16.14}$-= 7.1, p=0.02]). However there was no difference in the number of communal nests formed across treatment groups (nest site availability: χ^2=0.32, p=0.57; relatedness of competitors: χ^2=1.14, p=0.29, Table S5). Breeding females always chose to rear their offspring in protected nest sites, and in groups where both sisters within a pair produced litters, all pairs shared a nest, even when multiple protected nest sites were available.

Effects of nest site availability on activity and competitive signaling

We found significant differences in the index of activity for subjects according to cues of resource competition. That is, from birth of offspring to peak lactation, females with a single protected nest site left and re-entered the nest more frequently during the light (inactive) phase (Table 2a, factor 'Nest site availability' [$F_{1,15.65}$=7.92, p=0.01], Fig. 2a), with control for total time spent in the nest box (Table 2a, factor 'Time spent in nest box' [$F_{1,31.52}$=6.6, p=0.02]). By contrast, during the dark (active) phase, activity patterns were not related to the availability of protected nest sites (Table S6a).

The contrasting number of protected nest sites available between treatment groups also resulted in significant differences in scent marking behavior between subjects. Females with a single

protected nest site were more responsive to simulated intrusions, scent marking a larger area in response to intruder scent compared to a control test with water only, irrespective of the relatedness of the simulated intruder (Table 2b, factor: 'Stimulus x Nest site availability' [$F_{2,60}$=3.32, p=0.04]; Fig.3; for orthogonal contrasts see Table S7). However, while scent marks deposited by females with single protected nest sites covered a greater area during intruder presentation, the number of scent marks was not significantly different between treatment groups (Table S6b), indicating that females with single protected nest sites responded to intruders with larger scent marks.

Fitness consequences linked to competition for protected nest sites

Perceived competition for protected nest sites did not influence the number of offspring weaned among breeding females (Table 1, factor: 'Nest site availability' [$F_{1,12.97}$=0.18, p=0.68]; Single nest site: 5.67 ± 0.44, Multiple nest sites: 5.67 ± 0.58 [means ± SE]), or the productivity of communal nests, measured as the total number of weaned offspring per communal nest (Table 3a, factor: 'Nest site availability' [$F_{1,12}$=0.5 p=0.49]; Single nest site: 11.9 ± 1, Multiple nest sites: 12 ± 0.8 [means ± SE]; Fig. 4). However, we did find evidence for an effect of protected nest site availability on offspring size. That is, offspring raised by females with a single protected nest site had a lower mean body mass at weaning compared to those with access to multiple protected nest sites (Table 4 factor: 'Nest site availability', [$F_{1,17.55}$=5.15, p=0.04; Single nest site: 14.2 ± 0.45 g, Multiple nest sites: 15.4 ± 0.34 g [means ± SE]; Fig.2b). Irrespective of treatment, male offspring were larger than females (Table 4 factor 'Sex of offspring' [$F_{1,18.89}$=48.89, p<0.01]), and older mothers produced heavier offspring (Table 4 factor: 'Age of mother(s)' [$F_{1,17.83}$=9.6, p=0.01]). Although larger litters appeared to result in heavier average weaning mass, further inspection reveals that this was explained by one case in which a single offspring was weaned with an unusually low weaning mass (Supplementary results S1).

Social association between related and unrelated competitors

We found that unrelated females were less likely to associate together during the light (inactive) phase when given the option to sleep safely apart. That is, in groups with multiple protected nest sites, older and younger sister pairs were found less often in the same nest box when they were unrelated (Table S8 factor: 'Relatedness of competitors' [$\chi^2(1, N=10)=5.72$, $p=0.02$]; Fig. S2).

Fitness consequences linked to relatedness of competitors

We found no evidence for an increase in the productivity of communal nests when competitors were unrelated. Rather, total communal litter size was *lower* for communally nesting females with unrelated competitors compared to those with related competitors (Table 3a, factor: 'Relatedness of competitors' [$F_{1,12}=6.02$, $p=0.03$]; Related: 13 ± 0.7, Unrelated: 10.3 ± 0.8 [mean ± SE]; Fig. 4), and there was no significant difference in time spent with offspring according to relatedness of competitors (Table S9, factor: 'Relatedness of competitors' [$F_{1,9}=0.29$, $p=0.6$]; Fig. S3). Similarly, we found no evidence that reproductive skew between sisters sharing communal nests was lower in the presence of unrelated competitors (Table 3b factor: 'Relatedness of competitors' [$F_{1,11}=0.51$, $p=0.49$]).

Discussion

Our study reveals that social competition has direct fitness costs for breeding females, arising both when key resources are limited (but not limiting to immediate reproductive success) and when competitors are unrelated. However, contrary to our initial predictions, we found no evidence for increased cooperation between related females when local competitors were unrelated, which might have helped to mitigate such costs.

By manipulating the availability of protected nest sites for breeding and cues of social competition, we have demonstrated fitness costs of resource competition in female house mice. That is, the offspring of females that had access to only a single protected nest site were on average smaller at weaning compared to those produced by females with access to multiple protected nest sites, regardless of the relatedness of competitors. As discussed in more detail below, subjects with limited access to protected nest sites appear to be investing more strongly in resource defense. Our findings are thus consistent with the idea of a trade-off between female competitive behavior (nest defense) and maternal investment (Cain and Ketterson 2013).

As in other species that are dependent on a safe nest site for successful reproduction (Heinsohn et al. 2005; LeBas 2006; Stockley and Bro-Jorgensen 2011), female house mice are likely to be particularly sensitive to the risk of losing access to such a valuable resource. Under naturalistic conditions, non-breeding adult females persistently invade the territories of neighboring groups, and show particular interest in nest sites, despite aggressive defense by dominant males and breeding females (Hurst 1987; Hurst 1990). Increased effort may therefore be invested to defend the safe nest site and its associated breeding opportunity under conditions where availability is limited, for example through increased vigilance or other activity. Consistent with this expectation, we found that subjects with access to a single protected nest site showed increased levels of activity during the light (inactive) phase. This is suggestive of elevated vigilance behavior, similar to the behavior of lactating female bank voles (*Myodes glareolus*) that show greater levels of activity and leave their nests more often in response to a perceived risk of male infanticide (Breedveld et al. 2019). Although competitive physical interaction was prevented between older and younger sisters during the period when increased activity was recorded, subjects were continuously exposed to social odors of competitors, reinforced with controlled physical contact. The expression of competitive responses by house mice and other rodents are well documented under similar experimental conditions (Ramm and Stockley 2009; Lemaitre et al. 2011; Firman et al. 2013; Breedveld et al. 2019). In the current study, likely competitive responses of female house mice include patrolling the territory,

investigating social odors and refreshing scent marks, for example to advertise breeding status or social dominance (Stockley et al. 2013). As such behaviors were not inhibited under our experimental design, limiting direct physical contact to prevent infanticide during the reproductive phase of the experiment should not have significantly altered the normal expression of the competitive behaviors we quantified. By leaving the nest more frequently during the light (inactive) phase, female house mice are also likely to suffer a heightened predation risk under natural conditions, and their increased activity levels could impact sleep patterns and stress-related physiology (Hill et al. 2020).

Also consistent with increased resource defense, when access to protected nest sites was limited, subjects were more responsive to simulated intrusions by unfamiliar females from outside their social group, compared to when multiple protected nest sites were available. Moreover, as for our finding of reduced weaning mass under limited nest site availability, this response was independent of the relatedness of the simulated intruders and of competitors within the social group, indicating that when resources are limiting, competitive responses to kin can be similarly intense as those to non-kin (Clutton-Brock et al. 1982; Hoogland 1985; West et al. 2001; West et al. 2002). Although we found no evidence for an increase in the total number of individual scent marks deposited, the scent marks covered a larger total area when protected nest sites were limited. Female house mice typically produce fewer scent marks of more variable size compared to those produced by males, such that the area of scent deposited is likely to better reflect investment in competitive signaling than whether the scent is distributed across many small scent marks (see also Thonhauser et al. 2013). Subjects with limited access to protected nest sites again therefore appear to be investing more strongly in resource defense.

Kin selection theory predicts that the relatedness of competing females should influence the expression and fitness consequences of their behavior. Since house mice are able to discriminate kin (Green et al. 2015), and are adapted to living under diverse social conditions (Bronson 1979; Hurst 1987; König and Lindholm 2012), we expected subjects to vary their expression of cooperative

behavior according to the relatedness of competitors within their social groups (West et al. 2002). Consistent with previous evidence for kin discrimination between unfamiliar females (Green et al. 2015), we found that unrelated females were less likely to associate together when given multiple protected nest sites, compared to related females. Contrary to our original predictions, however, we found no evidence of increased propensity for sisters to form a communal nest when local competitors were unrelated. Rather, sisters consistently formed communal nests, regardless of the relatedness of other group members. Similarly, we found no evidence of more productive cooperation between sisters or increased reproductive investment based on time spent in the nest under such conditions. Instead, communal nests were less productive in the presence of unrelated competitors, with lower individual and combined fitness of sister pairs.

Overall then, it appears that having unrelated competitors within a social group is ultimately costly in terms of female reproductive success, and we found no evidence that cooperation between relatives compensates for this. Individual fitness costs of competition between unrelated females have previously been reported for mammals under both experimental and natural conditions (reviewed in Silk 2007; Stockley and Bro-Jorgensen 2011), although without manipulation of resource availability. Examples include cases of reduced fertility, smaller litter sizes, higher offspring mortality and reduced weaning success (e.g. Boonstra and Hogg 1988; Dobson et al. 2000). Here we have shown that social interactions between kin groups can be costly to reproductive success even when resources are not immediately limiting. This is consistent with the idea that inhibiting reproduction of others may be beneficial both to the future reproductive success of individual females, and to their future offspring and other relatives, as population density (and hence resource competition) increases.

