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INSECT BEHAVIOR

From Mechanisms to Ecological and Evolutionary Consequences

EDITED BY
ALEX CÓRDOBA-AGUILAR
DANIEL GONZÁLEZ-TOKMAN
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Alex Córdoba-Aguilar
Universidad Nacional Autónoma de México, México

Daniel González-Tokman
CONACYT, Instituto de Ecología A. C., México

Isaac González-Santoyo
Universidad Nacional Autónoma de México, México

OXFORD
UNIVERSITY PRESS

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Great Clarendon Street, Oxford, OX2 6DP,
United Kingdom

Oxford University Press is a department of the University of Oxford.
It furthers the University's objective of excellence in research, scholarship,
and education by publishing worldwide. Oxford is a registered trade mark of
Oxford University Press in the UK and in certain other countries

First Edition published in 2018

Impression: 1

British Library Cataloguing in Publication Data
Data available

Library of Congress Control Number: 2018932146

ISBN 978–0–19–879750–0 (hbk.)
ISBN 978–0–19–879751–7 (pbk.)

DOI: 10.1093/oso/9780198797500.001.0001

Printed in Great Britain by
Bell & Bain Ltd., Glasgow

Foreword

John Alcock

Emeritus Regents' Professor, School of Life Sciences, Arizona State University

Niko Tinbergen (1963) wrote a now famous paper in which he proposed that a complete study of animal behavior required research into the development of behavior, the physiological control of behavior, the adaptive value of behavior, and the evolutionary history of behavior. Given the broad range of the disciplines needed for a total picture of the causes of behavior, ranging from genetics to evolutionary biology, it is not surprising that most previous books on the subject of insect behavior have been largely limited to some portion of the four areas of research. So, for example, classic books by Vincent Dethier (1976) and Kenneth Roeder (1963) dealt with the physiology of behavior in certain insects while Choe and Crespi (1997) edited a book on the evolution of social behavior in insects. Evolutionary adaptations were the focus of a book that Thornhill and Alcock (1983) wrote, a book that was updated recently by Shuker and Simmons (2014). The second edition of the book on insect behavior by Matthews and Matthews (2010) did discuss both proximate and ultimate aspects of insect behavior, but almost a decade has passed since it was published and, moreover, the authors intended to reach undergraduates, rather than a more advanced audience. Therefore, previous books, whether edited or written entirely by one or two persons, left room for a modern survey of both proximate (developmental and physiological) and ultimate (adaptive and historical) causes of insect behavior.

The current edited compendium fills the need for a complete survey of the causes of insect behavior by taking advantage of the ability of specialists in all facets on insect behavior, including the relationship between behavior and pest control, as well as insect conservation, to communicate with readers about the most recent developments in their specialty, whether they be primarily proximate or ultimate in content. Graduate students in behavior and entomology will be the main beneficiaries inasmuch as many of the authors provide suggestions for additional research in their field. So, for example, Hunt and co-authors point out in this volume that, although genetic effects on the reproductive behavior of insects have been well documented, the relationship between natural selection and genes for elements of reproductive behavior requires much more work because many genes contribute both to reproduction and to the development of other important attributes. Sherratt and Kang suggest that use of the comparative method, a key tool for tracing the evolutionary history of attributes of interest, could help explain why, in groups of related species, some but not all exhibit certain characteristics, such as the brightly coloured underwings of certain *Catocala* moths. Olzer and her colleagues note that cryptic female choice in which females choose mates on the basis of their ability to manipulate stored sperm remains controversial and poorly studied. Vale and his co-authors examine the fascinating subject of parasites that change the behavior of infected insects, while cautioning that it is difficult to show that infected insects are preyed upon by the appropriate hosts of the parasites. Many additional examples of the kinds of useful future research are provided by the authors of this book's chapters providing interesting challenges for readers.

