

# Maternal effects in the large milkweed bug

## *Oncopeltus fasciatus*

Submitted by Devi Isadora Ramayanti Newcombe to the University of Exeter

as a thesis for the degree of

Doctor of Philosophy in Biological Sciences February 2013

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.

A handwritten signature in black ink, appearing to be 'D. I. R.', is centered within a light gray rectangular box.

Signature: .....

## Abstract

Maternal effects are the non-genetic contributions of mothers (or fathers) towards the phenotype of their offspring. Maternal effects are now well recognised as a facilitator for evolutionary change in offspring phenotypes and life history strategies which can have effects on population dynamics, population divergence and even speciation. Furthermore, maternal effects have been shown to have a heritable genetic basis and that they are genetically variable, which suggests that they contribute to maintaining phenotypic variation. Maternal effects may impede or accelerate responses to selection which has implications for adaptive evolution and making predictions about their evolutionary potential. The importance of their contribution to phenotypic variation and life history evolution has made maternal effects an important consideration in fields such as conservation and population biology, evolutionary ecology and evolutionary genetics.

The aim of this thesis is to investigate if maternal effects can influence offspring life history traits and fitness parameters through maternal resources via the egg. Main questions that are asked include: can maternal effects help facilitate transition to a novel host-diet (Chapter 2); does maternal diet influence egg composition and, if so, does this have an effect on offspring life-history parameters (Chapter 3); is there a genetic basis to egg composition and is there potential for egg composition to evolve (Chapter 4); and are defensive compounds from the diet transferred into the eggs, if so, are these uni- or biparentally transferred and does this offer protection against predation (Chapter 5)? To address these questions we used a specialist insect herbivore, the large milkweed bug *Oncopeltus fasciatus* (Hemiptera: Lygaeidae). In the wild, *O. fasciatus* feed on plants from the genus *Asclepias* (Apocynacea). However, *O. fasciatus* can be reared successfully in laboratories on sunflower seeds *Helianthus annuus*. For our experiments we used two populations of *O. fasciatus*, one population has been maintained on dry seeds of *A. syriaca* while the other population has been reared and maintained on sunflower seeds.

The results of Chapter 2 were suggestive of a maternal host-diet effect on egg mass and hatching success, but we did not find evidence that maternal host-diet was significant in influencing a transition to a novel host. In Chapter 3 we found that there was variation in the free amino acid profiles of the eggs between our treatments suggesting that amino acid profiles may be influenced by maternal diet. The results of our multivariate selection analysis to examine linear and nonlinear

(quadratic) relationships between maternal diet and the free amino acid profiles of the eggs suggest that there may be population-specific responses which can influence specific amino acid profiles in relation to hatchling mass. In Chapter 4 we used only the milkweed-adapted population to determine if there was a genetic basis to amino acid profiles in the eggs. We constructed a genetic variance-covariance (**G**) matrix to determine the strength and direction of the relationships between amino acids and to assess the potential for amino acid profiles to evolve. While we found genetic variation for amino acids, and that there was evidence for positive moderate to strong genetic correlations between many of them, we also found evidence for constraints for the potential for amino acid profiles to evolve as evidenced by the calculation of  $g_{\max}$  (which represents the linear combination of components that has the highest genetic variance and which is the most accessible to evolution). In Chapter 5 we found maternal, but not paternal, transmission of cardenolides into the eggs. However, this did not confer protection of all eggs against predation from larvae of the green lacewing *Chrysoperla carnea*.

Overall, results suggest that for our populations of *O. fasciatus*, maternal effects are significant in influencing early life history traits such as egg mass and hatchling mass. However, we did not find any significant effects on other offspring life history or fitness parameters that we measured. This may be surprising as positive, and negative, effects of non-genetic contributions of females (and males) to their offspring has been widely reported in many taxa. The patterns and implications of maternal resource allocation and their effects on offspring life history evolution are explored and discussed, as are the limitations of our experimental designs. I hope that this research can be used to stimulate further investigations into maternal effects and the relationships between host-plant, maternal allocation strategies and life history evolution.

