THE EFFECT OF RESOURCE QUALITY ON PARTNER COMPENSATION IN A BIPARENTAL SPECIES

by

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TABLE OF CONTENTS

LIST OF TABLES
LIST OF FIGURES
ABSTRACT
CHAPTER 1. INTRODUCTION
CHAPTER 2. MATERIALS AND METHODS 16
2.1 Source of Beetle Population
2.2 Experimental Design
2.3 Lysozyme-Like Activity (LLA) Testing
2.4 Phenoloxidase (PO) Testing
2.5 Statistical Analysis
CHAPTER 3. RESULTS
3.1 Changes in Maternal Investment
3.2 Changes in Paternal Investment
CHAPTER 4. DISCUSSION
REFERENCES

LIST OF TABLES

LIST OF FIGURES

Figure 2. Least-squares means (+/- 95% confidence interval) for female oral LLA differences (panel A), anal LLA differences (panel B), oral PO differences (panel C), and anal PO differences (panel D) across varied carcass type. Control treatment group is white and experimental treatment group is grey. 23

Figure 3. Least-squares means (+/- 95% confidence interval) for male oral LLA differences (panel A), anal LLA differences (panel B), oral PO differences (panel C), and anal PO differences (panel D) across carcass type. Control treatment group is white and experimental treatment group is grey.

ABSTRACT

Sexual conflict arises in biparental species because of the fitness payoffs of shifting the costs of care onto their partner. The negotiation model asserts that parents actively monitor their partner's level of investment and adjust their own in response generally resulting in partial compensation when a member of the pair reduces their level of investment. The willingness of one parent to compensate for the other's change is found to be widely variable. Habitat or resource quality available to pairs may explain such variation. It is predicted that the level of compensation by one partner will increase with decreasing resource quality. I tested this prediction with the biparental burying beetle, Nicrophorus orbicollis. Under natural conditions, burying beetles apply costly social immune molecules to carcasses of small vertebrates to preserve nutritional value for young. The goals of this research were (1) to determine if males immunologically compensate when females are immunologically handicapped; and (2) determine the impact of resource quality on compensation. Changes in lysozyme-like-activity (LLA) and phenoloxidase (PO) production in oral and anal secretions were used to quantify social immune investment. Pairs were provided a mouse carcass of one of three qualities: freshly thawed, aged for 3 days, or aged for 7 days. As expected, female LLA decreased once injected with lipopolysaccharide (LPS) irrespective of carcass quality. Injections caused significant down regulation of oral PO in females. Male LLA increased as the quality of carcasses decreased when paired with handicapped females. Males showed no changes in PO across treatments or carcass types. My results demonstrate that males compensate for a change in maternal investment and the level of compensation increases as resource quality decreases.

CHAPTER 1. INTRODUCTION

Conflict arises between parents in species that provide biparental care over the level of investment each parent provides during reproduction. This conflict occurs because the fitness each parent gains from a reproductive event is dependent upon the combined effort of the pair, but the costs from providing care are accrued independently by each (Trivers, 1996; Parker, 2006; Lessells, 2012). Thus, there is an incentive for individuals to shift reproductive costs of the current brood to their partner in order to increase its own lifetime reproductive success. (Johnstone and Hinde, 2006).

Game theory provides a powerful approach to evaluate the evolutionary outcome of conflict over the level of parental care (Lessells and McNamara, 2011). One of the first models to address this question is the sealed bid model (Houston and Davies, 1985). This model asserts that the level of effort each sex provides is determined independently over an evolutionary time scale (Houston and Davies, 1985). This early model predicts that if a parent is experimentally manipulated to either increase or decrease its level of investment, no change in its partner's level of investment is expected (Houston and Davies, 1985). A second model, the negotiation model, evaluates short-term interactions between pairs. The negotiation model asserts that parents actively monitor their partner's level of investment and adjust their own levels in response (McNamara et al., 1999). If one parent changes its level of investment, this model predicts that the partner would partially compensate for the change by adjusting their level of investment. This prediction is supported by a large number of empirical tests across a number of animal taxa (Hinde, 2005; Johnstone and Hinde, 2006; Akçay and Roughgarden, 2009; Harrison et al., 2009).