In explaining the proximate mechanisms underlying fitness costs of competing with non-kin, we are able to rule out the occurrence of infanticide between unrelated females as a factor contributing to reduced weaned offspring numbers. Female infanticide occurs in diverse taxa (Hausfater and Hrdy 1984) and is typically directed towards the offspring of unrelated competitors,

most likely as a way of reducing immediate or future resource competition (Tuomi et al. 1997; Lukas and Huchard 2019). Although prevented here, such behavior is likely to add significantly to fitness costs of competition between unrelated females under natural conditions (Schmidt et al. 2015). Given that infanticide between unrelated females was prevented, the most likely proximate explanation for our findings relates to differences in social tolerance within experimental groups, with higher levels of aggression or intolerance among unrelated females. Similar to females of many other taxa, female house mice do not typically express high levels of overt competitive aggression (Hurst 1987; Hurst 1990). However, low-level aggression or persistent social intolerance can still invoke stress responses in female mammals, with the potential to inhibit reproductive success (Wasser and Barash 1983). Our finding that unrelated females were less likely to share sleeping sites is consistent with reduced social tolerance among non-kin during the pre-reproductive phase of the study, when heightened stress could have impacted subjects' fertility. Moreover, even when kin groups were physically separated to prevent infanticide, breeding females may still have experienced stress in response to the social cues of unrelated competitors within the shared territory, with potential consequences for reproductive success.

In conclusion, by manipulating resource availability independently of relatedness of competitors, our study reveals that both resource competition and competition with unrelated females are costly to female reproductive success. Our findings thus provide experimental evidence that for group living animals: 1) resource defense can be costly for females, potentially trading-off against maternal investment, and 2) associating with kin is beneficial to avoid negative fitness consequences of competing with non-kin, in addition to more widely recognized kin-selected benefits. These findings have broad implications for understanding the selective pressures resulting from social competition in vertebrate animals.

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Statement of Authorship

Conceptualization: P.S., S.F. & J.L.H.; Funding acquisition: P.S. & J.L.H; Methods development/experimental design: S.F., C.D., A.J.D., R.B., J.L.H & P.S.; Data collection: S.F., C.D., R.B. & A.D.; Data analysis: S.F.; Writing – original draft: S.F. & P.S.; Writing – review & editing: S.F., C.D., A.J.D., R.B., J.L.H & P.S.

Data and code accessibility

All data and code to re-create the resuts and figures can be found on Dryad (https://doi.org/10.5061/dryad.j0zpc86h1; Fischer et al. 2022).

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Tables

Table 1: Predictors of number of offspring weaned by female house mice

Factors	Estimate ± SE	Num. D.F.	Den. D.F.	F-value	*P*-value
Number of offspring weaned					
Intercept	1.22 ± 0.23	-	-	-	-
Nest site availability	-0.1 ± 0.22	1	12.97	0.18	0.68
Relatedness of competitors	-0.15 ± 0.23	1	12.92	0.42	0.53
Communal or single nest	0.63 ± 0.24	1	16.14	7.1	**0.02**

Predictors of number of offspring weaned by female house mice across four treatment groups, with independent manipulation of access to protected nest sites ('Nest site availability') and relatedness of older and younger females within the social group ('Relatedness of competitors'). Results are shown for all breeding females, including those that combined litters in shared nests and those that reared young alone (Communal or single nest). Estimates are shown on a log scale and as differences to the reference levels 'Single nest sites' for factor 'Nest site availability', 'Related' for factor 'Relatedness of competitors' and 'Single nest' for factor 'Communal or single nest'. Maternal body mass and maternal age did not predict the number of weaned offspring and were removed from the final model. F-values and p-values refer to comparisons of models with or without the factor of interest. To obtain normally distributed residuals the dependent variable was log transformed. N=38 females in 19 trials. P values <0.05 are highlighted in bold.

Table 2: Differences in behavior of female house mice according to their competitive environment

Factors	Estimate ± SE	Num. D.F.	Den. D.F	F-value	*P*-value
a) Index of activity during the light phase					
Intercept	20.58 ± 5.03	-	-	-	-
Nest site availability	-0.8 ± 0.28	1	15.65	7.92	**0.01**
Relatedness of competitors	-0.16 ± 0.28	1	14.72	0.35	0.56
Time spent in nest box	-0.99 ± 0.39	1	31.52	6.6	**0.02**
b) Area scent marked					
Intercept	-2.13 ± 0.44	-	-	-	-
Nest site availability	-0.40 ± 0.53	1	13	6.27	**0.03**
Relatedness of competitors	0.29 ± 0.45	1	13	0.42	0.53
Stimulus (control, unrelated, or related)*	-	2	60	3.02	0.06
Stimulus x Nest site availability*	-	2	60	3.32	**0.04**

*For parameter estimates see Fig. 3

Subjects were from four treatment groups, with independent manipulation of access to protected nest sites ('Nest site availability') and relatedness of older and younger females within the social group ('Relatedness of competitors'). (a) Activity was recorded as the number of times that subjects entered and left the nest from PND 0-14. (b) To test the response of subjects to simulated territorial intrusion, each was presented with artificial scent marks on three occasions during the post-reproductive phase. In a control test, artificial scent marks consisting only of distilled water were presented. In simulated intruder tests, artificial scent marks were presented consisting of urine from an unfamiliar related or unrelated female, respectively (for more details see Methods). Subject responses to these artificial scent marks were analyzed for total area scent marked. Estimates are shown on a log scale and as difference to the reference levels 'Single nest sites' for the factor 'Nest site availability' and 'Related' for the factor 'Relatedness of competitors'. Estimates for factor 'Stimulus' and 'Stimulus x Nest site availability' in (b) are shown as orthogonal contrasts in Table S7 and in the legend of Fig. 3. F-values and p-values refer to comparisons of models with or without the factor of interest. To obtain normally distributed residuals the dependent variable in both models was log transformed. (a) N=36 females in 18 trials. (b) N=64 observations on 32 females in 16 trials. P values <0.05 are highlighted in bold.

Table 3: Predictors of reproductive success for communally breeding female house mice

Factors	Estimate ± SE	Num. D.F.	Den. D.F.	F-value	*P*-value
(a) Total litter size in communal nests					
Intercept	2.52 ± 0.08	-	-	-	-
Nest site availability	0.07 ± 0.1	1	12	0.5	0.49
Relatedness of competitors	-0.25 ± 0.1	1	12	6.02	**0.03**
(b) Reproductive skew of communally breeding females					
Intercept	0.36 ± 1.11	-	-	-	-
Nest site availability	0.35 ± 0.35	1	11	1.01	0.34
Relatedness of competitors	0.3 ± 0.42	1	11	0.51	0.49
Total communal litter size	0.02 ± 0.08	1	11	0.04	0.85

Predictors of reproductive success for communally breeding female house mice across four treatment groups, with independent manipulation of access to protected nest sites ('Nest site availability') and relatedness of older and younger females within the social group ('Relatedness of competitors'). To test for evidence that relatedness of competitors affects competitive or cooperative behavior of communally breeding females, we tested for effects of treatment group on: (a) total litter sizes within communal nests, as a measure of shared productivity, and (b) reproductive skew within communal nests, as a measure of conflict within the shared nest. Estimates are shown on a log scale and as differences to the reference levels 'Single nest site' for factor 'Nest site availability' and 'Related' for factor 'Relatedness of competitors'. F-values and p-values refer to comparisons of models with or without the factor of interest. To obtain normally distributed residuals the dependent variables of both models were log transformed. N=15 communal litters in 11 trials. P values <0.05 are highlighted in bold.

Table 4: Predictors of weaning mass for offspring produced by female house mice

Factors	Estimate ± SE	Num. D.F.	Den. D.F.	F-value	*P*-value
Intercept	2.3 ± 0.1	-	-	-	-
Nest site availability	0.1 ± 0.04	1	17.55	5.15	**0.04**
Relatedness of competitors	-0.04 ± 0.04	1	17.44	0.8	0.38
Sex of offspring	0.1 ± 0.01	1	18.89	48.89	**<0.01**
Age of mother(s)	$1e^{-3} \pm 3e^{-4}$	1	17.83	9.6	**0.01**
Litter size	0.02 ± 0.01	1	18.97	5.89	**0.03**

Predictors of weaning mass for offspring produced by female house mice across four treatment groups, with independent manipulation of access to protected nest sites ('Nest site availability') and relatedness of older and younger females within the social group ('Relatedness of competitors'). For the dependent variable, average weaning mass was calculated for each litter and for each sex. Litter size is included as a covariate, and refers to the total number of offspring weaned in either single or communal nests. Age of both mothers is the same for offspring in communal nests, since the sisters rearing them were always littermates. Estimates are shown on a log scale and as differences to the reference levels 'Single nest sites' for factor 'Nest site availability', 'Related' for factor 'Relatedness of competitors', and 'Females' for factor 'Sex of offspring'. To obtain normally distributed residuals the dependent variable was log transformed. F-values and p-values refer to comparisons of models with or without the factor of interest. N=43 observations in 23 litters. P values <0.05 are highlighted in bold.

Figure legends

Fig. 1: Schematic overview of the experiment. Older sister pairs are represented as larger than younger sister pairs, related females as the same color and pattern, protected nest boxes as dark rectangles and unprotected nest boxes as pale squares. (A) Upper left: older and younger sister pairs are related, and a single protected nest site is available (=*related competitors and single protected nest site*). Upper right: older and younger sister pairs are related, and four protected nest sites are available (=*related competitors and multiple protected nest sites*). Lower left: older and younger sister pairs are unrelated, and a single protected nest site is available (=*unrelated competitors and single protected nest site*). Lower right: older and younger sister pairs are unrelated, and multiple protected nest sites are available (=*unrelated competitors and multiple protected nest sites*). Each enclosure was divided into four equal-sized compartments, linked by entrances that could be blocked with wire mesh (indicated by dashed lines). Each enclosure contained two transponder readers that monitored the nest box attendance of subjects in occupied nest boxes during PND 0-14 (shown in the figure on the tunnel entrance to a protected nest box within each enclosure). (B) The four phases of the experiment (see Methods).

Fig. 2: Comparison of (A) index of activity during the light phase recorded between birth of offspring and peak lactation (PND 14), and (B) the average weaned body mass for offspring produced by female house mice under contrasting levels of social competition. Treatment groups differed according to availability of protected nest sites (Single versus Multiple nest sites) and the relatedness of potential competitors within social groups (Related versus Unrelated). Mean values of offspring weaning mass were calculated per litter (single or communal) produced within each treatment group, with male and female offspring combined. Shown are boxplots where boxes represent all data points within the lower .25 and higher .75 quartile. Thick horizontal lines represent the median and whiskers include all data points within 1.5*the length of the box (interquartile range). See Table 2a and Table 4 for statistical analysis.

