Social female preference in wild house mice

*(Mus musculus musculus)*

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<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
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<td>ANOVA</td>
<td>Variance analysis</td>
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<td>cm</td>
<td>Centimeter</td>
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<td>DA</td>
<td>Differential allocation</td>
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<td>GEE</td>
<td>Generalized estimating equations</td>
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<td>GLM</td>
<td>Generalized linear model</td>
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<td>MHC</td>
<td>Major histocompatibility complex</td>
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<td>NP</td>
<td>Non-Preference</td>
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<td>P</td>
<td>Preference</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>RC</td>
<td>Reproductive compensation</td>
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<td>SPSS</td>
<td>Statistical Package of the Social Sciences</td>
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1. Introduction

Many potential advantages of female choice are well-known: direct benefits such as paternal care and indirect, genetic benefits, such as good genes for the offspring (Jennions & Petrie 2000; Zeh & Zeh, 1997). Studies on experimental sexual selection were conducted to provide evidence that females show mating preferences which can result into benefits for the offspring (Anderson et al. 2007; Bluhm & Gowaty 2004a; Clutton-Brock & McAuliffe 2009; Jennions & Petrie 2000; Landry et al. 2001; Sandvik et al. 2000; Yasui 1998). In many of these studies mate preference assays were conducted, where the focal animal is paired with either its preferred (P) or a non-preferred (NP) mate and the following reproductive success and offspring performance are compared (see Hettyey et al. 2010). Studies have demonstrated that females are able to manipulate their investment into offspring according to the mating with either P or NP males (Gowaty et al. 2007; Harris & Uller 2009). However, there exists controversial hypothesis concerning the differential investment in response to mate quality.

Females can allocate resources into offspring, depending upon the quality of their mate (Harris & Uller 2009); however, two hypotheses provide opposite predictions about what to expect for maternal allocation: 1) differential allocation (DA) with a higher investment into more offspring produced with high-quality mates or P males (Burley 1986, 1988; Sheldon 2000) and 2) reproductive compensation (RC) which implies that females invest more into offspring produced with low-quality males or NP mates (e.g. when forced to mate) to counteract the negative effects on offspring fitness (Gowaty et al. 2003, 2007; Bluhm & Gowaty 2004b; Gowaty 2008). In the mallard (Anas platyrhyynchos) for example egg mass was heavier when mothers were paired with NP males compared to those paired with P males (Bluhm & Gowaty 2004a). Evidence for DA has been shown in Drosophila pseudoobscura, where offspring had greater viability when sired by P males compared to those of NP males (Anderson et al. 2007). Another study in mice showed that females mated with P males had a higher fitness and offspring were of higher viability compared to females mated to NP males, however partner preference had no significant influence on mean litter size (Drickamer 2000). This is surprising, as predicted by DA or RC hypothesis there should be also a higher investment into number of offspring. Therefore it is necessary to revisit the fitness
consequences of female mating preferences and taking the potential of female allocation strategies into account.

Female investment into sons and daughters may depend upon the quality of their mating partner. Trivers & Willard (1973) suggested that mothers should produce more sons or daughters depending upon their own condition or available resources (producing more sons only when they are in good condition and can afford the risks of producing male offspring), though the evidence for this hypothesis is mixed (Festa-Bianchet 1996; Cameron 2004; Sheldon & West 2004). For example in wild mice (*Mus musculus domesticus* and *Mus musculus musculus*) maternal condition and litter size had no detectable effect on sex ratios (Krackow 1997). Wright et al. (1988) on the other hand demonstrated in lab mice (CD1; *Mus domesticus*) that nutrition has an effect on sex ratio, as food deprived females produced female-biased litters compared to females with moderate food availability. However, it has not yet been tested whether female’s allocation into a specific sex is influenced upon partner preference.