Tests of the negotiation model have taken two general approaches. The first is parent removal studies in which a member of a breeding pair is removed and a response is measured in the other. Parent removal studies simulate if no effort is provided by a mate (i.e. parent disappeared from disease, predation or abandonment). Lendvai and Chastel (2008) demonstrated in a parent removal experiment in house sparrows (*Passer domesticus*) that mate-removed females exhibited higher levels of stress hormones as well as increased the number of food deliveries to young than biparental controls. These results showed only partial compensation as mate-removed mothers reared smaller, less competitive chicks in comparison to control young (Lendvai and Chastel, 2008). Partial compensation has been generally observed in other parent removal studies (Hunt and Simmons 2002; Lendvai et al., 2009; Harrison et al., 2009; Bulla et al., 2019). Although parent removal studies have been used to test the negotiation model, it may not be the best approach as real-time negotiations are not possible when a member of the pair is removed.

The second approach to test the negotiation model has been to manipulate (decrease or increase) the level of investment of one parent and observe the response of its partner. Taking this approach, Stoehr and Hill (2000) found that female house finches (*Haemorhous mexicanus*) had significantly higher previsioning rates than control pairs when paired with a male that was manipulated to decrease parental investment. The results of empirical tests of the negotiation model have generally supported the prediction that parents respond to changes in their partner's effort but the magnitude and direction of partner compensation has been highly variable (Hinde, 2005; Johnstone and Hinde, 2006; Akçay and Roughgarden, 2009; Harrison et al., 2009).

Studies where a parent is manipulated to decrease their levels of parental investment found results ranging from full compensation (Sanz et al., 2000; Stoehr and Hill, 2000; Wiebe, 2010) to partial compensation (Schwagmeyer et al., 2005; Lendvai et al., 2009; Creighton et al., 2015) to no compensation (Mazuc et al., 2003; Suzuki and Nagano, 2009; Wiebe, 2010). Studies where a parent is manipulated to increase their level of parental investment found also varying results, with

partners acting as predicted, by reducing their level of effort (Kosztolányi et al., 2009) and by matching the increase in effort by the manipulated parent which is contradictory to predications (Hinde, 2006).

Some variability could simply be from differences in methodology and/or model system studied (Houston et al., 2005; Harrison et al., 2009). Experiments have been conducted on a variety of model systems including mice (*Peromyscus californicus*) (Cantoni and Brown, 1997; Gubernick and Teferi, 2000), fish (*Heros nigrofasciatus*) (Raadik et al., 1990) (*Archocentrus nigrofasciatus*) (Itzkowitz et al., 2001), and invertebrates (*Ips pini*) (Robertson, 1998; Smiseth et al., 2005) (*Nicrophorus vespilloides*) (Jenkins et al., 2000) (*Onthophagus taurus*) (Hunt and Simmons, 2002) (*Nicrophorus orbicollis*) (Rauter and Moore, 2004) (*Nicrophorus quadripunctatus*) (Suzuki and Nagano, 2009). However, an overwhelming majority of these studies use socially monogamous birds (54 experiments in Harrison et al., 2009 meta-analysis). Differences in life history strategies across taxa would be expected to yield varying results when testing models, such as the negotiation model.

Methods of manipulation studies also vary widely. Manipulation methods can be used to either decrease or increase parental investment of the manipulated individual. Handicap experiments are a common practice to decrease care as it makes providing care more energetically demanding via added weights or plumage clippings (Wright and Cuthill, 1990; Sanz et al., 2000; Griggio et al., 2005; Paredes et al., 2005; Suzuki and Nagano, 2009; Wiebe, 2010; Creighton et al., 2015). Males in avian systems have been hormonally manipulated to reduce parental care through testosterone implants (Ketterson et al., 1992; Stoehr and Hill, 2000; Van Roo, 2004; Schwagmeyer et al., 2008), increased availability of nesting sites (Smith, 1995), and changes in sexual signaling plumage (Qvarnstrom, 1997; Nakagawa et al., 2007). Parental investment has been increased through brood-size manipulations (Wright and Cuthill, 1990; Sanz, 1997; Rauter and Moore 2004; Balden et al., 2019) and playback experiments where extra chick begging calls were played (Hinde, 2006, McDonald et al., 2009). However, even within the same species and identical methodology, variation in compensation exists across latitudinal (Lendvai et al., 2018) and altitudinal gradients (Lejune et al., 2019). Partners may be more willing to compensate for their mates at different latitudes and altitudes due to differences in habitat quality associated with those differing latitudes and altitudes.