Fig. 3: Comparison of the area scent marked by female house mice in response to simulated intruders within their home territory, according to availability of preferred nest sites (Single or Multiple nest sites available). To test the response of subjects to simulated territorial intrusion, each was presented with artificial scent marks on three occasions. In a Control test, artificial scent marks consisting only of distilled water were presented. In simulated intruder tests, artificial scent marks were presented consisting of urine from an unfamiliar related female (Related urine) or unfamiliar unrelated female (Unrelated urine), respectively (for more details see Methods). Shown are boxplots where boxes represent all data points within the lower .25 and higher .75 quartile. Thick horizontal lines represent the median, whiskers include all data points within 1.5*the length of the box (interquartile range [IQR]) and dots represent outliers that are outside of 1.5*the IQR. See Table 2b for statistical analysis. Estimates for marginal means (in cm^2+ 95% confidence interval) for Multiple nest sites, control condition = 0.09 (0.04-0.2); Multiple nest sites, related condition = 0.09 (0.04-0.19); Multiple nest sites, unrelated condition = 0.09 (0.04-0.2); Single nest site, control condition = 0.14, (0.06 – 0.3); Single nest site, related condition = 0.35 (0.16-0.75); Single nest site, unrelated condition = 0.45 (0.21-1).

Fig 4: Comparison of number of offspring weaned by sister pairs that formed communal nests under contrasting levels of social competition. Treatment groups differed according to availability of protected nest sites (Single versus Multiple nest sites) and the relatedness of potential competitors within social groups (Related versus Unrelated). Shown are boxplots where boxes represent all data points within the lower .25 and higher .75 quartile. Thick horizontal lines represent the median and whiskers include all data points within 1.5*the length of the box (interquartile range). See Table 3a for statistical analysis.

Figure 1

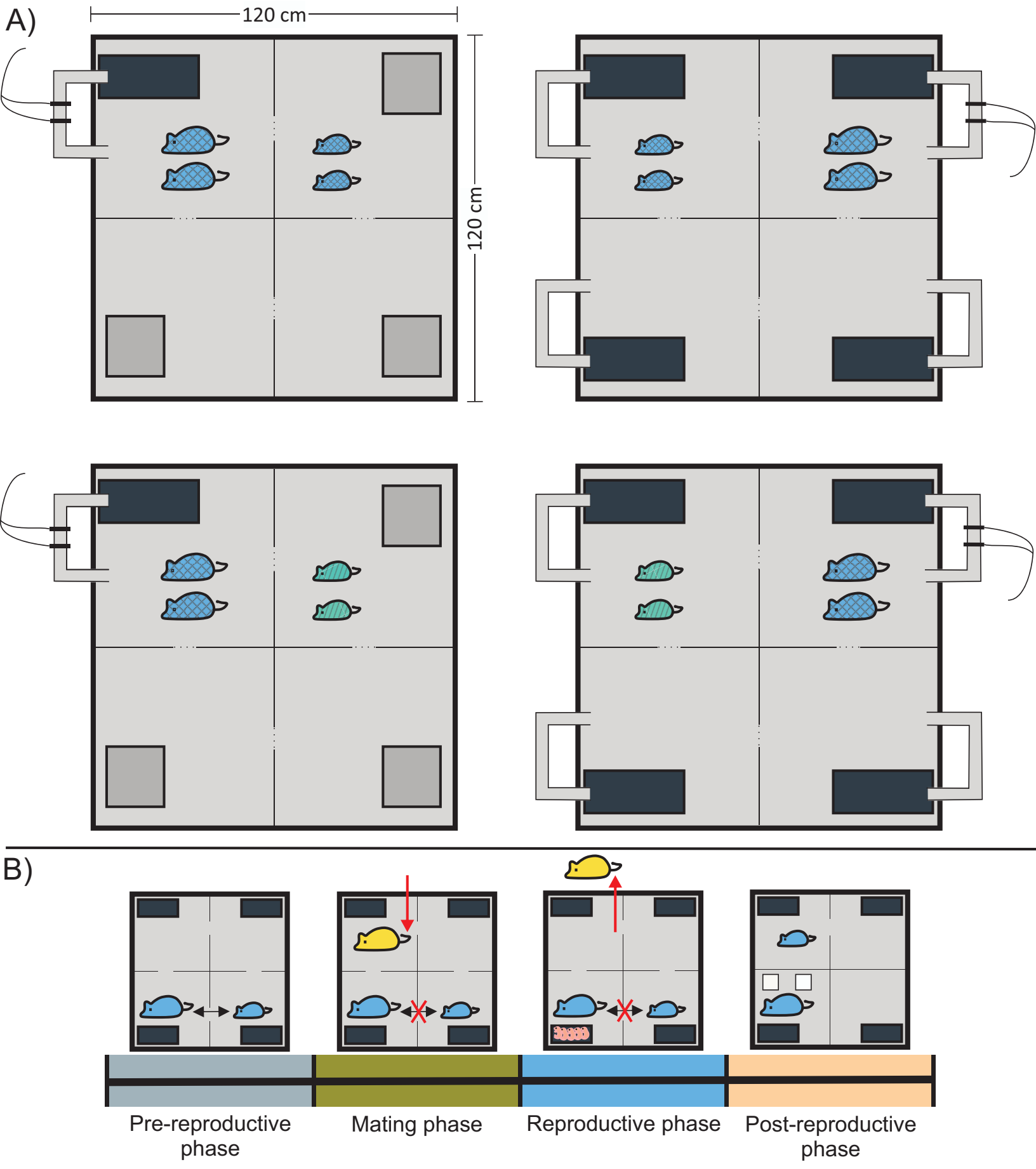
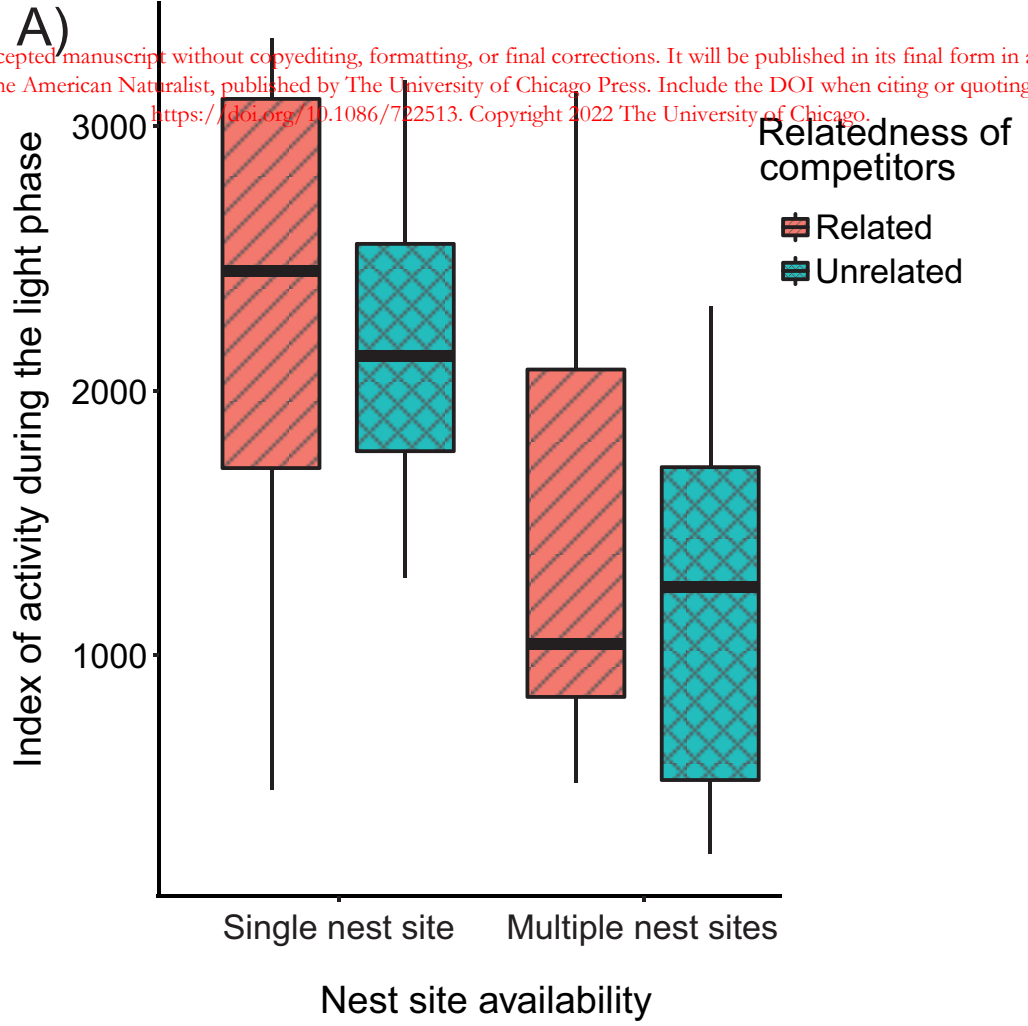