# Table of Contents

<b>Abstract</b> .....	2
<b>List of tables and figures</b> .....	8
<b>List of plates</b> .....	10
<b>Author's declaration</b> .....	11
<b>Chapter 1 Maternal effects: a general introduction</b> .....	13
1.1 Defining maternal effects .....	13
1.2 Why study maternal effects? .....	15
1.2.1 Maternal provisioning: linking maternal diet, reproductive investment and offspring performance .....	17
1.3 Thesis outline .....	21
1.4 Study System .....	23
<b>Chapter 2: Evolving an expanded diet: can maternal effects facilitate transition to a novel host?</b> .....	26
2.1 Abstract .....	27
2.2 Introduction .....	28
2.3 Materials and methods .....	29
2.3.1 Study system .....	29
2.3.2 Experimental populations & rearing .....	30

2.3.3 Statistical analyses.....	32
2.4 Results .....	34
2.4.1 Effects on egg mass.....	34
2.4.2 Effects on offspring development .....	35
2.4.3 Effects on offspring survival .....	36
2.5 Discussion .....	36
<b>Chapter 3: Effect of diet on free amino acids in eggs of <i>Oncopeltus fasciatus</i></b> .....	<b>48</b>
3.1 Abstract .....	49
3.2 Introduction .....	50
3.3 Materials and methods.....	53
3.3.1 Study system.....	53
3.3.2 Experimental populations & rearing .....	54
3.3.3 Experimental design .....	54
3.3.4 Free amino acid extraction .....	55
3.3.5 Quantification of free amino acid composition.....	57
3.3.6 Statistical analysis .....	57
3.3.7 Egg amino acid profiles and effects on offspring performance traits .....	57
3.4 Results .....	59
3.4.1 Offspring performance .....	59

3.4.2 Free amino acid profiles of eggs .....	60
3.4.3 Effects on egg free amino acid profiles.....	61
3.4.4 Amino acid profiles of eggs and offspring performance.....	61
3.5 Discussion .....	62
3.5.1 Maternal diet and egg mass – quantity or quality?.....	62
3.5.2 Maternal diet and amino acid profiles of eggs .....	64
3.5.3 Egg amino acid profiles and relationship with offspring performance .....	66
<b>Chapter 4: Quantitative genetics of maternal allocation of amino acids into eggs of an insect herbivore .....</b>	<b>76</b>
4.1 Abstract .....	77
4.2 Introduction .....	78
4.3 Materials and methods.....	80
4.3.1 Study species .....	80
4.3.2 Breeding design.....	80
4.3.3 Amino acid extraction .....	81
4.3.4 Quantification of free amino acid composition.....	82
4.3.5 Statistical analyses.....	83
4.4 Results .....	84
4.5 Discussion .....	85

<b>Chapter 5: Maternal, not paternal, transmission of cardenolides into eggs of the large milkweed bug <i>Oncopeltus fasciatus</i> (Dallas)</b> .....	96
5.1 Abstract .....	97
5.2 Introduction .....	98
5.3 Materials and methods.....	101
5.3.1 Experimental populations and rearing.....	101
5.3.2 Parental diet treatment groups .....	102
5.3.3 Bioassay: predation of eggs by green lacewing larvae <i>Chrysoperla carnea</i> .....	103
5.3.4 Quantification of cardenolide content of eggs using High Performance Liquid Chromatography (HPLC).....	104
5.3.5 Statistical analyses.....	105
5.4 Results .....	106
5.4.1 Predation bioassays .....	106
5.4.2 Cardenolide content of eggs .....	106
5.5 Discussion .....	107
Chapter 6: General discussion.....	114
6.1 Maternal effects and novel host-diets.....	114
6.2 Maternal diet and egg composition .....	116
6.3 Genetics of maternal allocation.....	119
6.4 Male contributions.....	121