Habitat or resource quality directly impacts parental fitness (Bernstein et al., 1991, Pulliam, 2000), and parental care strategies are predicted to change as available reproductive resources change (Davis et al., 1999). Empirical studies support this prediction as substantial plasticity in parental care behaviors has been found in a number of different taxa when resources vary (Creighton, 2005; Creighton et al., 2009; Paul et al., 2015; Smith et al., 2015; Barbasch et al., 2020). Assuming the relationship between habitat or resource quality and fitness accrued is sigmoidal, the relative rate of change in fitness depends on the quality of the habitat or resource (hereafter referred to as resource quality). Small changes in high quality resources yield a smaller fitness payoff than intermediate quality resources. Further, if changing the level of parental investment alters the quality of the resource, changes in investment in intermediate quality habitats would have a greater fitness payoff (see Figure 1). In the context of partner compensation, higher levels of partner compensation are predicted with intermediate quality resources than high quality resources because of the higher fitness payoff. In this study, I tested this prediction by experimentally manipulating both resource quality and level of parental investment in the burying beetle, Nicrophorus orbicollis.



Figure 1. Change in fitness across a range of resource quality. Assuming an increase in parental investment increases resource quality, scenario A represents the increase in fitness on a high quality resource as a result of increased parental investment. Scenario B represents the increase in fitness on an intermediate quality resource as a result of same level of increased parental investment. Relative amount of fitness gained for the same level of increased investment is significantly greater. For biparental species, an increase in the level of compensation results in a higher fitness payoff when breeding under an intermediate level of resource quality.

Burying beetles utilize small vertebrate carcasses as a reproductive resource to rear their young. Both parents provide extensive parental care both before and after the young hatch. The parents aid in pre-larvae hatching care by removing all feathers or fur and rolling the carcass into a sphere while applying oral and anal secretions on it (Eggert and Müller, 1997; Hoback et al., 2004). These secretions have antimicrobial molecules and represent a form of social immunity (Cotter and Kilner, 2010). The secretions increase survivorship of young (Arce et al., 2012) and are costly for parents to produce (Cotter et al., 2010). The larvae provisioning stage begins when the larvae arrive on the carcass between five to seven days after the pair begin carcass preparation. The behaviors associated with post-hatching care includes guarding and maintaining the carcass

as well as providing partially digested regurgitations to the young (Fetherston et al., 1990; Rauter and Moore, 1999).

A number of studies have used burying beetles as a model system to test the negotiation model. These experimental studies have measured parental compensation in both the pre-hatching (Smiseth et al., 2006; Creighton et al., 2015) and post-hatching care periods (Fetherston et al., 1994; Rauter and Moore, 2004; Smiseth et al., 2006; Suzuki and Nagano, 2009). Both partner removal and manipulation studies have generally found that males compensate for a reduction in their partner's effort in both periods (Fetherston et al., 1994; Rauter and Moore, 2004; Smiseth et al., 2006; Cotter and Kilner, 2010; Creighton et al., 2015). Females, however, have been found to not compensate, largely because they are already working at nearly full capacity (Kosztolányi et al., 2009; Suzuki and Nagano, 2009; Creighton et al., 2015). Across burying beetle studies, there is broad similarity in qualitative results. However, it is not known how differences in resource quality affects the level of compensation.