Figure 2



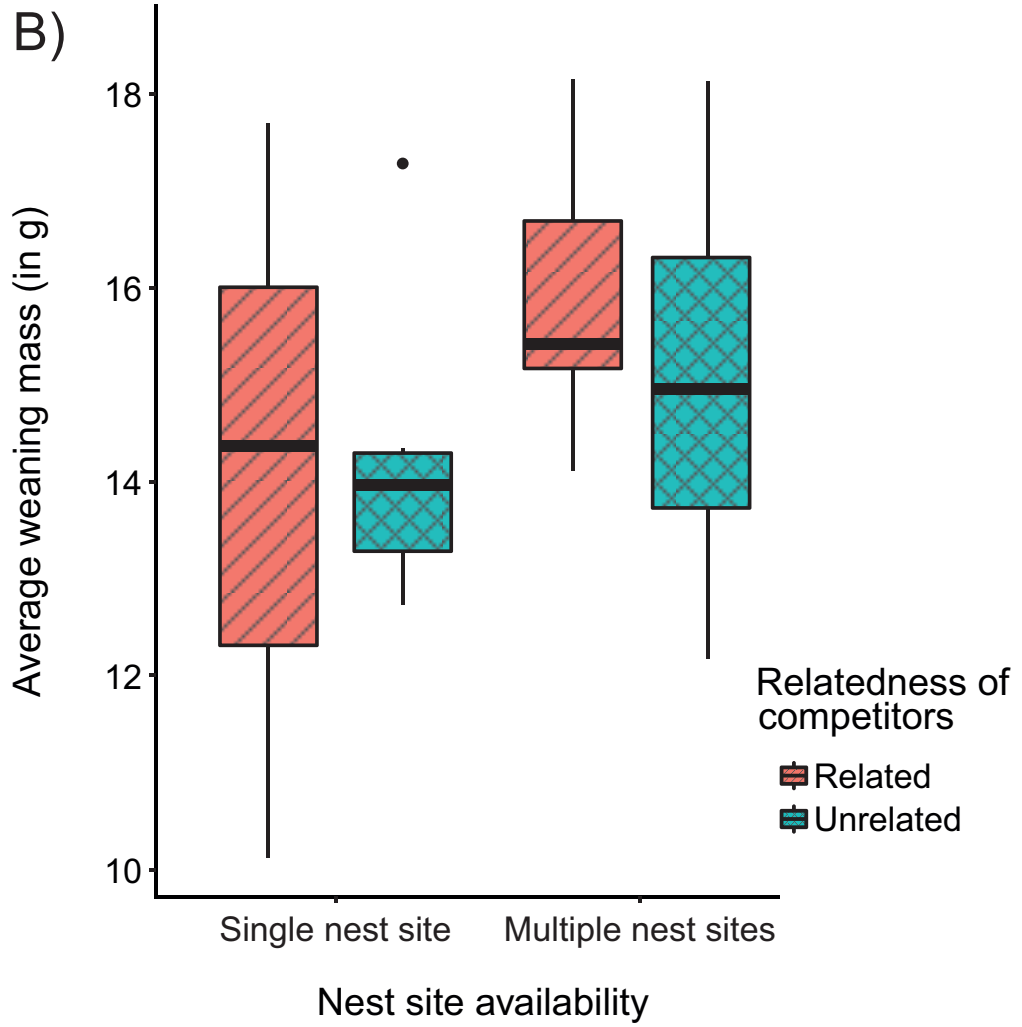


Figure 3

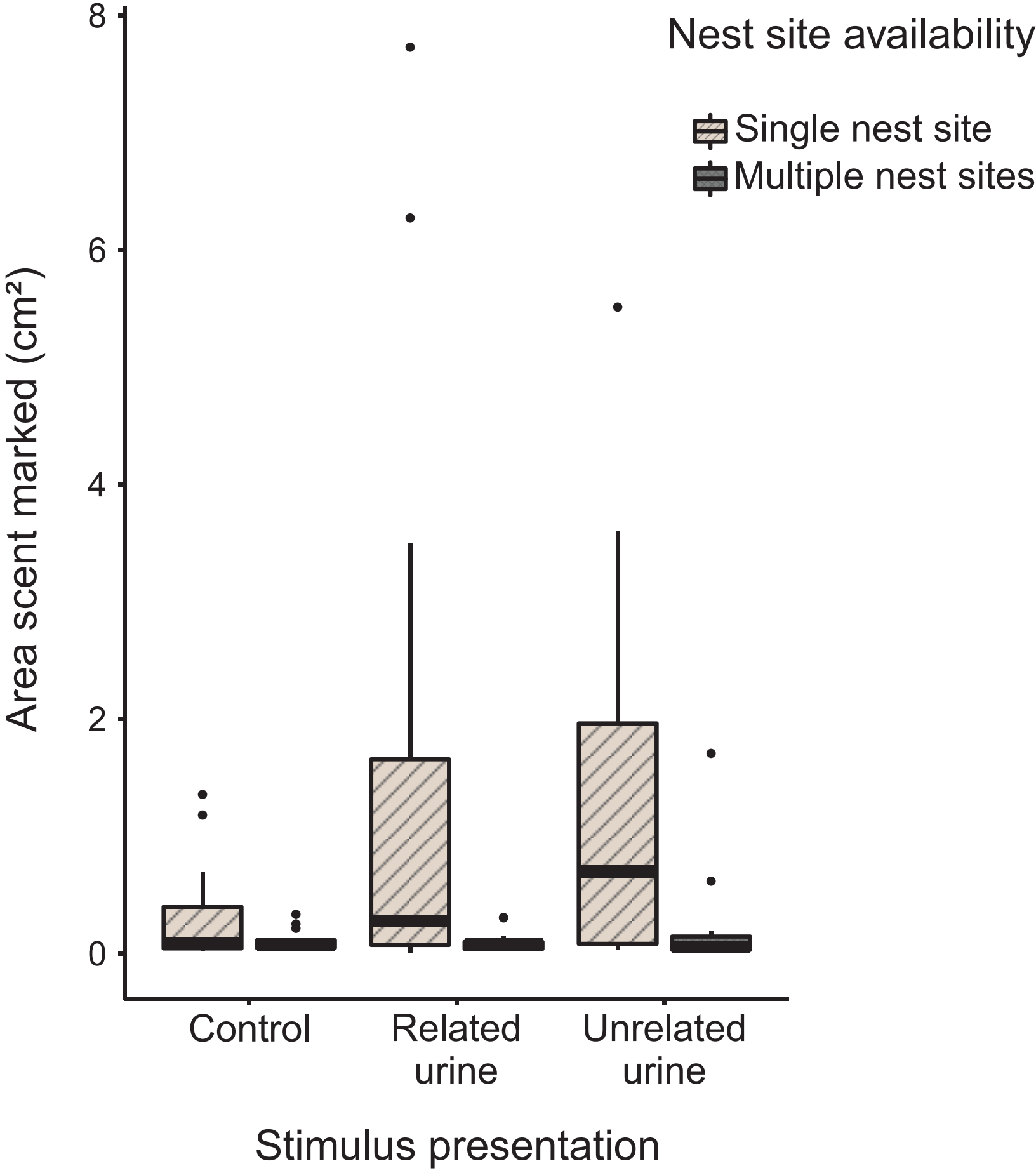
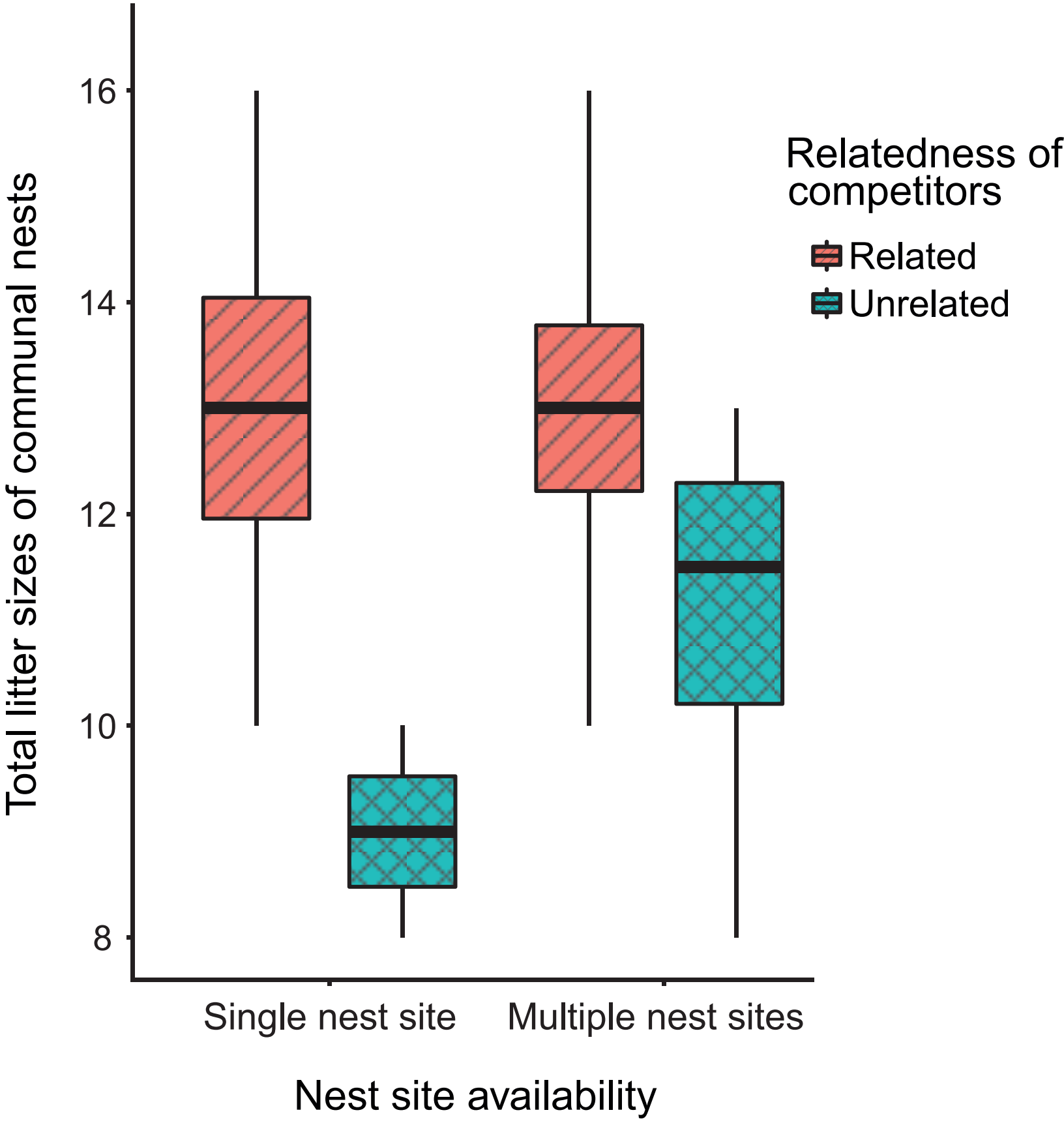


Figure 4



Electronic supplementary material for:

Fitness costs of female competition linked to resource defence and relatedness of competitors

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The following supplemental material contains details of methods applied to manage the social interactions of female house mice during the experiment (see main text for more details), additional details of experimental methods and procedures applied during each trial, details of each of the statistical models presented in the results section, plus additional results, tables and figures to support the conclusions presented in the main body of the manuscript.