Although graduate students could clearly gain by reading this book, all behavioral biologists and entomologists would do well to peruse the book's chapters. Insects, of which there are more than a millions species, are not only extremely diverse

behaviorally, but the ever increasing number of first rate research reports means that the task of keeping abreast of new developments related to insect behavior is ever more difficult. This book will do much to help in this regard particularly since one of the recurrent themes of the book is the importance and utility of investigating the connection between proximate mechanisms and the evolution of behavior, a still imperfectly studied phenomenon. So despite the fact that much has been done with insects, as this book documents, much more remains for inspired researchers to examine. The authors of this collection help us identify what still needs to be done if we are to more fully understand the behavior of the small-brained, but behaviorally complex inhabitants of our world.

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Acknowledgements

Many coincidences shape our life and decisions. For the case of this book, one coincidence is that we all editors happen to be good friends. When you can trust another person in shared tasks (such as producing a book), your life becomes much easier and this is why we not only made this adventure possible but also had fun during the process. Moreover, a huge task such as reviewing biological aspects of the most diverse animal group can become a hopeless adventure had not we had the help of quite a few fellows. We would therefore like to thank all people that contributed, including reviewers of chapter drafts and people who made suggestions of varying nature: John Alcock, Wolf Blanckenhorn, Bruno Buzatto, Adolfo Cordero-Rivera, Federico Escobar, Thomas Flatt, James Gilbert, Michael Greenfield, Johnattan Hernández-Cumplido, Héctor Méndez-Maldonado, Salvador Hernández-Martínez, Humberto Lanz-Mendoza, Zenobia Lewis, Germán Octavio López-Riquelme, Pierre-Olivier Montiglio, Miguel Moreno-García, Roberto Munguía-Steyer, Fernando Noriega, Diana Pérez-Staples, Bernard Roitberg, Gianandrea Salerno, Marla Sokolowski, Robert Srygley, Kate Umbers and Maren Wellenreuther. A big "thank you" for devoting their time and knowledge. Our acknowledgement also goes to Ian Sherman and Bethany Kershaw from Oxford University Press for your encouragement and patience.

Our granting agencies also deserve a place for indirectly supporting this book. We would therefore like to thank the following funding sources: PAPIIT UNAM IN203115 and IN206618 to AC-A, Project CONACYT Ciencia Básica 257894 to DG-T, PAPIIT UNAM IA209416 and Project CONACYT Ciencia Básica 241744 to IG-S.

Finally, AC-A wishes to thank his family: Ana, Rodrigo and Santiago.

Alex Córdoba-Aguilar
Daniel González-Tokman
Isaac González-Santoyo

Contents

List of contributors

Patrick Abbot Department of Biological Sciences, Vanderbilt University, Nashville, TN, USA

Sandra A. Allan Center for Medical, Agricultural and Veterinary Entomology, ARS/USDA, Gainesville, FL, USA

Giovanni Benelli Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy

Wolf U. Blanckenhorn Evolutionary Biology & Environmental Studies, University of Zürich-Irchel, Zürich, Switzerland

Jason W. Chapman Centre for Ecology and Conservation, and Environment and Sustainability Institute, University of Exeter, Penryn, Cornwall, UK

Gregory T. Chism Entomology and Insect Science Program, University of Arizona, Tuscon, AZ, USA

Alex Córdoba-Aguilar Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, CDMX, México

Reuven Dukas Department of Psychology, Neuroscience and Behaviour, McMaster University, Hamilton, Ontario, Canada

Rebecca L. Ehrlich Department of Ecology, Evolution, and Behavior, University of Minnesota, Twin Cities, St. Paul, Minnesota, USA

Jennifer Fewell School of Life Sciences, Arizona State University, Tempe, AZ, USA

Mark R. Forbes 209 Nesbitt Bldg, Carleton University, 1125 Colonel By Drive, Ottawa ON K1S 5B7 Canada

José Antonio De Fuentes-Vicente Universidad Pablo Guardado Chávez, Tuxtla Gutiérrez, Chiapas, Mexico

Isaac González-Santoyo Departamento de Psicobiología y Neurociencias, Facultad de Psicología, Universidad Nacional Autónoma de México, CDMX, México