In this study, I examine the effect of resource quality on male compensation during the prehatching period. Previous work has shown that during this period, males compensate behaviorally by increasing the proportion of time spent preparing the carcass when paired with a physically handicapped female (Creighton et al., 2015) and immunologically by increasing social immunity investment, through increased lytic concentration in anal secretions when females are removed (Cotter and Kilner, 2010). Specifically, I: (1) test the negotiation model by experimentally reducing female social immunity contributions and measuring the males' response. I reduce female social immunity by administrating a personal immune challenge to females, which reduces their level of social immune contribution to carcass preparation (Cotter et al., 2013; Reavey et al. 2014); and (2) determine the impact of resource quality on the level of male compensation. I simulated differences in resource quality by providing pairs with one of three different carcasses types. Pairs were provided either a high quality carcass (freshly thawed mouse), or an aged carcass (mouse aged for three days or seven days at room temperature). I predict that (1) males partnered with handicapped females should compensate by increase their level of social immunity investment; and (2) the level of male compensation should increase as carcass quality decreases.

CHAPTER 2. MATERIALS AND METHODS

2.1 Source of Beetle Population

Burying beetles used to generate a laboratory population for this experiment were captured May of 2019 and August 2020 in Big Falls, Wisconsin using pitfall traps baited with aged chicken. Wild-caught beetles were placed upon 20 g mouse carcasses within plastic containers (18 cm x 15 cm x 10 cm) two-thirds full with commercial topsoil. The laboratory population was housed individually in small plastic containers (6 cm x 6 cm x 5 cm), fed *ad libitum* raw chicken liver on a 14:10 h light/dark cycle.

2.2 Experimental Design

My experimental design consisted of mouse carcasses (30 g \pm 1 g) aged to create three levels of quality: carcasses that were recently thawed (high quality), carcasses aged at room temperature for three days (intermediate quality) and carcasses aged for seven days (low quality). Carcasses were aged by placing them singly on topsoil inside plastic containers (10 cm x 7 cm x 6 cm) in a fume hood at room temperature (~21°C). Aged carcasses have shown to reduce reproductive success and growth rates of larval young, and burying beetles actively choose fresh carcasses over aged ones (Rozen et al., 2008).

Unrelated virgin beetles (19 - 45 days old males and females) were randomly paired. Members of each pair had their mass and pronotum width measured and were then randomly assigned to one of the three carcass types. The pairs and corresponding carcass were then placed within plastic containers (18 cm x 15 cm x 10 cm) two-thirds full with commercial topsoil. After 72 hours (approximately halfway through the carcass preparation phase), initial oral and anal secretions were collected from each member of the pair to establish a base-line measurement. Pairs were eliminated from the experiment if there was no sign of carcass preparation. After initial secretions were collected, pairs were assigned randomly to a control (further notated as FC, 3C, and 7C) or to a manipulated treatment (further notated as FE, 3E, and 7E) across all three carcass types. Manipulated treatment females were immune challenged by injecting 5 µL lipopolysaccharide (LPS) solution (concentration: 1.0 mg/mL \times 1 μ L) via sterilized Hamilton syringe between their second and third sterna of the abdomen. A personal immune challenge of this type has been shown to reduce social immunity contributions (Cotter et al., 2013; Reavey et al. 2014). Prior to injection, females were placed on ice for five minutes to aid in administering the injection. All other beetles were also placed on ice to control for possible effects of icing on secretion production. By handicapping females immunologically, the potential issue of changing perceived mate quality through physically handicapping individuals is minimized (Griggio et al., 2005). Oral and anal secretion samples were collected again after a one-hour adjustment period. All samples collected were diluted to a 1:5 ratio of secretion to phosphate buffer solution (PBS) and stored in a -80°C freezer until analysis could be carried out. Of my 232 total trials, any brood that did not produce young (number from each treatment: FC 1, 3C 0, 7C 3, FE 4, 3E 11, 7E 6) or had high loads of nematodes (number from each treatment: FC 1, 3C 4, 7C 3, FE 2, 3E 3, 7E 8) were removed from my analysis.