Table of contents

Supplementary methods S1: Maternity analysis

DNA was extracted from a 5 mm ear punch (sampled post-mortem) using a QIAGEN DNeasy Blood & Tissue Kit (QIAGEN, West Sussex, UK). Haplotypes were established by genotyping parents and offspring, using 11 microsatellite markers (D2Mit277, D4Mit17, D4Mit139, D4Mit164, D4Mit217, D6Mit138, D11Mit4, D14Mit132, D17Mit22, D17Mit126, D17Mit234) selected from Firman and Simmons (2008) and Sherborne et al. (2007). The forward primer for each marker was 5'-fluorescently labelled with 6-FAM, PET, NED or VIC. The loci were organized into four multiplex loading groups, containing mixed loci from 7 regions. PCR amplification reactions were performed in a 10 µl volume of ~20 ng DNA, 0.25- 0.5 µM primer and 5 µl of BioMix Red reaction mix (Bioline, London, UK). The PCR protocol steps were: an initial denaturation for 5 min at 94 °C, 35 cycles of denaturation at 95 °C for 30 s, annealing at 60°C -56°C for 30 s, extension at 72 °C for 30 s, and after the 35 cycles were complete a final extension at 72 °C for 5 min. The PCR reactions were then diluted to 16- to 25-fold (depending on primer set) and multiplexed in formamide with GeneScan LIZ500 size standard (Applied Biosystems). Haplotype size was determined with an Applied Biosystems Genetic Analyser 3500XL and GeneMapper v3.0 software (Applied Biosystems). To assign maternity we compared markers carried by offspring to those carried by known sires and unknown mother(s).

Supplementary methods S2: Enclosures

Enclosures were distributed evenly around the same environmentally controlled and ventilated animal room (temp 20-21°C, relative humidity 45-65%, 20 room air changes per hour and a reversed 12:12 light-dark cycle). Spacing of enclosures and directional ventilation minimised any odour contamination between groups.

Each enclosure was divided into four equal-sized square compartments (0.6 x 0.6 m), linked by two circular entrance holes per connecting wall that could be blocked with wire mesh covers to restrict movement of subjects between compartments when necessary (for example, to temporarily isolate subjects for behavioural assays). To allow females the choice to nest individually or communally, each of the four compartments was provided with a nest box (protected or unprotected – see below), as well as a PVC tunnel and paper wool nest material for enrichment, and access to food and water (Fig. 1A).

Protected nest boxes consisted of opaque plastic boxes (11.8cm x 19.8cm x 9cm [W x L x H) with ventilation holes, nesting material, a lid, and a single entrance hole. These were placed in the corner of enclosures with access via a clear plastic sheltered entrance tunnel. Non-protected nest boxes were open opaque plastic boxes (11.8cm x 11.8cm x 6cm [W x L x H]) with nesting material but no lid. These were placed away from walls, in the same position as protected nest sites but with no sheltered entrance. The position of the single protected nest box and unprotected nest boxes was balanced between trials.

Within each enclosure, two transponder readers monitored the entrance tunnel to a protected nest box where older sister pairs were nursing their litters, allowing us to discriminate a mouse entering or leaving the nest. Movements were monitored between the birth of offspring and peak lactational investment at postnatal day (PND) 14. Nests were checked daily for pups and recording was initiated on detection of the first litter born within an enclosure and continued for 15 days. In cases where females moved their litters between nest boxes, the transponders were relocated accordingly. Each

set of readers was connected to a customized data logger (Francis Scientific Instruments, Cambridge, United Kingdom) that saved the time stamp of each RFID reading onto a USB drive. The sequence of movements for each subject was subsequently analysed blind to treatment group (software written by JLH) to give the total time spent by each subject within the nest box, and the number of times that each subject entered and left the nest box during the total recording time, as an index of activity.

Supplementary methods S3: Managing social interactions

Introduction and Pre-reproductive phase

Female house mice typically do not show high levels of overt competitive aggression once established within social groups, although variable levels of aggression may be exhibited when meeting unfamiliar animals. To establish social groups, unfamiliar sister pairs were therefore initially housed in separate halves of the enclosure. This allowed gradual introduction with remote monitoring of social interactions via an overhead camera to prevent escalated aggression using an ethical protocol when animals were in direct social contact. During this process, four identical initial nest boxes (9.0cm x 13.5cm x 7.5cm [W x L x H]) were provided as shelter for subjects being introduced. The ethical protocol involved intervening immediately if a chase was observed that lasted for 10 seconds, or if a direct attack (body contact that could result in biting) or fight was initiated. If this occurred the animals were interrupted by approaching the enclosure to induce a flight response, or (unusually) if unsuccessful, by direct intervention. If two interventions were required within the 30-minute observation period, the unfamiliar animals were separated using mesh dividers. In two trials aggression persisted and subjects were replaced with same-aged full siblings from the stock population prior to the start of the experiment, following the same process of gradual introduction. One of these trials involved unrelated sister pairs and one related sister pairs. It was necessary to replace the females involved because under natural conditions the recipients of persistent aggression would be forced to leave the territory, which was not possible in a captive situation.

Once relationships between group members had stabilised, subjects were given free access to the whole enclosure and the identical nest boxes initially provided were replaced by protected and unprotected nest boxes, according to treatment group. The experiment was then initiated and groups were left to interact for on average 15.6 days [range 7-22 days], during a pre-reproductive phase (Fig. 1B).

Mating phase

To prepare for mating, subjects were primed twice with the scent of an unfamiliar unrelated adult male (using cage substrate soiled with the male's odour), 7 and 4 days before introducing the male to the enclosure. During the second presentation, the male was placed within its home cage inside the enclosure for approximately 8 hours to allow protected social contact with female groups. Prior to releasing males, the younger sister pairs in blocks 2-5 were restricted to an MB1 cage inside the enclosure to prevent them from mating (see main text and Table S1). The enclosures were remotely monitored for the first 30 min after introduction of males, following an ethical protocol to prevent escalated aggression (see above). In block 1, males were left in the enclosures for 18 days. However, following confirmation of high breeding success rates, this was shortened to 7 days in blocks 2-5.

Reproductive phase

After males were removed, cage substrate soiled with the mated male's odour was added to the enclosures weekly to maintain exposure to social cues from a dominant male as would occur under natural conditions. Females were managed according to whether all (block 1), or older subjects only (blocks 2-5), had mating opportunities (Table S1). In all cases, older and younger sister pairs were separated shortly before giving birth to prevent risk of infanticide, again with some differences between blocks 1 and 2-5 (Table S1). Controlled social contact was maintained between pairs during pup rearing via contact through wire-mesh barriers and scent cues. Where younger sister pairs were restricted to an MB1 cage within the enclosure, this was twice weekly repositioned. Younger sisters were also released twice weekly for 1-2 h to maintain their social odours throughout the enclosure. Thus although direct physical interaction was prevented between older and younger sisters during this period, subjects were continuously exposed to social odours of competitors, reinforced with controlled physical contact. The presence of other females (and their odours) in the subjects' territory was maintained to create a perceived competitive risk: 1) that subjects could lose exclusive use of valuable safe nest sites, and 2) that their offspring may not gain access to safe nest sites.

Post-reproductive phase

After weaning, all offspring were removed from the enclosures and integrated into the stock colony. During this post-reproductive phase (Fig. 1), subjects remained in their enclosures for a scent marking assay. Older and younger sister pairs were reintroduced following an ethical protocol. If aggression persisted, younger sisters were kept within an MB1 cage inside the enclosure, and released regularly to refresh their social odours around the enclosure, as described above (see Table S1).

Supplementary methods S4: Scent marking assays

Stimulus donor females used for the same subject were of similar age (related stimulus: 8.43 ± 2.89; unrelated stimulus: 8.48 ± 2.75 [mean age in months ± SD]). Depending on urine availability, in most trials (N=16) we used the same stimulus animal (related or unrelated) for both subjects in the same social group. Stimulus animals were not re-used in different trials, except for two that were used twice. The order of tests was balanced between subjects. Females were given no more than two trials per day, with a minimum of 4h between trials.

Supplementary methods S5: Statistical analysis

Residuals and Q/Q-plots of all LMs and LMMs were visually inspected, and the distributions of residuals were compared to a normal distribution using Kolmogorov-Smirnov and Shapiro tests. If residuals were not normally distributed, a log transformation was applied, and residuals again checked. All GLMs and GLMMs were checked for over-dispersion, but none required a correction. All models included protected nest site availability (single or multiple protected nest sites) and competitor relatedness (related or unrelated competitors) as fixed effects. In cases of repeated measures, we included individual identity or sister pair identity as random effects in all models.

For analysis of scent marking assays, we tested the total number and the total area of scent marks deposited by subjects on both tiles that were presented simultaneously (see main methods). We used orthogonal contrasts to minimize Type I error when conducting pairwise comparisons of the significant interaction term 'Stimulus x Nest site availability' (see Abdi and Williams 2010). To investigate this interaction there are five orthogonal contrasts available which do not require a p-value correction (Crawley 2007). Using these contrasts we were able to ask: 1) if the availability of protected nest sites influenced the scent marking activity of subjects, irrespective of the stimulus presented, 2) if subjects with access to a) single and b) multiple protected nest sites scent marked more in response to simulated scent marks from intruders (related and unrelated female stimuli combined) than during control tests, and 3) if subjects with access to a) single and b) multiple protected nest sites scent marked more in response to simulated scent marks from unrelated females than to related females (for further details see Table S7).

We expected reproductive success of communally breeding sisters to be more equal (less skewed) when local competitors were unrelated. To test this, we analysed the absolute difference in the number of weaned offspring produced by communally breeding sisters. We also tested if the relatedness of competitors influences the total amount of time spent by communally breeding

sisters in the nest, using time spent with pups as a proxy for reproductive investment. For models testing hypotheses for communal nests only, we excluded the interaction term 'nest site availability x relatedness of competitors' from the full models due to the low number of communal nests when separated into the four treatment combinations (see Table S3).