The aim of this diploma thesis was to examine whether an experimentally assignment of the female with the P or NP males in wild derived house mice (*Mus musculus musculus*) has an influence on maternal allocation and either correspond to the DA or RC hypothesis. Furthermore, I tested whether sex ratio was affected by partner preference. Therefore I investigated whether 1) females that were paired with P males had a greater reproductive success (e.g. litter size, mean pup weight and sex ratio) compared to NP males assignments, and whether 2) P male pairings resulted into a higher male biased sex than NP male pairings.
2. Materials and Methods

2.1 Animal housing

The mice used in this experiment were first generation descendants of wild trapped house mice *Mus musculus* in Vienna Forest (48° 12’ 38” N; 16° 16’ 54” E). The parental mice were trapped with live traps at 14 different locations within a 500 m radius. The resulting F1 generation mice were weaned after 21 days and were housed individually in type II cages (26.5×20.5×18 cm). All cages contained bedding, nesting material (Abedd) and a nest house for environmental enrichment, additionally food (Altromin, Germany) and water were provided *ad libitum*. Cages were kept under a 12:12 h light condition: dark cycle (lights on at 7:00 a.m.) with a room temperature ranging from 20 to 25 C°.

2.2 Study design

To test partner preference females (N=30) were allowed to choose freely between two unrelated males (N=60). All females were experienced in terms of mating and had given birth to one litter prior to this experiment to avoid any confounding factors due to female experience. Males were sexually mature (16.52 ± 0.35, mean ± SE), however had no mating experience. On the day of the experiment all mice were weighed. Five sets were tested per day (each: one female, two males). The sets were brought to the testing room at 12:00 to allow the animals to acclimate for six hours. Male mice were stimulated with 5 µl of pooled female’s urine (collected and mixed from seven females over five consecutive days), which was placed on filter paper in the male’s cage when brought to the test room. The experiment started in the dark phase (under red light conditions) and was conducted from 18:00 to 22:00. All trials were recorded with a D-link camera (DCS-3710 Day & Night WDR network camera) sensitive to red light. To avoid any observer bias all experiments were simultaneously observed outside the experimental room on a monitor connected to the video camera system. Later, I analyzed the recorded videos with the program Noldus Observer XD 9.0 to verify
1) the female’s partner preference (P or NP male) and consequently 2) the following decision after the 30 minutes on the experimental on assignments of female and male, which were based on direct observations.

The experimental apparatus consisted of three cages (Fig. 1a, b): two ‘male’ cages (left and right) (36.5 x 20.5 x 14 cm) each divided into two compartments (i,ii) using an acrylic glass with holes to allow odor and sniffing exchange between sexes during the trials, however preventing a mating event. The middle ‘female’ cage (26.5 x 20.5 x 14 cm) was connected with the two male cages using plastic tubes (Fig. 1a). First, the males were individually placed into their compartments (i), immediately afterwards bedding material from the male’s original cage was scattered in the other compartment (ii) of the ‘male’ cages to enable odor cues for the female (Fig. 1a). Afterwards the female was placed in the middle cage. The connecting tubes were closed with regular inflated balloons to prevent the female from 1) entering the male cages before five minutes of habituation ended and 2) obtaining odor cues from the male’s bedding material. After the five minutes of acclimatization, the recording began and the balloons were simultaneously deflated to make the tubes (and cages) accessible for the female. The female was considered to have entered or left a cage when her nose was visible in the cage. The experiment started once the female had visited both male cages. This procedure ensured that the female was aware of both males. Each trial was 30 minutes long and divided in ten minute units. The time the female had spent in each male cage was recorded every ten minutes using a stopwatch. For this study only the first ten minutes were considered, as this time unit is a good predictor for partner preference (Drickamer et al. 2000). The male where the female spend 60% or more of her time was defined as her preferred male (following Drickamer et al. 2000). After having identified the females’ partner preference half of the females were randomly paired either with their preferred (P) or non-preferred (NP) males in a new cage. All animals were returned to their housing room. After five days the male and female were separated. As soon as the females gave birth, the litter size, individual pup weight, and sex ratio were recorded at weaning.
Fig. 1. Experimental set-up. a) The middle female cage (A) was connected with the two “male compartments” (B1, B2) by connecting tubes which were closed in the beginning. After five minutes of habituation the barriers were removed (C) to enable the female (A) to access the two male compartments. Two perforated plastic dividers (d1, d2) (one per “male compartment”) allowed odor cue exchange, as well as acoustic and visual communication between female and male, however preventing a mating event. Each male could only stay in their area (i) of the compartment and the female mouse had free access to the male’s compartment until the separation (ii). b) Experimental set up by daylight Picture: © Vetmeduni Vienna/Wassermann
2.3 Statistical analyses