6.5 Concluding remarks .....	122
<b>Acknowledgements</b> .....	124
<b>References</b> .....	126

## List of tables and figures

### Chapter 2

**Table 2.1** Analysis of Variance (ANOVA) table for effects of treatments (population, maternal diet, offspring diet and sex) on development time (days from hatch to adult eclosion) of nymphs .....42

**Table 2.2** ANOVA table for effects of treatments (population, maternal diet, offspring diet) on offspring pronotum width.....43

**Figure 2.1** Mean egg mass of eggs of females from two populations (KY and LAB) on milkweed seeds and sunflower seeds.....44

**Figure 2.2** Development time (days) of nymphs to adult ecdysis in **(A)** the milkweed-adapted population and **(B)** the sunflower-adapted population.....45

**Figure 2.3** Adult size of offspring from the two populations on both diets for **(A)** females and **(B)** males.....46

**Figure 2.4** Survivorship of offspring (from hatch to final adult eclosion).....47

### Chapter 3

**Table 3.1** Principal Component Analysis (PCA) of the free amino acid composition of eggs in *O. fasciatus*.....69

**Table 3.2** Multivariate Analysis of Covariance (MANCOVA) and univariate analysis of covariance (ANCOVA) examining effects of maternal diet on free amino acid profiles of eggs from two different populations of *O. fasciatus*.....70

**Table 3.3** Selection analysis to determine linear and nonlinear (quadratic) selection of amino acid profiles on **(A)** hatching success and **(B)** hatchling mass.....71



**Table 3.4** The M matrix of eigenvectors from the canonical analysis of  $\gamma$  for the four PCs describing the amino acid composition of the eggs in *O. fasciatus* and their effects on (A) offspring hatching success and (B) hatchling mass.....72

**Figure 3.1** Mean egg mass of eggs from our populations of *O. fasciatus* on milkweed and sunflower diets.....73

**Figure 3.2** Mean hatchling mass ( $\pm$ SE) across the Kentucky and Laboratory populations when females reproduce on milkweed or sunflower diets.....73

**Figure 3.3** Mean PC scores ( $\pm$ SE) describing the amino acid composition of eggs across the Kentucky and Laboratory populations when females reproduce on milkweed or sunflower diets...74

**Figure 3.4** Thin-plate spline (A) perspective and (B) contour view visualization of performance surface to assess the relationships between amino acid profiles and hatchling mass.....75

## Chapter 4

**Figure 4.1** Quantitative genetic design for investigating the evolutionary potential of maternal allocation of amino acids into eggs of *O. fasciatus* females from the Kentucky population fed milkweed.....90

**Table 4.1** Descriptive statistics, heritabilities and evolvabilities of amino acids in eggs of *O. fasciatus* females from the Kentucky population fed milkweed.....91

**Table 4.2** Phenotypic correlations for all amino acids found in eggs of *O. fasciatus* from females from the Kentucky population fed milkweed.....92

**Table 4.3** Genetic correlations of amino acids in eggs of *O. fasciatus*.....93

**Table 4.4** Additive genetic variance-covariance matrix (**G**) of amount of free amino acids allocated to eggs.....94

**Table 4.5** Eigenvectors of the **G** matrix for amino acid allocation in the eggs of *O. fasciatus* indicating amino acid combinations of highest genetic variance and which are the most accessible to evolution.....95

## Chapter 5

**Figure 5.1** Box plots of cardenolide content of eggs of *O. fasciatus* from different parental diet treatments.....112

## List of plates

<b>Plate 1.4.1</b> <i>Oncopeltus fasciatus</i> nymph feeding on milkweed seeds.....	25
<b>Plate 1.4.2</b> Milkweed bugs mating.....	25
<b>Plate 1.4.3</b> Female milkweed bug ovipositing eggs in cotton wool.....	25
<b>Plate 1.4.4</b> Larva of green lacewing <i>Chrysoperla carnea</i> consuming an <i>O. fasciatus</i> egg.....	25
<b>Plate 5.1</b> Chromatogram representative of cardenolide peaks found in eggs of <i>O. fasciatus</i> .....	113