Supplementary results S1

On testing if the availability of protected nest sites influenced average offspring weaning mass per litter (see Results section in main text), we found, unexpectedly, that average offspring weaning mass increased with litter size, whereas usually the opposite relationship of reduced average weaning mass per pup would be expected within larger litters (König et al. 1988). Further inspection revealed that this result was explained by one case in which a single offspring was weaned with an unusually low weaning mass. This individual was 56% smaller than other single offspring produced in the experiment. An additional model without this data point showed that litter size did not influence weaning weights ($F_{1,11.48}$=0.16, p=0.7). Nevertheless, removing this data point did not qualitatively change the result that offspring from females with access to a single protected nest site were smaller than offspring from females with access to multiple protected nest sites ($F_{1,15.52}$=4.61, p=0.04). Thus, the result that average weaning mass increased with litter size, as presented in the main text, should be treated with caution due to one unexpectedly small individual.

Supplementary figure S1

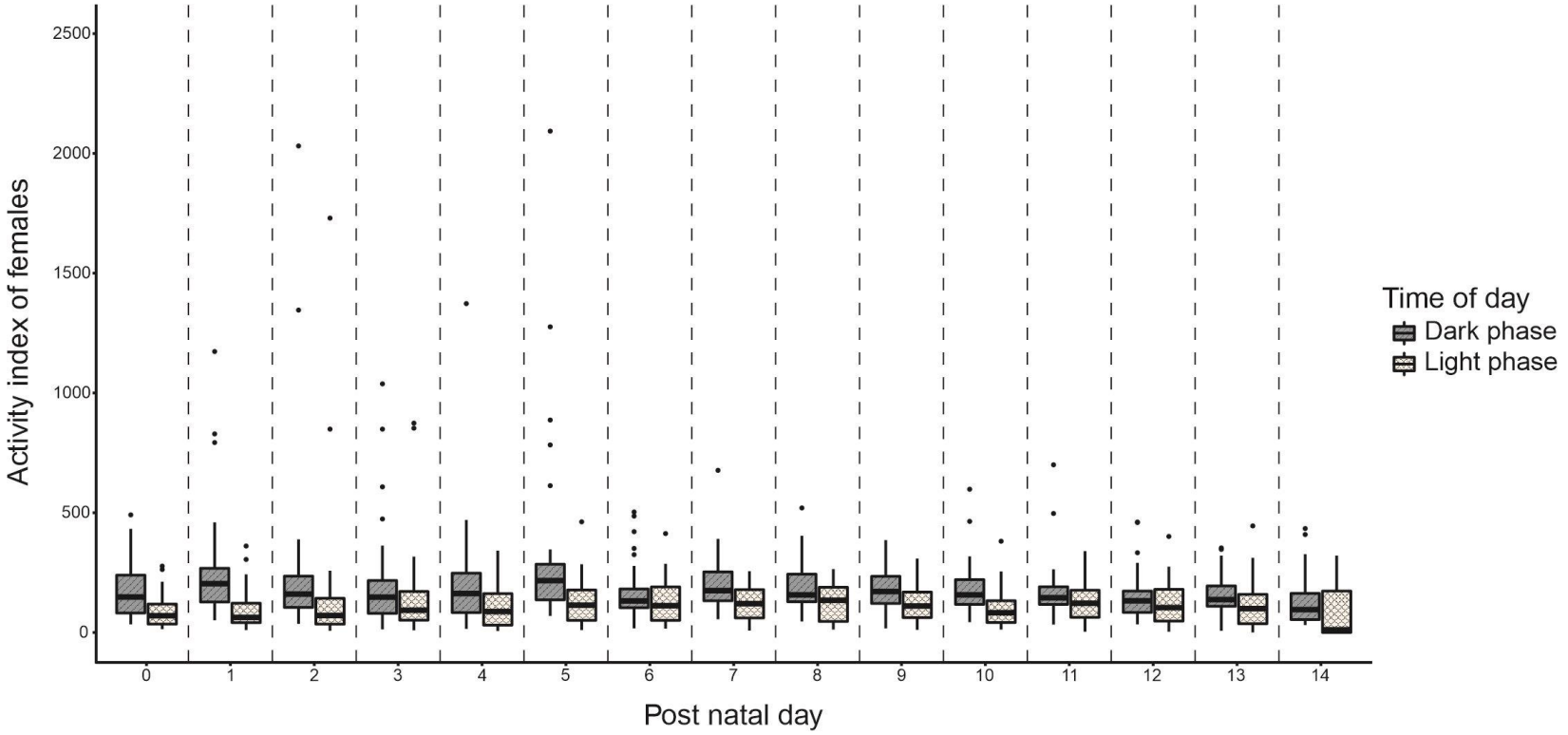

Figure S1: Activity of female house mice during the period of maternal investment from post-natal day (PND) 0-14, separated into the dark and light phase. Activity was measured continuously during the period from birth of the first litter to PND 14, using transponder readers that monitored the number of times each female left and-re-entered the nest. The total number of times that females entered and left the occupied nest is shown, over the total period of 360 hours (180 hours in each of the light and dark phases respectively). Females were more active during the dark phase but there was no obvious change in activity from PND 0-14. Shown are boxplots where boxes represent all data points within the lower .25 and higher .75 quartile. Thick horizontal lines represent the median, whiskers include all data points within 1.5*the length of the box (interquartile range [IQR]) and dots represent outliers that are outside of 1.5*the IQR.

Supplementary figure S2

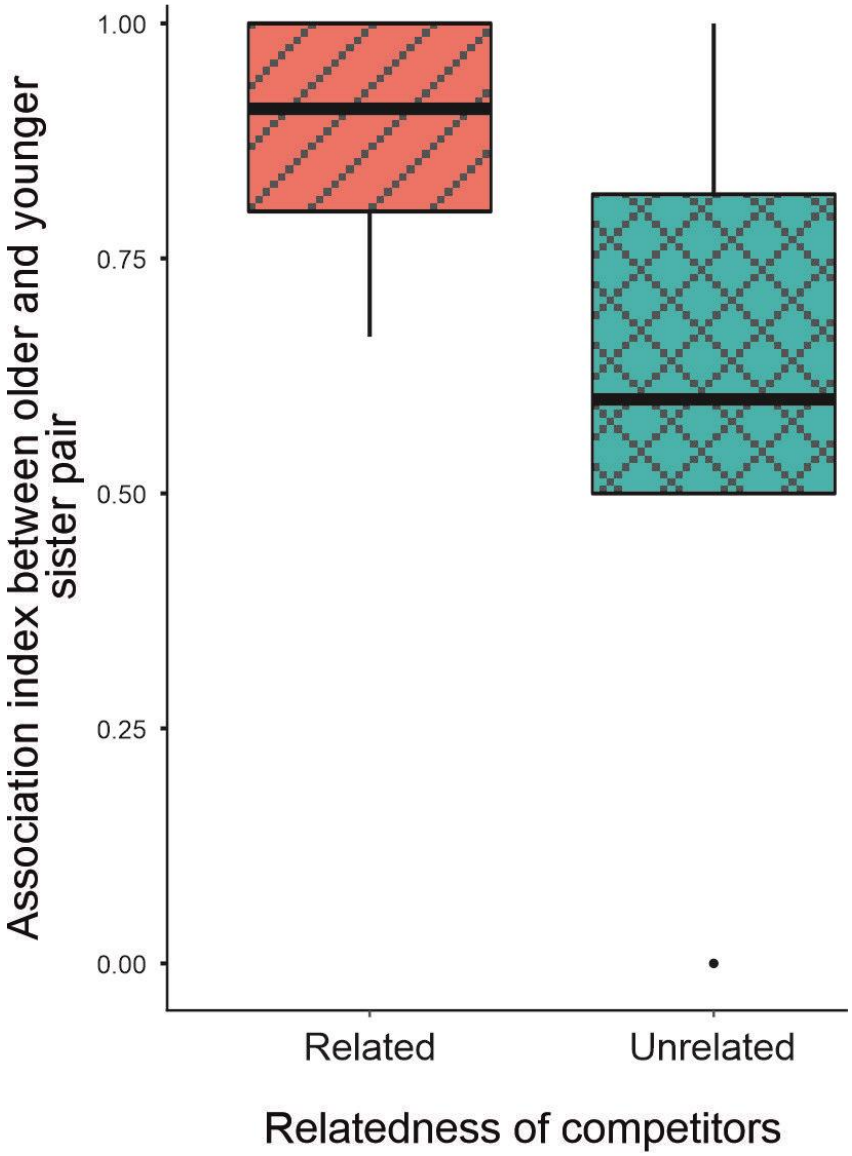

Figure S2: Comparison of the association index of older and younger sister pairs measured during the pre-reproductive phase of the experiment. Shown are results collected during the light (inactive) phase for subjects provided with multiple protected nest sites, and hence a choice of where to sleep safely. The association index was calculated by counting the number of times at least one of the older females was found with one of the younger females in the same nest box divided by the total number of observations (on average 5.8 observations per trial [range: 3-11]). Older and younger sister pairs were found more often sleeping together in the same nest box when competitors were related. Shown are boxplots where boxes represent all data points within the lower .25 and higher .75 quartile. Thick horizontal lines represent the median, whiskers include all data points within 1.5*the length of the box (interquartile range [IQR]) and dots represent outliers that are outside of 1.5*the IQR. See Table S8 for statistical analysis.