Daniel González-Tokman CONACYT. Red de Ecoetología, Instituto de Ecología, A. C. Xalapa, México

Ana E. Gutiérrez-Cabrera CONACYT-Centro de Investigación sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, México

Justa L. Heinen-Kay Department of Ecology, Evolution, and Behavior, University of Minnesota, Twin Cities, St. Paul, Minnesota

Clarissa House School of Science and Health and the Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW, Australia

John Hunt Centre for Ecology and Conservation, University of Exeter, Cornwall, UK. School of Science and Health and the Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW, Australia

Changku Kang Department of Biosciences, Mokpo National University, Muan, Republic of Korea

Carl N. Keiser Department of Biology, University of Florida, Gainesville, Florida, United States

Darrell J. Kemp Department of Biological Sciences, Macquarie University, North Ryde, New South Wales, Australia

Emily Laub Department of Biology, Duke University, Durham, NC, USA

James L.L. Lichtenstein Department of Ecology, Evolution and Marine Biology, University of California at Santa Barbara, Santa Barbara, CA, USA

Glauco Machado LAGE do Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, SP, Brazil

André Morrill Department of Biology, Carleton University, Ottawa, Canada

Tim R. New Department of Ecology, Environment and Evolution, La Trobe University, Victoria, Australia

H. Frederik Nijhout Department of Biology, Duke University, Durham, NC, USA

James C. O'Hanlon School of Environmental and Rural Science, University of New England, Armidale, Australia

Rachel Olzer Department of Ecology, Evolution, and Behavior, University of Minnesota, Twin Cities, St. Paul, Minnesota

Anne C. von Philipsborn Danish Research Institute of Translational Neuroscience (DANDRITE), Aarhus University, Aarhus C, Denmark

Jonathan N. Pruitt Department of Psychology, Neuroscience and Behaviour, McMaster University, Hamilton, Ontario, Canada

James Rapkin Centre for Ecology and Conservation, University of Exeter, Cornwall, UK

Don R. Reynolds Natural Resources Institute, University of Greenwich, Chatham, Kent, UK

Carlos Ribeiro Champalimaud Research, Champalimaud Centre for the Unknown, Lisbon, Portugal

Bernard D. Roitberg Biology, Simon Fraser University, Burnaby, Canada

Heiner Römer Zoology, University of Graz, Austria

Thomas N. Sherratt Department of Biology, Carleton University, Ottawa, Canada

Stephen J. Simpson Charles Perkins Centre, The University of Sydney, NSW, Australia

Jonathon A. Siva-Jothy Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Scotland, UK

Marla B. Sokolowski Department of Ecology and Evolutionary Biology, University of Toronto, Canada

Jessie Tanner Department of Ecology, Evolution, and Behavior, University of Minnesota, Twin Cities, St. Paul, Minnesota, USA

Stephen T. Trumbo Department of Ecology and Evolutionary Biology, University of Connecticut, Waterbury, CT, USA

Kate D.L. Umbers School of Science and Health Western Sydney University, Hawkesbury, Richmond, NSW, Australia

Hawkesbury Institute for the Environment, Western Sydney University, Hawkesbury, Richmond, NSW, Australia

Pedro F. Vale Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Scotland, UK

Thomas Walker Department of Disease Control, London School of Hygiene and Tropical Medicine, London, UK

Thomas E. White School of Life and Environmental Sciences, The University of Sydney, Sydney, New South Wales, 2006, Australia

Karen D. Williams Department of Ecology and Evolutionary Biology, University of Toronto, Canada

Colin M. Wright Department of Ecology, Evolution and Marine Biology, University of California at Santa Barbara, Santa Barbara, CA, USA

Marlene Zuk Department of Ecology, Evolution, and Behavior, University of Minnesota, Twin Cities, St. Paul, Minnesota, USA

CHAPTER 1

Introduction

Daniel González-Tokman[1], Isaac González-Santoyo[2], and Alex Córdoba-Aguilar[3]