Statistical analyses were performed using IBM SPSS® version 19 (SPSS Inc., Chicago, Illinois) software. The comparison between litters produced when paired with P and NP male after the trial was analyzed using a $\chi^2$ test. The litter size was analyzed using an ANOVA analysis with pairing (with P or NP male, categorical effect) as a fixed factor and female body mass (continuous effect) as a covariate. As a further measurement mean pup weight (offspring quality) for individual reproduction success, was calculated as the total weight of litter/litter size at weaning. This dependant variable was analyzed using a one-way ANCOVA for all 30 females. Pairing (with P or NP male, categorical effect) was entered into the model as a fixed factor and female body mass (continuous effect) as a covariate. In addition sex ratio was measured per female as male proportion at weaning (number of male pups within a litter divided by total litter size). Sex ratio was analyzed using a Generalized linear model (GLM) (Binomial; logit function) with pairing (with P or NP male, categorical effect) as a fixed factor (categorical variable) and female body mass (continuous effect) as a covariate.

All two-way interactions were included into initial models and a backward stepwise removal procedure was applied (Grafen & Hails 2002) to avoid problems because of the inclusion of non-significant term (Engqvist 2005). Removed variables were re-entered one by one to the final model to obtain relevant statistics.
3. Results

There was a trend but females (N=15) mated with P males were not significantly more likely to produce a litter (14 litters from 15 pairings or 93% success) compared to females (N=15) mated with NP males (11 litters from 15 pairings or 73 %) ($\chi^2=2.088$, df=1, $p=0.165$). Yet, overall, litter size was increased when females were paired with P males ($F_{1, 12} = 5.545$, $p = 0.036$, Fig. 2) and heavier females produced more pups ($F_{1, 12} = 5.487$, $p = 0.037$, Fig.3), whereas the interaction was not significant ($F_{1, 11} = 0.909$, $p =0.656$). Furthermore, mean pup weight (litter weight/litter size) was not affected by pairing with P or NP male ($F_{1, 11} = 0.301$, $p = 0.594$; female weight: $F_{1, 11} = 0.161$, $p = 0.696$), and the remaining two-way interaction remained non-significant ($F_{1, 11} =0.230$, $p= 0.641$).

Sex ratio (male within a litter/litter size) at weaning was significantly affected by the main effects of pairing with P or NP males and female weight: pairing with the NP-male resulted in more daughters compared to P-pairings (Fig.4) and heavier females produced more sons compared to lighter females (Generalized linear model, pairing with P or NP male: Wald $\chi^2=4.889$, df=1, $p=0.027$, female weight: Wald $\chi^2=10.964$, df=1, $p=0.001$). The remaining interaction pairing with P or NP male*female weight had no effect on sex ratio (Wald $\chi^2=1.542$, df= 1, $p= 0.214$).
Fig. 2. Females that were paired with preferred (P) males produced a larger litter size compared to females assigned to non-preferred (NP) males.
Fig. 3. Litter size was significantly associated with female body mass. Heavy females produced more pups compared to light females.
Fig. 4. Females that were paired with NP males produced more daughters compared to P assigned pairings.
4. Discussion

The findings of this study support the DA hypothesis, as assignments with P male resulted into 1) a greater litter size and 2) in NP-pairings more daughters were produced compared to P pairings. Furthermore heavy females had an increased litter size, whereas mean pup weight was not affected by partner preference and female weight. Several studies have demonstrated a positive correlation between female reproductive investment and indicators of male quality (e.g. Petrie & Williams 1993; Wedell 1996; Reyer et al. 1999; Kolm 2001; Gowaty et al. 2003; Olsson et al. 2005; Head et al. 2006), however, other studies have found consistency with RC (e.g. Bluhm & Gowaty 2004a; Gowaty et al. 2007), whereas, others have produced contradictionary or negative results (e.g. Oksanen et al. 1999; Mazuc et al. 2003; Rutstein et al. 2004; Galeotti et al. 2006; Nakagawa et al. 2007). Offspring sired by P males can obtain certain advantages, for example it has been shown in mice that they were better nest builder and had an increased survival (Drickamer et al., 2000). Additionally, adult sons sired by P males were more dominant compared to sons from NP assignments (Drickamer et al., 2000), which may represent an ideal situation to enhance a reproductive success for these young, as females mate preferentially with the dominant (territorial) males (Bronson 1979; 1989). In this study, I found that females paired with P males not only produced more offspring, but also had more sons when paired with the P male (quality) compared to NP pairings. This combination may lead to higher overall fitness.