Supplementary figure S3

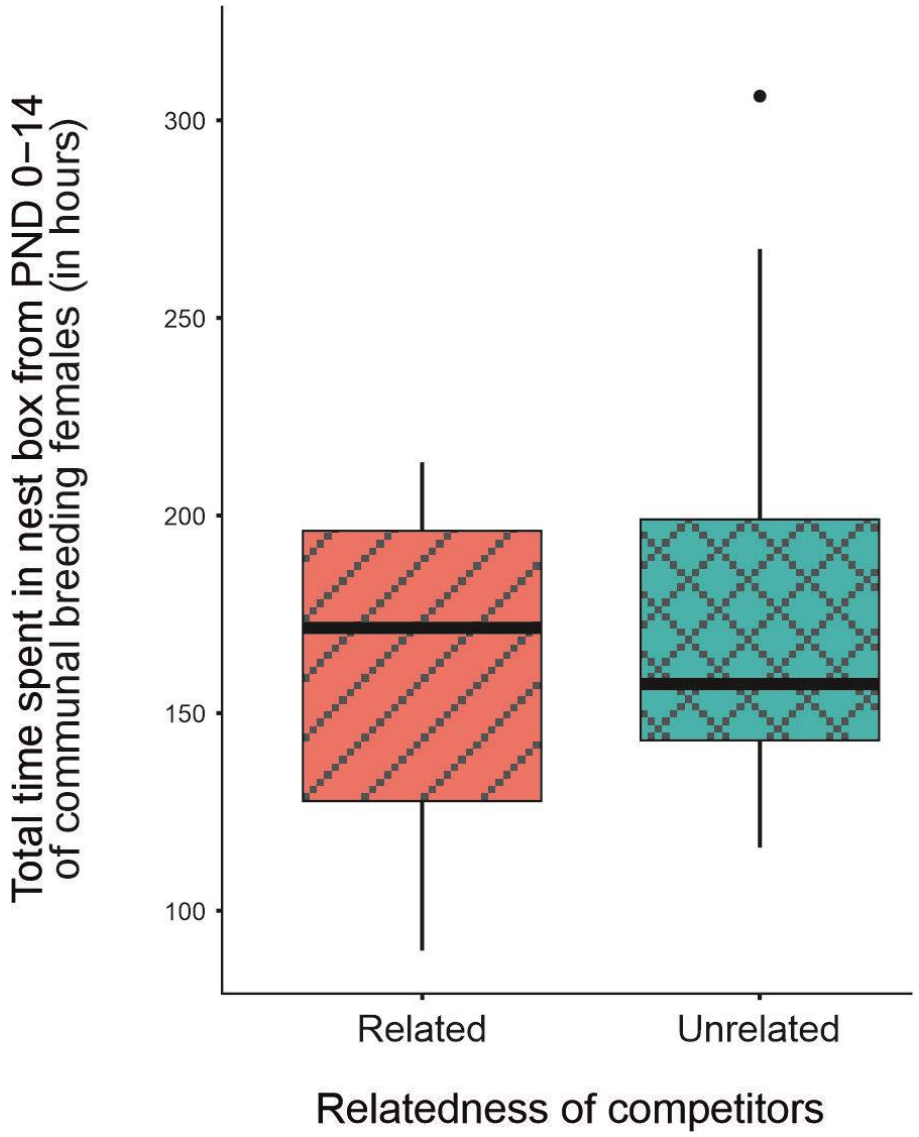

Figure. S3: Total time (in hours) spent in nest box by communal breeding females from postnatal day (PND) 0 to PND 14 (combined for dark and light phase). Subjects were housed either with related or unrelated competitors, and direct physical contact with competitors was prevented during the period when offspring where present in the nest, to prevent infanticide. Shown are boxplots where boxes represent all data points within the lower .25 and higher .75 quartile. Thick horizontal lines represent the median and whiskers include all data points within 1.5*the length of the box (interquartile range). See Table S9 for statistical analysis.

Supplementary table S1

Block	Trial	Treatment	Age difference (days)	Number females that bred / number females given opportunity to breed		Number communal nests	Managing social interactions to prevent escalated aggression and avoid infanticide		
				Older sisters	Younger sisters		Post-mating period	Pre-birth separation	Post-weaning period
1	1	SN/RC	20	2/2	2/2	2	FA	Split enclosure	FA
1	2	MN/RC	25	2/2	2/2	2	FA	Split enclosure	FA
1	3	MN/UC	38	2/2	2/2	2	FA2	Split enclosure	FA2
1	4	SN/RC	57	2/2	2/2	2	FA2	Split enclosure	FA2
2	5	SN/RC	106	1/2	0/0	0	FA2	Caged younger females	FA2
2	6	SN/UC	139	0/2	0/0	0	FA2	Caged younger females	RA
2	7	MN/UC	136	1/2	0/0	0	FA2	Caged younger females	FA2
2	8	MN/RC	146	1/2	0/0	0	RA	Caged younger females	RA
3	9	MN/UC	243	2/2	0/0	1	RA	Caged younger females	RA
3	10	MN/RC	251	1/2	0/0	0	FA2	Caged younger females	RA
3	11	SN/UC	263	1/2	0/0	0	RA	Caged younger females	RA
3	12	SN/UC	106	2/2	0/0	1	RA	Caged younger females	RA
3	13	MN/UC	269	1/2	0/0	0	FA2	Caged younger females	RA
3	14	SN/RC	253	1/2	0/0	0	RA	Caged younger females	FA2
4	15	MN/RC	58	2/2	0/0	1	RA	Caged younger females	RA
4	16	SN/UC	55	2/2	0/0	1	RA	Caged younger females	RA
5	17	SN/RC	56	2/2	0/0	1	RA	Caged younger females	RA
5	18	MN/RC	54	2/2	0/0	1	RA	Caged younger females	RA
5	19	MN/UC	56	2/2	0/0	1	RA	Caged younger females	RA
5	20	SN/RC	57	1/2	0/0	0	RA	Caged younger females	RA

Table S1: Summary of the age difference of older and younger sister pairs, the number of breeding females, the number of communal nests and how social interactions were managed to prevent escalated aggression and avoid infanticide for each trial. Key: *Age difference* = age difference in days between older and younger sisters; *Number communal nests* = number of communal nests formed (communal nests were always shared by two littermates –older or younger sister pairs respectively); *SN* = single protected nest site; *MN* = multiple protected nest site; *RC* = related competitors; *UC* = unrelated competitors; *FA* = all females allowed free access to whole enclosure (no separation during mating period); *FA2* = all females allowed free access to whole enclosure after testing for aggression following separation during mating period; *RA* = younger females given restricted access to whole enclosure after testing for aggression following separation during mating period; maintained within an MB1 cage which was twice weekly repositioned and released for 1-2h inside the larger enclosure, without direct contact with the older females to avoid aggressive interactions, while maintaining odour cues throughout the

enclosure; *Split enclosure*: older and younger sister pairs were restricted to separate halves of the enclosure prior to giving birth, to avoid risk of infanticide between pairs; *Caged younger females*: younger sister pairs were restricted to an MB1 cage within the enclosure prior to older sister pairs giving birth and throughout gestation. Younger sister pairs were released twice weekly for 1-2h without direct contact with the older females and their offspring to avoid risk of infanticide, while maintaining odour cues throughout the enclosure.

Supplementary table S2

Block	Trials	Treatment groups			
		Single protected nest Related competitors	*Multiple protected nests Related competitors*	*Single protected nest Unrelated competitors*	*Multiple protected nests Unrelated competitors*
1	1-4	2	1	0	1
2	5-8	1	1	1	1
3	9-14	1	1	2	2
4	15,16	0	1	1	0
5	17-20	2	1	0	1
Total	20	6	5	4	5

Table S2. Summary of how treatment groups were distributed across five blocks in the experiment. Each trial consists of a social group of four female mice, made up of two sister pairs. There were four treatment groups, with either a single protected nest site or multiple protected nest sites (to manipulate resource availability), and related or unrelated sister pairs making up the social group (to manipulate relatedness of competitors).

Supplementary table S3

Dependent variable	Factors	Covariates	Random factor	Transformation or Link	Interactions	Final model
Number of weaned offspring	Nest site availability Competitor relatedness Communal or single nest	Maternal body mass Age	Sister pair ID Block ID	Log (LMM)	Nest site availability × Competitor relatedness	Table 1
Activity index of females during the light phase	Nest site availability Competitor relatedness	Time spent inside the nest box during the light phase	Sister pair ID	Log (LMM)	Nest site availability × Competitor relatedness	Table 2a
Area scent marked	Nest site availability Competitor relatedness Stimulus	none	Sister pair ID Individual ID	Log (LMM)	Nest site availability x Stimulus Competitor relatedness x Stimulus Nest site availability × Competitor relatedness × Stimulus	Table 2b
Total communal litter size	Nest site availability Competitor relatedness	none	none	Log (LM)	none	Table 3a
Reproductive skew	Nest site availability Competitor relatedness	Total communal litter size	none	Log (LM)	none	Table 3b
Weaning masses	Nest site availability Competitor relatedness Sex of offspring	Age Average maternal body mass Total communal litter size	Sister pair ID Block ID	Log (LMM)	Nest site availability × Competitor relatedness	Table 4
Whether a female weaned a litter (yes, no)	Nest site availability Competitor relatedness	none	Sister pair ID Block ID	Logit link (GLMM)	Nest site availability × Competitor relatedness	Table S4
Whether a communal nest was formed (yes/no)	Nest site availability Competitor relatedness	none	Block ID	Logit link (GLMM)	Nest site availability × Competitor relatedness	Table S5
Activity index of females during the dark phase	Nest site availability Competitor relatedness	Time spent inside the nest box during the dark phase	Sister pair ID	Log (LMM)	Nest site availability × Competitor relatedness	Table S6a

Number of scent marks	Nest site availability Competitor relatedness Stimulus	none	Sister pair ID Individual ID	Log (LMM)	Nest site availability x Stimulus Competitor relatedness x Stimulus Nest site availability × Competitor relatedness × Stimulus	Table S6b
Sleeping net site associations between older and younger sister pairs	Competitor relatedness	none	none	Logit link (GLM)	none	Table S8
Total time spent in nest by communal breeding sisters	Competitor relatedness	none	Sister pair ID	Log (LMM)	none	Table S9

