

Genetic Influences on Parental Care in *Nicrophorus vespilloides*.

Submitted by Chloe. J. Bird, to the University of Exeter as a thesis for the Degree of Doctor of Philosophy in Biological Sciences, July 2010.

This thesis is available for Library use on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signed: Chloe. J. Bird

.....

ACKNOWLEDGEMENTS

There are so many people to thank for their help over the last four years, needless to say without all these people I would never have made it this far.

First, I would like to thank my supervisors Allen Moore and Trish Moore for their patience, support and advice throughout my PhD.

I also need to thank Nick Royle and Mike Ritchie, for taking the time to read this and agreeing to be my examiners. I owe extra thanks to Mike for sharing the sequence of *Nyfor*, without which my thesis would be somewhat slimmer.

Since arriving at the CEC as an undergraduate I have benefited from the support and knowledge of the academic staff, but I owe particular thanks to Tom Tregenza and Dave Hosken for having the confidence in me to think that doing a PhD was a good idea in the first place.

I am also indebted to all the people who have given practical advice and taught me many of the methods I have used: Yannick Pauchet, Stuart Hinchliffe, Michelle Hares, Nicola Chamberlain, Paul Wilkinson, Andrea Dowling, Richard French-Constant, Damian “I smoke crack rocks” Smith and The Amanda Bretman. I also owe thanks to Craig Walling, Clare Stamper and Clarissa House for getting me started with the burying beetles.

All the inhabitants of PhD/Postdoc offices (past and present): Thank you all for your friendship, good humour and amazing tolerance of Distraction Corner.

The Coven: you know who you are. You are all awesome, do I need to say any more? Matthew Witt and Chris Mitchel for putting up with my bizarre hours, and beetle themed rants. Best housemates ever.

All the members of the Hupo Wushi Kung Fu club, particularly Austin and Arlo, for the stress-busting and banter. Xie xie!

Last, but by no means least, I need to thank my family for all their love and support, I couldn't have done it without you!

ABSTRACT

The burying beetle (*Nicrophorus vespilloides*) has unusually highly developed parental care; parents prepare and maintain a food resource (thereby providing indirect parental care), feed through direct provisioning by regurgitation, and protect their larvae. Parental care is highly variable and can be uniparental female care, uniparental male care, or biparental. There are genetic components to the parenting behaviour of the burying beetle, the amount of direct and indirect care given, and the size of the brood are heritable and therefore genetic traits.

In this thesis I have focused on two candidate genes that I predicted would influence parental care behaviour. The first is *foraging*, which has been shown to influence a range of social and reproductive behaviours in other insect species. Using QRT-PCR and pharmacological manipulations I have investigated the role of *Nvfor* in adult and juvenile burying beetles. The second gene is *inotocin*, the insect orthologue of oxytocin. Oxytocin has been shown to influence social behaviour as well as many behaviours associated with reproduction in vertebrates and invertebrates, however the effects of inotocin have not yet been investigated in insects. I have used pharmacological manipulations to investigate the role of inotocin in parental behaviour in female burying beetles.

Collectively my results demonstrate the central role of *Nvfor* in the control of direct parental care and the association with major behavioural changes in both adult and larval burying beetles. I have also demonstrated the possible involvement of oxytocin in the control of aggression towards conspecific larvae. These insights suggest the controlling mechanism for the behavioural changes seen in burying beetles is complex and involves interactions between many genes. Combined with previous research on these genes, it is clear they are key components in the evolution of sociality. Finally, my research indicates the power of the candidate gene approach, and suggests additional components of the related pathways that could be investigated.

TABLE OF CONTENTS

TITLE PAGE&DECLARATION	1
ACKNOWLEDGEMENTS	2
ABSTRACT	3
CONTENTS	4
FIGURES AND TABLES	6
AUTHOR'S DECLARATIONS	9
CHAPTER 1: INTRODUCTION-A GENE FOR PARENTAL CARE	10
CHAPTER 2: THE <i>FORAGING</i> GENE – GENE EXPRESSION IN ADULT BEETLES, <i>Nicrophorus vespilloides</i>	30
INTRODUCTION	31
METHODS	36
RESULTS	43
DISCUSSION	45
CHAPTER 3: PHARMACOLOGICAL MANIPULATION OF cGMP – EFFECTS ON PARENTAL CARE, <i>Nicrophorus vespilloides</i>	51
INTRODUCTION	52
METHODS	55
RESULTS	59
DISCUSSION	64
CHAPTER 4: THE <i>FORAGING</i> GENE – GENE EXPRESSION IN LARVAE, <i>Nicrophorus vespilloides</i>	69

