Social Immunity in a Sub-social Insect: Influence of Parental Care and Exposure to Microbes During Development

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Introduction

Exposure to microbes during development can have long-lasting impacts later in life (McLean et al. 2014). For example, exposure to microbes early in life allows an individual to develop an efficient immune system which can quickly mount an immune response when the organism encounters the same or similar microbe later on in life. This process is called ontogenetic priming (Moreno-García et al. 2015).

The impact of early exposure to microbes, however, can also have long-lasting negative effects. It can have a negative impact on growth resulting in adults with reduced reproductive performance due to smaller size or worse body condition (McLean et al. 2014). In many cases mitigating factors such as high food availability or parental care can reduce or even eliminate any fitness costs (Demas & Nelson 2012).

The **aim of this study** was to determine:

1. Whether exposure to microbes during development causes ontogenetic priming.
2. Whether parental care functions as a mitigating factor in reducing long-term costs caused by exposure to microbes during development.

Study Organism

In this study we used the burying beetle *Nicrophorus marginatus,* a beetle that provides extensive parental care to its offspring and is regularly exposed to microbes during development and as adults. Burying beetles reproduce on small carrion and feed the larvae with regurgitated carrion. Larvae can self-feed, but develop faster and get larger when they receive parental care.

Nicrophorus marginatus

Three-day old *N. marginatus* larvae on carcass.

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Experiment

To test simultaneously 1) whether microbe exposure during development causes ontogenetic priming and 2) whether parental care functions as a mitigating factor in reducing long-term costs caused by exposure to microbes during development, we conducted a 2x2x2-factorial experiment where we manipulated the exposure to microbes of developing beetles in the presence or absence of parental care as well as the exposure to microbes of these beetles as adults during reproduction. As response, we determined the antimicrobial potency of the females' anal secretions and their reproductive success.

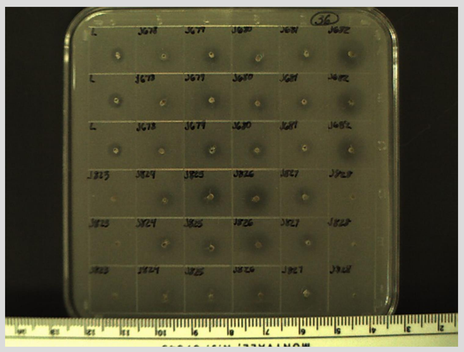
Manipulation of Exposure to Microbes during Development in the Presence and Absence of Parental Care The exposure to microbes during larval development was manipulated by dipping the carcass (i.e. a thawed mouse) either into sterile tryptic soy broth (TSB) (i.e. no additional microbes treatment) or into TSB inoculated with the common soil bacterium *Micrococcus lysodeikticus* (i.e. additional microbes treatment).

To manipulate parental care, we either removed the female beetle (one day after the first egg had been observed; i.e., no parental care treatment) or left the female with eggs and respective carrion (i.e. parental care treatment). Brood size of all broods was standardized at 13 larvae at the time when larvae hatched.

Manipulation of Exposure to Microbes during Reproduction
Once the larvae had reached sexual maturity, females were mated with unrelated males that had experienced the same treatments in regard to exposure to microbes and parental care during development. One day after mating, the males were removed and half of the females received a freshly thawed mouse dipped in sterile TSB (i.e. no additional microbes treatment), while the other half received a freshly thawed mouse TSB inoculated with the common soil bacterium *M. lysodeikticus* (i.e. additional microbes treatment).

Microbial Secretions of Adult Females The antimicrobial potency of secretions from adult females during reproduction was determined with a standard lytic zone assay (Cotter & Kilner 2010). We collected anal secretions from each female at the following stages during reproduction: right before we added the carcass, 24-hours after adding the carcass, 1-day after the first egg had been observed, 1-day after the first larvae have hatched, and at dispersal (i.e., when the larvae leave the brood chamber to pupate in the surrounding soil).

Using ImageJ, we measured the area of the clearing (i.e. lysis of *M. lysodeikticus)* caused by the antimicrobial compounds of the anal secretions. As standard we used lysozyme (1mg/μL) and antimicrobial potentials of anal secretions are expressed in equivalents of the lysozyme standard.



Lytic assay plate. Each well was loaded with 1 μL of a female's anal secretions. The larger the clearing around a well, the larger the anti-microbial potency of the anal secretions. L indicate wells loaded with 1 μL lysozyme standard (concentration: 1μL /mL).

Reproductive Success
The reproductive success was measured by brood mass, the number of larvae, and mean larval mass (i.e. brood mass/number of larvae) were recorded when larvae were dispersing (i.e., leaving the brood chamber).

Results

Antimicrobial Potency of Anal Secretions was neither influenced by exposure to microbes during development ($F_{1,100}$ = 0.18 ; P = 0.68) nor was it affected by the exposure to microbes during reproduction ($F_{1,100}$ = 0.00 ; P = 0.95). Presence or absence of parental care during development also had no effect on the antimicrobial potency of anal secretions ($F_{1,100}$ = 0.00 ; P = 0.95). All interactions of the three main factors were not significant.

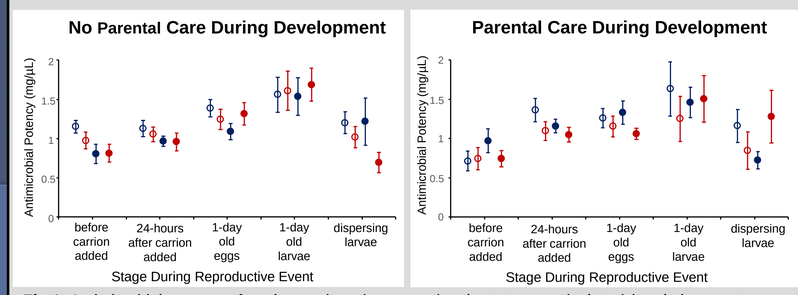


Fig 1. Antimicrobial potency of anal secretions (expressed as lysosome equivalents) in relation to stage during reproductive event. Open circles: no exposure to additional bacteria during reproduction; Closed circles: exposure to additional bacteria during reproduction; blue open and closed circles: no exposure to additional bacteria during development; red open and closed circles: exposure to additional bacteria during development

Reproductive Success None of the three measures of reproductive success was affected by exposure to microbes during development or during reproduction, or by the presence or absence of parental care during development.

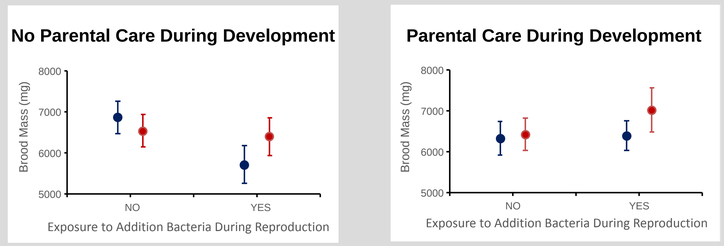


Fig 2. Brood mass (mean ± SE) of female beetles with and without exposure to additional bacteria during reproduction. Blue circles: no exposure to additional bacteria during development; red circles: exposure to additional bacteria during development.

Conclusion

The lack of a significant effect of microbe exposure during development on the antimicrobial potency of secretion during reproduction suggests that **no ontogenetic priming** has occurred.

Similarly, the lack of an effect of exposure to parental care during development on antimicrobial potency of secretion or reproductive success indicates **no modifying effect of parental care** and that developmental carry-over effects are negligible.

These results corroborate findings by Trumbo (2016) and provide additional evidence that competition with bacteria over carrion is not as important as originally proposed (Janzen 1977).

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