[1] *CONACYT, Red de Ecoetología, Instituto de Ecología, A. C. Xalapa, México*

[2] *Departamento de Psicobiología y Neurociencias, Facultad de Psicología, Universidad Nacional Autónoma de México. CdMx México*

[3] *Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México. CdMx, México*

1.1 Introduction

With over 1 million described species and 4–6 million species hypothesized to exist (Schowalter 2016), insects are the most diverse group of animals in the world, and such diversity is reflected in their behavior. Such vast diversity has propelled studies to put forward explanations of its basis, patterns, and consequences. Due to this, the study of insect behavior has attracted not only geneticists, physiologists, ecologists, evolutionary biologists, entomologists, and agronomists, who may be directly interested in the causes and consequences of insect behavior, for academic reasons or simple personal fascination, but also psychologists, nutritionists, economists, and mathematicians that have based or are currently basing their own research on the knowledge generated with insects. This attraction has found a fertile ground for another reason—insects can be great study subjects as they allow a fairly easy manipulation of key variables to investigate their behavior. This practical property has permitted insect behavior to be studied at all levels of analysis, from proximal causes, such as physiology, genetic regulatory mechanisms, and development, to the ultimate consequences, such as evolution and ecology. This is the reason why studies using several insect systems have championed our understanding of biological phenomena. To put a simple example that illustrates such 'insect strength', the 2017 Nobel prize was given to Jeffrey C. Hall, Michael Rosbash, and Michael W. Young, for elucidating the molecular mechanisms underlying circadian rhythms, a research fundamentally carried out in *Drosophila* flies.

Scientific knowledge of insect biology expands every day and this urgently needs updated reviews that facilitate our access to such information, especially for 'newcomers' in the insect behavior discipline. This feeling of an empty niche emerged several years ago, when we taught different graduate and postgraduate courses. These made use of different sources that never really captured an updated and/or summarized version of insect behavior at all levels. With this in mind, the aim of this book was to create a textbook of insect behavior for both students (mainly) and researchers that provides the key classical and modern concepts and approaches to understanding insect behavior, all

González-Tokman, D., González-Santoyo, I., and Córdoba-Aguilar, A., *Introduction.* In: *Insect Behavior: From mechanisms to ecological and evolutionary consequences.* Edited by Alex Córdoba-Aguilar, Daniel González-Tokman, and Isaac González-Santoyo: Oxford University Press (2018).
DOI: 10.1093/oso/9780198797500.003.0001

set within a multidimensional framework—from genes to ultimate evolutionary and ecological consequences. As it was highly likely that the target may be missed if this book were to include topics outside our study fields, it was decided to produce a multi-authored work, where experts in each aspect of insect behavior could provide specialist views of the field.

As it is usual in science, this book did not start from scratch, but uses Matthews and Matthews' (2009) and Alcock's (2013) magnificent books as fundamental starting points. Thus, several specialists were asked to provide a clear and concise state-of-the-art review of their fields directed to new generations in areas that are or have become fruitful grounds for research, including traditional (e.g. genetics, hormonal control) and topical (e.g. personality, parasite-induced insect behavior), or even fields that are usually included in other areas, such as global change, and pest and vector management. The authors are fully aware that this treatise is by no means complete. On one hand, there are issues that this book does not cover, but that are fortunately found in other textbooks, such as the history of insect behavior (Matthews and Matthews 2009), multitrophic interactions (e.g. herbivory, predation, and pollination; Rosenthal and Berenbaum 1992; Price et al. 2011), and thermoregulation behavior (Matthews and Matthews 2009) to quote a few. On the other hand, there are issues that could not be covered for reasons of space limitation, but that remain pending for future treatises, such as oviposition behavior and behavioral adaptations of insects as predators (a counterpart of Chapter 9). Other topics included in the selection were written with some explicit limitations, as the field was too vast to be reviewed in fewer than 7000 words [e.g. Chapter 2 used only reproductive behavior, rather than all behaviors to illustrate the genetic basis or Chapter 21 where only three vectors (two of which are insects) are explained].