In many taxa it has been shown that females in better body condition produce more offspring (Reznick 1985; Smith & Fretwell, 1974), especially in mammals where lactation is a very costly physiological process (Speakman 2008). My findings support this, as heavier females produced more offspring. Surprisingly, mean pup weight was not affected by female body mass or partner preference. Weight at weaning is known to be an indicator for survival in offspring and therefore it is considered as a quality trait (e.g. Hall et al. 2001). Thus it seems that females face a trade-off between numbers vs. quality (e.g. weight) of produced offspring. Moreover, heavier females produced more sons, which is in accordance with the Trivers and Willard hypothesis (1973). The Trivers-Willard hypothesis (Trivers & Willard 1973) predicts greater investment in males by parents in good conditions and greater investment in females
by parents in poor conditions. Sex ratio may be affected by ecological physiological factors, such as age, dominance, nutrition, social stress or body condition (Cameron et al. 2008; Rosenfeld & Roberts 2004). For example in red deer (Cervus elaphus) high ranking females gave birth to more sons as females (Hewison & Gaillard 1999). Older and more experienced females in Antarctic fur seals (Arctocephalus gazella) were greater than that of younger, inexperienced animals (Lunn et al. 1994). Meikle and Drickamer (1986) demonstrated that female house mice that are deprived of food gave birth to fewer male young. However, another study have found no effect of food deprivation on sex ratio in outbred house mice (Mus musculus) but argue that deprivation had worse affects on male offspring more than on daughters (Meikle & Westberg 2001). My findings as expected show that females in good condition invested into males (the costly sex), however, additionally I found that also partner preference (see above) play an important role and affected sex ratio in mice.

Despite following a standard protocol to measure partner preference, more studies are needed to test whether other parameters beside time spent in the male’s cage play a role in female decision making (partner and allocation). For example it would be important to investigate whether the major histocompatibility complex (MHC), which has been shown to influence partner preference (females prefer to mate with males carrying dissimilar MHC genes) (e.g. Penn, 2002; Yamazaki et al. 1988) has an impact on female’s investment regarding reproductive success. Furthermore, it has been shown that also male partner preference play a major role, as males that were paired with the P female sired more litters, viable offspring and dominant sons compared to NP female pairings (Gowaty et al. 2003). Therefore it may be important to consider male behavior and allocation in such experimental sexual selection studies. For example, sexual mature males display ultrasonic vocalization (USV) in mice to courtship (Nyby, 1983; Musolf et al., 2010). In this study no USV was recorded, however, it would be significant to know whether P males are the better “singers” and thus have a higher courtship investment and quality than NP males. Combining the findings of this study with such additional experiments would be a next step to identify more partner preference criteria for females and males and to examine its implication on offspring or reproductive success.
5. Summary

Numerous studies have measured mating preferences and its consequences in many taxa and have shown that mate choice may allow females to obtain indirect, genetic benefits for their offspring. However, there are surprisingly few direct tests of individual variation in mating preferences and female reproductive investment decisions. In this diploma thesis I investigated whether females invested differently into their offspring when paired with the preferred or non-preferred male in wild derived house mice (*Mus musculus musculus*). I tested two allocation hypotheses 1) differential allocation (DA, higher investment into reproductive success when paired with preferred males) vs. 2) reproductive compensation (RC, greater production when being assigned with the non-preferred male). Furthermore, I investigated whether sex ratio was affected by partner preference.

This study shows that litter size was increased with larger females and when they were paired with P males, which support the DA hypothesis. However, females mated with P males produced not more litters (93.3%) than females mated with NP males (73%). Additionally, mean pup weight per litter (quality) was neither affected by partner preference nor body mass of the female. Furthermore, I found that heavier females produced more sons, as predicted by the Trivers-Willard hypothesis; furthermore sex ratio was female biased when females were paired with their NP males.

This diploma thesis gave insight to female’s preference and the impact of its choice. The study confirms that partner preference has an implication on female allocation strategies and that body condition plays a major role when it comes to reproductive allocation of number of offspring and sex ratio within a litter.
Literature


FESTA-BIANCHET, M. (1996): Offspring sex ratio studies of mammals: Does publication depend upon the quality of the research or the direction of the results? Ecoscience, **3**, 42-44.


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