Lysozyme-like-activity (LLA) and phenoloxidase (PO) levels were measured in oral and anal secretions to evaluate changes in social immunity investment. After receiving a personal immune challenge, burying beetles down-regulate lysozyme in oral and anal secretions, and PO is upregulated within the hemolymph (Cotter et al., 2013; Reavey et al., 2014). LLA is an indirect measure of lysozyme, which is an induced antimicrobial enzyme found in both oral and anal secretions. It plays an important role in carcass preservation (Acre et al., 2012; Palmer et al., 2016). PO is a constitutive immune enzyme that aids in encapsulation and melanization of foreign bodies, in wound healing and in bacterial defense (Hiruma and Riddiford 1988; Bidla et al., 2005; Haine et al., 2007; Eleftherianos and Revenis, 2011). PO is downregulated when beetles begin carcass preparation and returns to normal levels after breeding, suggesting a trade-off between personal immunity and social immunity (Steiger et al., 2011; Cotter et al., 2013; Reavey et al., 2014; Reavey et al., 2015). PO is arguably the best indicator for insect personal immunity due to its prevalence in both insect cellular immunity and hormonal immunity (Reavey et al., 2015). However, PO is found within secretions applied to the carcass, and Steiger et al. (2011) quantified PO present within secretions as a social immune contribution as PO also promotes the production of antimicrobial components, such as quinones (Gillespie et al., 1997).

2.3 Lysozyme-Like Activity (LLA) Testing

Lytic plates were made with agarose, lyophilized cells of *Micrococcus lysodeikticus* (Sigma-Aldrich M3770), *Streptomycin*, and Triton X-100. The 1.5 mm diameter wells were loaded with 1 μ L of sample and incubated at 27°C for 48 hours. As a control, 1 μ L of 1% lysozyme isolated from egg whites (Sigma-Aldrich L6876) was added to one well on each plate. Image J software (http://rsweb.nih.gov/ij/index.html) was used to measure the zone of clearance of each sample. The ratio of the sample to control was used in all measurements to standardize across all plates.

2.4 **Phenoloxidase (PO) Testing**

All oral and anal secretions collected from parents were tested for total PO. PO assays were run in a 96-welled flat bottom microplate using 2 μ L of sample with 100 μ L of a 1% by volume LPS solution (Sigma-Aldrich L6876) and 100 μ L of 5 mM L-Dopa (3,4-Dihydroxy-L-phenylalanine from Sigma Aldrich D9628). L-Dopa is a known substrate for both PO (Cotter et al., 2010) as well as tyrosinase (Sigma-Aldrich T3824). Tyrosinase was the positive control (Duckworth and Coleman, 1970). My negative control was 2 wells of L-Dopa on each plate lacking tryrosinase. A BioTek Synergy 2 microplate reader incubated the plates at 30°C, measuring the absorbance of light (wavelength: 490 nm) passed through the reaction every minute for 60 minutes. Total PO was quantified by the final absorbance reading measured at the end of the 60-minute cycle.

2.5 Statistical Analysis

I used Analysis of Covariance (ANCOVA) with a factorial design in a general linear model (Proc GLM in SAS; SAS 9.4; SAS Institute, Cary, NC, USA) to determine effects of carcass quality and immune challenge on immune response of females (immune challenge target) and their male partner. I measured immune response as LLA and PO from both oral and anal secretions in both females and males. This resulted in four immune responses measured for both females and males: oral LLA, anal LLA, oral PO, and anal PO. I measured these four immune responses before the immune challenge and after the immune challenge, so our response variables for the model are the difference between before and after measures for each of the immunity measures. I calculated these differences as post-immune challenge values minus pre-immune challenge values. A positive difference value indicates upregulation of LLA or PO and a negative value indicates downregulation. Main effects (predictors) were immune challenge (two levels: challenged or control) and carcass quality (three levels: 0, 3, 7 days), and the interaction between immune challenge and carcass quality. In addition, I included three covariates as predictors in the analysis to account for variation from inadvertent or procedural variation not associated with the main effects. These three covariates were size of the individual measured as pronotum width in mm, age of the individual since eclosion measured in days, and timing of the experiment measured as the

month when this replicate was started with one being the first month of experiments and 12 being the last month. I inspected plots of the distribution of residuals versus predicted values to assess whether the raw data met the assumptions of normality of residuals and equal variances across treatments. Raw data met these assumptions, so I did not transform the data for analysis. I tested for significant differences in immune response of males and females in main effects and in the interaction between main effects. To illustrate observed differences, I plotted least squares means and their 95% confidence intervals for each combination of the main effects. Differences in number of degrees of freedom for oral secretions in comparison to anal secretions can be attributed to the difficulty in collecting oral section samples. In addition, one female (both oral and anal PO) and one male (oral LLA) are missing in my analysis due to insufficient sample volume.