Besides this introductory chapter, the book includes further 21 chapters, which are divided in three main sections. The first section includes four chapters about the interacting mechanisms controlling behavior—genes, hormones, and the nervous system. The second section, which is the core of the book, includes thirteen chapters about the diversity of behaviors, and their ecological and evolutionary consequences, incorporating emerging topics that have traditionally been studied in other animal groups, such as learning, cognition, and animal personality. The final section of four chapters comprises the application of insect behavior, including the importance of climate change on insect behavior, management of crop pests and disease vectors, and the importance of behavior in insect conservation. Since this book was conceived essentially for students, readers will find a glossary section at the end of the book, where concepts used throughout all chapters have been defined by contributing authors. These concept terms can be found in bold the first time they are mentioned in the text.

Finally, the aim of this book will not be achieved if the reader does not find this book an indispensable part of their library. Thus, the authors are open to feedback in case anyone wants to reach them. Meanwhile, the authors wholeheartedly expect their readers to enjoy each chapter in the same fashion as they did as they paved their way to publication.

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CHAPTER 2

The genetics of reproductive behavior

John Hunt[1,2], James Rapkin[1], and Clarissa House[2]

[1] *Centre for Ecology and Conservation, University of Exeter, Cornwall Campus, Penryn, TR10 9EZ, UK*
[2] *School of Science and Health and the Hawkesbury Institute for the Environment, Western Sydney University, Hawkesbury Campus, Locked Bag 1797, Penrith, NSW, Australia*

2.1 Introduction

Understanding the relative contribution of genes and the environment to observed variation in behavior has been the central aim of behavioral geneticists for nearly six decades. This is clearly an important endeavour as behavior must have a genetic basis if it is to evolve and drive key evolutionary processes such as adaptation and speciation (Boake 1994). However, after decades of empirical research on this topic, it is safe to say that the majority of researchers would agree that most (if not all) behaviors have a genetic basis, but are also influenced, to some degree, by the environment. Consequently, the question is no longer whether behavior is under genetic control, but rather what is the distribution of genetic effects for behavior (many genes with a small effect or few genes with a large effect), how do these genes interact with each other, with genes for other traits, and with the environment, and what are the wider implications of this complex genetic architecture to the evolutionary process?

Insects have played a key role in the understanding of how genes influence behavior. This is for three main reasons. First, many behaviors in male and female insects are highly stereotyped, meaning they are performed the same way each time. This enables behavior to be easily and accurately quantified for a large number of individuals. Secondly, the short generation times and high fecundity of many insect species (relative to vertebrates) makes them well suited to a variety of quantitative genetic breeding designs that span a few (parent–offspring regression, full and half-sibling designs) or multiple generations (i.e. artificial selection). Furthermore, many insect species can be inbred without a substantial decline in fitness, enabling inbred and iso-female lines to be easily created and used in various crossing designs (e.g. diallel) to estimate **non-additive genetic variance** and to create mapping populations for genomic analysis. Finally, many insects have a relatively simple genome (compared with vertebrates) that is well-annotated, notable examples include *Drosophila melanogaster*, the silk moth (*Bombyx mori*), and honey bees (*Apis mellifera*). This increases the ease and effectiveness of genomic studies investigating the specific gene(s) that regulates

Hunt, J., Rapkin, J., and House, C., *The genetics of reproductive behavior.* In: *Insect Behavior: From mechanisms to ecological and evolutionary consequences.* Edited by Alex Córdoba-Aguilar, Daniel González-Tokman, and Isaac González-Santoyo: Oxford University Press (2018).
DOI: 10.1093/oso/9780198797500.003.0002

behavior in insects (e.g. Mackay et al. 2005). Given these features, it is not surprising that the genetic basis of a large variety of different behaviors have been investigated in insects (e.g. foraging, Page et al. 1995; personality, Løvlie et al. 2014; courtship and mating, Gaertner et al. 2015; division of labour, Smith et al. 2008; learning, Dunlap and Stephens 2014; and grooming, Hamiduzzaman et al. 2017), making this topic an incredibly broad one.