INTRODUCTION	70
METHODS	72
RESULTS	75
DISCUSSION	75
CHAPTER 5: THE EFFECT OF OXYTOCIN ON PARENTAL CARE	
BEHAVIOUR IN ADULT BEETLES, <i>Nicrophorus vespilloides</i>	78
INTRODUCTION	79
METHODS	83
RESULTS	86
DISCUSSION	87
CHAPTER 6: DISCUSSION AND CONCLUSIONS.	92
APPENDIX I	100
BIBLIOGRAPHY	105

FIGURES AND TABLES

CHAPTER 1: INTRODUCTION-A GENE FOR PARENTAL CARE

No figures or tables

CHAPTER 2: THE *FORAGING* GENE – GENE EXPRESSION IN ADULT BEETLES, *Nicrophorus vespilloides*

- Fig. 1: a Japanese beetle trap and diagram of how the trap was prepared and baited for collection of *N. vespilloides*. 37
- Fig. 2. Timing of mating and collection of the treatment groups to ensure a matched final age 40
- Fig 3. Partial sequence of *Nyfor*, the section highlighted in blue is the region amplified by the *Nyfor* primers. 42
- Fig. 4. Relative expression of *Nyfor* in three caring stages of female *N. vespilloides*. 44
- Fig. 5. Relative expression of *Nyfor* in three caring stages of male *N. vespilloides*. 45

CHAPTER 3: PHARMACOLOGICAL MANIPULATION OF cGMP – EFFECTS ON PARENTAL CARE, *Nicrophorus vespilloides*

- Table 1: ingredients for beetle Ringers buffer 56
- Fig. 1: injection site in *N. vespilloides* 57
- Fig. 2: Total time spent by females providing direct care to the first brood by the four treatment groups. 60
- Fig. 3: Total time spent by males providing direct care to the first brood by the four treatment groups. 60
- Fig. 4: Total time spent by females providing indirect care to the first brood by the four treatment groups. 61

Fig. 5: Total time spent by males providing indirect care to the first brood by the four treatment groups.	61
Fig. 6. Proportion of individuals providing direct care to the second brood.	62
Fig. 7. Total time spent providing direct care to the second brood.	63
Fig. 8. Proportion of individuals that cannibalised larvae from the second brood.	63
Fig. 9. Proportion of individuals providing direct care to the second brood.	64
Fig. 10. Total time spent providing direct care to the second brood.	64
Fig. 11. Proportion of individuals that cannibalised larvae from the second brood.	65

CHAPTER 4: THE *FORAGING* GENE – GENE EXPRESSION IN LARVAE, *Nicrophorus vespilloides*

Fig. 1. Relative expression of <i>Nyfor</i> in three larval stages of <i>N. vespilloides</i> .	75
--	----

CHAPTER 5: THE EFFECT OF OXYTOCIN ON PARENTAL CARE BEHAVIOUR IN ADULT BEETLES, *Nicrophorus vespilloides*

Table 1: Structures of vasopressin, oxytocin, and some selected vasopressin- and oxytocin-like peptides (taken from Stafflinger <i>et al</i> 2008)	82
Table 2: ingredients for beetle Ringers buffer.	84
Fig. 1: injection site in <i>N. vespilloides</i> .	84
Fig. 2: Mean numbers of larvae killed 12 hours after treatment, each beetle started with a brood of 15 larvae.	86
Fig. 3: mean time spent performing indirect care by each treatment group	87
Fig. 4: Schematic representation of the appearance of the major orders of holometabolous insects and the occurrence of the inotocin hormonal system (highlighted in red). This hormonal system has been conserved only in the evolutionary lines leading to basal	

holometabolous insects: Coleoptera (beetles) and Hymenoptera (wasps). The inotocin system must have been abandoned at least two times during the evolution of the Holometabola (taken from Stafflinger *et al* 2008)

90

CHAPTER 6: DISCUSSION AND CONCLUSIONS.

No figures or tables

AUTHOR'S DECLARATIONS

During the research contributing to this thesis I was supported by a studentship from The University of Exeter.

All of the chapters presented in this thesis were written by Chloe J. Bird, with comments and editing from A. Moore.

Chapter 3: I developed the injection protocol in collaboration with Amy Simpson, who also ran some of the observation experiments that are included in my data set.