CHAPTER 3. RESULTS

3.1 Changes in Maternal Investment

Females changed their level of parental investment when presented with a personal immune challenge. Handicapped females significantly reduced LLA in both oral secretions (F = 14.06; df = 1/39; p = 0.001) and anal secretions (F = 11.12; df = 1/80; p = 0.001), while control females showed no significant changes in oral or anal LLA production (Table 1, Figure 2A & B). However, there was no effect of carcass quality nor was there a significant carcass quality by treatment interaction (Table 1).

The personal immune challenge also impacted PO production in female beetles. Handicapped females experienced a significant reduction in oral PO production (F = 4.13; df = 1/38; p = 0.049), although not significant in anal PO. In contrast, control females showed no significant changes in oral or anal PO production (Table 1, Figure 2C & D). There were no differences in PO production across carcasses qualities; nor were there a significant interaction effect between carcass quality and treatment (Table 1). Time had a significant effect on oral PO differences although slope was minimal ($\beta_2 = 0.013$).

Table 1. Analysis of covariance table for oral LLA, anal LLA, oral PO, and anal PO differences
for female beetles. Pronotum size, age of beetle, and month of trial (time) are all considered
covariates. Statistically significant observations (p<0.05) are highlighted bold.

Response Variable	Effect	Num df / Den df	F	р
Oral LLA Difference				
	Carcass Quality	2/39	0.96	0.3917
	Treatment	1/39	14.06	0.0006
	Quality*Treatment	2/39	0.41	0.6644
	Pronotum	1/39	0.08	0.7732
	Age	1/39	0.11	0.7468
	Time	1/39	0.02	0.8871
Anal LLA Difference				
	Carcass Quality	2/80	1.80	0.1726
	Treatment	1/80	11.12	0.0013
	Quality*Treatment	2/80	0.05	0.9523
	Pronotum	1/80	0.24	0.6268
	Age	1/80	0.68	0.4117
	Time	1/80	0.00	0.9608
Oral PO Difference				
	Carcass Quality	2/38	1.68	0.2004
	Treatment	1/38	4.13	0.0493
	Quality*Treatment	2/38	1.73	0.1902
	Pronotum	1/38	0.89	0.3501
	Age	1/38	0.27	0.6035
	Time	1/38	6.26	0.0168
Anal PO Difference				
	Carcass Quality	2/79	1.17	0.3146
	Treatment	1/79	2.38	0.1270
	Quality*Treatment	2/79	0.24	0.7851
	Pronotum	1/79	0.04	0.8433
	Age	1/79	0.58	0.4478
	Time	1/79	0.00	0.9656



Figure 2. Least-squares means (+/- 95% confidence interval) for female oral LLA differences (panel A), anal LLA differences (panel B), oral PO differences (panel C), and anal PO differences (panel D) across varied carcass type. Control treatment group is white and experimental treatment group is grey.

3.2 Changes in Paternal Investment

Males paired with handicapped females increased their level of parental investment by increasing their production of LLA in both oral secretions (F = 9.87; df = 1/25; p = 0.004) and anal secretions (F = 23.03; df = 1/96; p = <0.0001) (see Table 2 and Figure 3). Carcass quality did not have a significant effect on LLA production but there was a significant carcass quality-treatment interaction with increasing compensation with decreasing carcass quality in oral secretions (F = 4.84; df = 2/25; p = 0.018) and marginally significant interaction in anal secretions (F = 2.58; df = 2/96; p = 0.081). These interaction effects were primarily the result of increased compensation of males paired with handicapped females in the aged carcasses (oral LLA in the 7E group (p = 0.004) and anal LLA in both the 3E group (p = 0.029) and 7E group (p = 0.001)) (see Figure 3A & B). Control males showed no change in oral or anal LLA production across the varying carcass types. Pronotum size was found to have a significant effect on oral LLA production, with large beetles showing greater increase in production. Time also had a significant effect on anal LLA production among males, although the slope was minimal ($\beta_2 = 0.009$).