Table S3: Information on the linear mixed models (LMM), linear models (LM), general linear models (GLM) and generalized linear mixed models (GLMM) analysed during this study, including the respective dependent variables, fixed factors, covariates and random factors, eventual data transformations performed to obtain normally distributed residuals, and any interaction terms included in the initial, full models. If data included zeros and log transformations had to be applied, we added a constant value to all data points (i.e. 1, or in cases with data points below 1, the value closest to 0). To obtain final models, non-significant interactions and covariates were stepwise removed. Explanations of dependent variables: 'Number of weaned offspring': number of weaned offspring produced by focal females; 'Activity index of females during the light/dark phase': the activity index of females during the light or dark phase, measured as the number of times a female left and re-entered the nest from post-natal day (PND) 0 to PND 14; 'Area scent marked': the sum of the area scent marked on both tiles by focal females during each trial in the behavioural assays; 'Total communal litter size': combined litter size within communal nests; 'Reproductive skew: the absolute difference of the number of weaned offspring produced by females within the same communal nest; 'Weaning masses': average weaning mass per offspring sex for litters produced by focal females; 'Whether a female weaned a litter': whether a female weaned a litter (yes/no) in a given trial; 'Whether a communal nest was formed': whether a communal nest was formed (yes/no) in a given trial; 'Number of scent marks': the sum of the number of scent marks deposited on both tiles by focal females during each trial in the behavioural assays; 'Sleeping nest site associations between older and younger sister pairs': an association index indicating how often older and younger sister pairs shared a sleeping nest site, calculated as the number of times when at least one of the older females was sharing a nest with at least one of the younger females divided by the total number of observations; 'Total time spent in nest by communal breeding sisters': the total time (dark and light phase combined) communal breeding sisters spent in the nest during PND 0 to PND 14. Factor names: 'Nest site availability': whether females had access to multiple or single protected nest sites. 'Competitor relatedness': whether older sister pairs were housed with related or unrelated unfamiliar younger sister pairs (for details see Methods section); 'Communal or single nest': whether a communal or single nest was formed within a given trial; 'Stimulus': the type of stimulus presented during the behavioural assays (control, related or unrelated); 'Sex of offspring': whether male or female offspring. Covariate names: 'Maternal body mass': body mass of focal female on the day her offspring were weaned or, if the focal female was not pregnant, on the day the offspring of her familiar sister were weaned; 'Age': age of focal females; 'Time spent inside the nest box during dark/light phase': the absolute duration of time spent

inside the nest box from PND 0 to PND 14 during the dark or light phase; 'Average maternal body mass': in cases where a communal nest was formed, average maternal body mass was calculated of the two focal sisters in the same trial; in cases where a single nest was formed the maternal body mass of the respective mother was used; 'Total communal litter size': combined litter size within communal nests. Random factor names: 'Sister pair ID': identification number of sister pairs sharing the same nest or enclosure; 'Individual ID': individual identification to correct for multiple observations on the same animal; 'Block ID': identification number of blocks 1-5.

Supplementary table S4

Factors	Estimate ± SE	X^2-value	*P*-value
Intercept	1.56 ± 1.11	-	-
Nest site availability	0.68 ± 0.85	0.7	0.41
Relatedness of competitors	0.08 ± 0.9	0.01	0.93

Table S4: Whether a female weaned a litter (yes/no) in a given trial across four treatment groups, with independent manipulation of access to protected nest sites ('Nest site availability') and relatedness of older and younger females within the social group ('Relatedness of competitors'). Estimates are shown on a logit scale and as differences to the reference levels 'Single nest sites' for factor 'Nest site availability' and 'Related' for factor 'Relatedness of competitors'. X^2-values and p-values refer to comparisons of models with or without the factor of interest. N=48 females in 20 trials.

Supplementary table S5

Factors	Estimate ± SE	X^2-value	*P*-value
Intercept	0.2 ± 2.25	-	-
Nest site availability	0.77 ± 1.39	0.32	0.57
Relatedness of competitors	1.74 ± 1.87	1.14	0.29

Table S5: Whether a communal nest was formed (yes/no) in a given trial across four treatment groups, with independent manipulation of access to protected nest sites ('Nest site availability') and relatedness of older and younger females within the social group ('Relatedness of competitors'). Estimates are shown on a logit scale and as differences to the reference levels 'Single nest sites' for factor 'Nest site availability' and 'Related' for factor 'Relatedness of competitors'. X^2-values and p-values refer to comparisons of models with or without the factor of interest. N=24 sister pairs.

Supplementary table S6

Factors	Estimate ± SE	Num. D.F.	Den. D.F	F-value	*P*-value
a) Index of activity during the dark phase					
Intercept	7.79 ± 2.31	-	-	-	-
Nest site availability	-0.21± 0.16	1	14.87	1.78	0.2
Relatedness of competitors	-0.02 ± 0.15	1	14.33	0.02	0.89
Time spent in nest box	0.02 ± 0.18	1	23.68	0.01	0.9
b) Number of scent marks					
Intercept	1.96 ± 0.24	-	-	-	-
Nest site availability	-0.28 ± 0.26	1	13	1.18	0.3
Relatedness of competitors	0.02 ± 0.26	1	13	0.01	0.94
Stimulus (control, unrelated, or related)	-	2	62	2.36	0.1
Related stimulus	0.19 ± 0.15	-	-	-	-
Unrelated stimulus	0.33 ± 0.15	-	-	-	-

Table S6: Testing if behavioural responses of female house mice differ according to their local competitive environment. Subjects were from four treatment groups, with independent manipulation of access to protected nest sites ('Nest site availability') and relatedness to younger females within the social group ('Relatedness of competitors'). (a) An activity index of females was measured using automated transponder readers from post-natal day (PND) 0 to PND 14 during the dark phase. (b) To test the response of subjects to simulated territorial intrusion, each was presented with artificial scent marks on three occasions. In a control test, artificial scent marks consisting only of distilled water were presented. In simulated intruder tests, artificial scent marks were presented consisting of urine from an unfamiliar related or unrelated female, respectively (for more details see Methods). Subject responses to these artificial scent marks were analysed for total number of scent marks deposited. Estimates are shown on a log scale and as differences to the reference levels 'Single nest site' for the factor 'Nest site availability', 'Related' for the factor 'Relatedness of competitors' and 'Control' for the factor 'Stimulus'. F-values and p-values refer to comparisons of models with or without the factor of interest. To obtain normally distributed residuals the dependent variable in both models was log transformed. (a) N=36 females in 18 trials. (b) N=64 observations on 32 females in 16 trials. P values <0.05 are highlighted in bold. See Table 2 for analysis of activity during the light phase and area scent marked.

Supplementary table S7

Contrast	Estimate ± SE	t-value	*P*-value
Intercept	1.85 ± 0.22	-8.36	**<0.01**
(a) Single nest site versus multiple nest sites present			
(SN-C, SN-RE, SN-UR) vs. (MN-C, MN-RE, MN-UR)	-0.56 ± 0.22	-2.56	**0.02**
(b) Control versus stimulus urine presentation (single nest site treatment only)			
SN-C vs. (SN-RE, SN-UR)	0.35 ± 0.1	3.47	**<0.01**
(c) Related urine versus unrelated urine presentation (single nest site treatment only)			
SN-RE vs. SN-UR	0.13 ± 0.17	0.76	0.45
(d) Control versus stimulus urine presentation (multiple nest site treatment only)			
MN-C vs. (MN-RE, MN-UR)	-0.01 ± 0.1	-0.13	0.89
(e) Related urine versus unrelated urine presentation (multiple nest site treatment only)			
MN-RE vs. MN-UR	0.04 ± 0.17	0.22	0.83

Table S7: The total area scent marked by subjects during control and stimulus presentations from related or unrelated stimulus females. Orthogonal contrasts are presented which were performed after confirming that the interaction between nest site availability and stimulus presentation was significant in Table 2b. Intercept estimate represents the grand mean of all treatments. First, we set the contrast of the model to compare the mean of all single protected nest site treatments with the mean of all multiple protected nest site treatments [(SN-C, SN-RE, SN-UR) vs. (MN-C, MN-RE, MN-UR)]. Second, we compared control presentations with the mean of both stimulus presentations for the single nest site treatment only [SN-C vs. (SN-RE, SN-UR)]. Third, we compared stimulus presentations from related females with those from unrelated females for the single nest site treatment only [SN-RE vs. SN-UR]. Fourth, we compared control presentations with the mean of both stimulus presentations for the multiple nest sites treatment only [MN-C vs. (MN-RE, MN-UR)]. Fifth, we compared stimulus presentations from related females with those from unrelated females for the multiple nest sites treatment only [SN-RE vs. SN-UR]. Note that mean values of treatments presented in round brackets were used in the comparisons. Orthogonal comparisons of treatments (a-e) are displayed as: SN-C = single nest site – control presentations; SN-RE = single nest site – stimulus presentations with urine from related females; SN-UR = single nest site – stimulus presentation with urine from unrelated females; MN-C = multiple nest sites – control presentation; MN-RE = multiple nest sites – stimulus presentations with urine from related females; MN-UR = multiple nest sites – stimulus presentations with urine from unrelated females. The direction of comparison within a contrast is left to right and the estimate value always refers to the treatment(s) to the right. If treatments are combined in parentheses, mean values of these treatments are used in the comparisons. N=64 observations on 32 females in 16 trials; P-values < 0.05 are highlighted in bold. Further details on the statistical analysis are provided in the Methods section.

Supplementary table S8

Factors	Estimate ± SE	X^2-value	*P*-value
Intercept	2.2 ± 0.61	-	-
Relatedness of competitors	-1.61 ± 0.73	5.72	**0.02**

Table S8: Testing if associations between older and younger sister pairs during the pre-reproductive phase differ according to the relatedness of competitors in treatments where multiple safe nest sites were available. We modelled the association index between older and younger females as the ratio between the number of occasions when at least one of the older females shared a nest with at least one of the younger females divided by the total number of observations (on average 5.8 observations per trial [range: 3-11]). Estimates are presented on a logit scale and as difference to the reference level 'Related' for factor 'Relatedness of competitors'. X^2-values and p-values refer to comparisons of models with or without the factor of interest. N=10 observations in 10 trials.

Supplementary table S9

Factors	Estimate ± SE	Num. D.F.	Den. D.F	F-value	*P*-value
Intercept	5.1 ± 0.12	-	-	-	-
Relatedness of competitors	0.1 ± 0.18	1	9	0.29	0.6

Table S9: Testing for an effect of the relatedness of competitors on the total time that communal breeding females spent in the nest with offspring. Communal breeding females did not vary the amount spent with pups depending on the relatedness of competitors. Estimates are shown on a log scale and as difference to the reference level 'Related' for factor 'Relatedness of competitors'. To obtain normally distributed residuals the dependent variable was log transformed. F-values and p-values refer to comparisons of models with or without the factor of interest. N=22 observations in 11 communal nests.

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