Neither carcass quality nor treatment influenced male oral or anal PO production. Nor was there a significant interaction between the two (Table 2). Control males also showed no change in oral or anal PO production across the varying carcass types (see Figure 3C & D).

Table 2. Analysis of covariance table for oral LLA, anal LLA, oral PO, and anal PO differences
(after manipulation – base line) for male beetles. Pronotum size, age of beetle, and time are all
considered covariates. Statistically significant observations (p<0.05) are highlighted bold.

Response Variable	Effect	Num df / Den df	F	р
Oral LLA Difference				
	Carcass Quality	2/25	1.33	0.2815
	Treatment	1/25	9.87	0.0043
	Quality*Treatment	2/25	4.84	0.0167
	Pronotum	1/25	7.23	0.0126
	Age	1/25	1.55	0.2242
	Time	1/25	4.10	0.0537
Anal LLA Difference				
	Carcass Quality	2/96	0.95	0.3896
	Treatment	1/96	23.03	<0.0001
	Quality*Treatment	2/96	2.58	0.0807
	Pronotum	1/96	0.04	0.8380
	Age	1/96	1.05	0.3081
	Time	1/96	20.55	<0.0001
Oral PO Difference				
	Carcass Quality	2/26	0.76	0.4786
	Treatment	1/26	0.06	0.8119
	Quality*Treatment	2/26	2.22	0.1290
	Pronotum	1/26	1.19	0.2859
	Age	1/26	0.20	0.6552
	Time	1/26	0.28	0.5994
Anal PO Difference				
	Carcass Quality	2/96	1.1	0.3321
	Treatment	1/96	1.26	0.2636
	Quality*Treatment	2/96	1.50	0.2285
	Pronotum	1/96	3.59	0.0612
	Age	1/96	3.19	0.0774
	Time	1/96	0.07	0.7876



Figure 3. Least-squares means (+/- 95% confidence interval) for male oral LLA differences (panel A), anal LLA differences (panel B), oral PO differences (panel C), and anal PO differences (panel D) across carcass type. Control treatment group is white and experimental treatment group is grey.

CHAPTER 4. DISCUSSION

I found that challenging the female immune system reduced their level of parental investment by lowering LLA levels in their oral and anal exudates. Therefore, the manipulation produced a significant difference between groups. This is consistent with Cotter et al. (2013) and Reavey et al. (2014) who found personal immune challenges reduce social immunity contributions in Nicrophorus vespilloides. Importantly, the absence of a significant carcass quality or carcass quality by treatment interaction effect demonstrates that the reduction in social immunity by females was due only to the personal immunity manipulation and not some aspect of carcass quality. I also found significant reductions in PO production within female oral secretions but not in anal secretions. This is consistent with Steiger et al. (2011) as they found no changes in anal PO production among immune challenged females. Unlike, Steiger et al. (2011) I included non-manipulated females for comparison as well as oral secretion samples in my analysis where I did find a significant reductions, although these significant reductions were found only in aged carcasses. Fresh carcasses may not be a challenge to female N. orbicollis and thus might have not adjusted their secreted PO production in response to a personal immune challenge.

Consistent with the prediction of negotiation model males paired with handicapped females increased their level of parental investment by increasing their production of LLA whereas males paired with control females did not. This result is consistent with other tests of the negotiation model (Fetherston et al., 1994; Rauter and Moore, 2004; Schwagmeyer et al., 2005; Smiseth et al., 2006; Lendvai et al., 2009; Cotter and Kilner, 2010; Creighton et al., 2015). However, this occurred only when pairs were on an aged carcass. To my knowledge, this is the first demonstration that burying beetles compensate immunologically for handicapped partners by adjusting the antimicrobial properties of social immune contributions. Consistent with my prediction of the effect of resource quality, the level of male compensation increased as the quality of the carcass decreased. Females reduced social immunity contributions irrespective of carcass quality to the same degree, and thus, any variability in the level of compensation by males can be attributed to the quality of the reproductive resource. To my knowledge, this is the first clear demonstration that the level of compensation is dependent on the perceived quality of the breeding habitat or resource.

The mechanism(s) of how mates determine the level of parental investment their partner provides has been controversial; whether it be through direct cues (such as observations of feeding rates) or indirect cues (such as begging rates of young). This has yet to be formally explored in burying beetles. It is known that males devote more time towards parental behaviors when paired with physically handicapped females (Creighton et al., 2015) and increase LLA production in anal exudates when females are removed (Cotter and Kilner, 2010) and now with immunologically handicapped females during the carcass preparation phase. In addition to these compensation studies, there are also no offspring during the carcass preparation phase and there is a lack of paternal investment during the larvae provisioning phase (Smiseth et al, 2005; Suzuki and Nagano, 2009). Taking all this into account, the evidence suggests that direct monitoring rather than indirect signals of female investment influence male burying beetle behavior, although additional experimentation may be necessary. Whether it is physical stimuli (witnessing reduced physical effort of female) or olfactory stimuli (higher concentration of decomposition cues due to reduced social immunity investment of female), or a combination of the two, males recognize when their mate reduces parental investment and adjusts their own in response during the carcass preparation phase.

There is a clear sexual difference in how males and females burying beetles react to a lack of their partner's effort (Suzuki and Nagano, 2009; Creighton et al. 2015). Also, sexes differ in response to the method of reducing partner effort (partner removal vs manipulation) (Suzuki and Nagano, 2009). Interestingly, the presence or absence of their partner also appears to influences how females respond to personal immune challenges. My results show that when males are present females reduce social immune contributions when their personal immunity is challenged. Cotter et al. (2013) found consistent results while males were removed. Conversely, Reavey et al. (2015) found that females maintained social immunity investment while males were removed, although this was only in the high microbial dosage group. More specifically, female show behavioral plasticity while facing varied dosage immune challenges when males are removed, similar to the terminal investment hypothesis. Suzuki and Nagano (2009) is the only empirical study comparing the impacts of mate removal and manipulation methods within burying beetles. Further exploration into the timing of mate removal as well as Nicrophorus species comparisons in response to removal vs manipulation would provide useful understanding in why there is variability in partner compensation.

The development of game theoretical models on biparental care sexual conflict resolution go through a process of consistent revision when current models fail to predict behaviors observed in empirical studies. Recent modifications of the negotiation model have provided additional insight into compensation patterns. For example, Johnstone and Hinde (2006) incorporated imperfect information about brood needs and brood value perceived by parents into their model. Under these conditions, parents were predicted to match their partner's effort instead of partially compensating as predicted by the negotiation model. While these models are useful, they are limited in capacity to predict sexual conflict resolutions in biparental species as the overwhelming majority of these models' predictive frameworks are constructed and tested with socially monogamous birds (Harrison et al., 2009). Further diversification of taxa used to test these models would be useful in future development of game theoretical models.

Empirical studies on wild populations have only just begun to connect the dots on how the external environment impacts sexual conflict resolutions (Perry and Rowe, 2018). A number of environmental and ecological variables have a significant impact on parental care decisions (Lejune et al., 2019). For example, abiotic factors, such as temperature, elevation, and latitude (Badyaev and Ghalambor, 2001; Lejune et al., 2019; Bründl et al., 2020; Plesnar-Bielak and Lukasiewicz, 2021) as well as ecological factors such as food abundance (Dunn and Robertson, 1992; Ortigosa and Rowe, 2002), population density/sex ratios (Perry and Rowe, 2018), habitat type (Lejune et al., 2019) and risk of predation (Rowe, 1992; Steyaert et al., 2016) all influence behavior, and thus potentially influence how sexual conflict is resolved. My study demonstrates that resource quality affects compensation rates in biparental species and incorporating resource quality into the negotiation models would potentially be insightful as well.

In summary, quality of resources or habitat available to biparental species can play an important role in how sexual conflict over parental investment is resolved. I have demonstrated that male burying beetles immunologically compensate for handicapped females by upregulating social immune molecules used in carcass preservation, and the degree of compensation depends upon the quality of the reproductive resource. Consistent with my prediction, males showed greater levels of compensation on lower quality carcasses than on high quality carcasses. Future theoretical models on sexual conflict resolutions in biparental care should incorporate external factors such as environmental and ecological variables.

30

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