In an attempt to help narrow this extensive list, this chapter will focus exclusively on the genetics of insect reproductive behavior. It takes a broad view of 'behavior' as 'the response of an individual to a particular stimulus' and 'reproductive behavior' as 'any behavior that influences reproduction in either sex'. The view presented here includes obvious reproductive behaviors, such as choice of mate, courtship and mating displays, and oviposition preference, as well as a range of traits in males and females that are not traditionally viewed as behaviors. This includes fecundity that can be increased by females in response to a variation in male quality (e.g. Kotiaho et al. 2003) or with impending mortality (e.g. Staudacher et al. 2015). Likewise, it includes several sexual traits in males, such as cuticular hydrocarbons (e.g. Kent et al. 2008) and acoustic signals (e.g. Kasumovic et al. 2012), that can be rapidly altered in response to changes in the social environment.

We cover a range of topics in this chapter that are considered to be fundamental to the understanding of the genetics of insect reproductive behavior. In the first section, it is argued that the majority of insect reproductive behaviors are governed by many genes (i.e. polygenic), that each have a small effect, and use empirical evidence from quantitative genetic studies and genomic approaches to support this argument. Section 2.2 examines the exception to this general polygenic rule, where insect reproductive behavior is determined by a small number of genes of major effect. Although empirical support for genes of major effect is currently weak for reproductive behavior and limited to species with well-annotated genomes, it is suspected that this view may change as the number of genomic studies increase. Section 3 shows that genes for insect reproductive behavior are often associated with genes for a diversity of other important traits, including those involving morphology and life-history. This suggests that reproductive behaviors are unlikely to be free to evolve independently. Section 4 examines the importance of non-additive genetic effects (dominance and epistasis) to insect reproductive behavior. While greatly under-studied, it is likely that dominance and epistasis make important contributions to the observed variation in insect reproductive behaviors. Section 5 shows that the genes for reproductive behavior in many insect species interact with both the abiotic and social environment. This indicates that the influence of genes on reproductive behavior is likely to be highly context-dependent, with **genotype-by-environment (GEI)** and **genotype-by-social environment** interactions both complicating the link between genotype and phenotype. The final section focuses on the wider implications that genetic architecture has for the evolution of insect reproductive behavior, as well as outline some future research directions that the authors view as exciting and deserving of more attention.

2.2 Reproductive behaviors in insects are polygenic and each gene has a small effect

Most behaviors in animals, especially those associated with reproduction, are complex quantitative traits that show considerable variation along a continuous (and normal) distribution of phenotypes. Early theoretical models for the inheritance of quantitative traits assume that they are controlled by an infinite number of loci, each having an infinitely small effect; the so-called 'infinitesimal' model (Fisher 1918; Bulmer 1980; Barton et al. 2016). Under this model, the genome is treated as a 'black box' with genetic effects described through statistical parameters (such as variances and covariances), rather than focusing on the effects of individual loci, which are considered to be small and unmeasurable (Falconer and Mackay 1996; Barton and Keightley 2002; Conner and Hartl 2004).

2.2.1 Exploring genetic variation in insect reproductive behavior using quantitative genetics

Before we can discuss how **genetic variances** and covariances can be used to describe the importance

of genetic effects on quantitative traits, we must first provide a brief overview of the basic principles of quantitative genetics. Quantitative genetics posits that the phenotype, P, of an individual is the sum of the effects of genes, G, and the environment, E (Falconer and Mackay 1996; Lynch and Walsh 1998):

$$P = G + E \quad [2.1]$$

Quantitative geneticists, however, typically focus on partitioning phenotypic variation within a population to genetic and environmental sources, rather than focusing on the phenotype of specific individuals. Therefore, Eqn [2.1] can be expressed in terms of population variances as:

$$V_P = V_G + V_E \quad [2.2]$$

where V_P is the **phenotypic variance**, V_G is the genetic variance, and V_E is the **environmental variance** in the population. Eqn [2.2] represents the simplest way that V_P can be partitioned into genetic and environmental sources, and is most useful when considering clonal (or highly self-fertilizing) organisms because the parental diploid genotypes are replicated in the offspring. It is less useful in sexually reproducing organisms where novel genotypes are created in each offspring by a random combination of one allele from each parent at each locus. In these species, we need to further partition V_G:

$$V_G = V_A + V_D + V_I \quad [2.3]$$

where V_A is the **additive genetic variance**, V_D is the **dominance variance**, and V_I is the epistatic variance. V_A is the most important form of genetic variation for sexually reproducing organisms because only the additive effects of genes are transmitted directly from parents to offspring and, therefore, contribute to changes in phenotype across generations. V_D and V_I are collectively referred to as non-additive genetic variance. Unlike V_A, V_D, and V_I are not directly transmitted from parents to offspring.

Historically, the environment was considered to have a 'random' (and non-genetic) effect on phenotype and is viewed as a source of variation that reduces the resemblance between parents and offspring. When the environment influences phenotype in this way it generates **general environmental variance** (V_{Eg}). However, in many cases, the environment is provided by other individuals in the population. This social environment is often experienced non-randomly by a given individual, for example, as occurs when a parent provisions their offspring. Collectively, the effect of the social environment on phenotype is referred to as **special environmental variance** (V_{Es}) and we discuss this source of variance further in section 6. Just like V_G, V_E can therefore also be further partitioned as:

$$V_E = V_{Eg} + V_{Es} \quad [2.4]$$

Finally, Eqns [2.1] and [2.2] assume that genes and the environment have independent effects on phenotype, which is unlikely to ever be the case. GEIs exist whenever genotypes respond differently to environmental variation and this can also represent an important source of variance in phenotype (V_{GEI}).

Eqn [2.2] can now be extended to include all of the previously mentioned sources of phenotypic variation in the population:

$$V_P = V_A + V_D + V_I + V_{Eg} + V_{Es} + V_{GEI} \quad [2.5]$$

2.2.2 Estimating genetic variance and heritability for phenotypic traits

The central aim of quantitative genetics is to estimate the variance components outlined in the above equations, especially V_G and V_A, and a variety of different breeding designs that use individuals of known relatedness are used to achieve this aim. A commonly used metric to describe the importance of genes to phenotypic variation is the **heritability**. Heritability is simply the ratio of genetic variance to total phenotypic variance and, therefore, estimates theoretically range from 0 to 1. Importantly, however, it can be measured in two ways: as a broad-sense estimate (H^2) or a narrow-sense estimate (h^2). H^2 is estimated as V_G/V_P, whereas h^2 is estimated as V_A/V_P. Consequently, h^2 is more informative as an agent of evolutionary change than H^2. The benefit of both metrics, however, is that dividing V_A and V_G by V_P means that the relative importance of genes can be compared across different traits and studies. However, it is important to remember that because H^2 and h^2 are ratios, changes in both the numerator (V_A or V_G) and the denominator (V_P) can influence the magnitude of these parameters. Thus, it is possible that differences in H^2 and h^2 may also reflect differences in V_D, V_I, V_E and/or V_{GEI}.

Next, some of the commonly used laboratory approaches to estimate the genetic contribution to phenotypic variation are outlined, placing particular emphasis on their strengths and weakness.

- *Common garden experiment*: This is the simplest way to demonstrate that a phenotypic trait has a genetic basis. Individuals from different populations that exhibit natural variation in a given phenotypic trait are collected and reared under the same environmental conditions in the laboratory, and the divergence in phenotype is again assessed across populations. If the populations are still divergent then the phenotypic trait has a genetic basis, whereas if differences are no longer apparent then this initial divergence is due to the environment. While this approach is relatively simple to implement, it does not allow key genetic parameters (such as h^2) to be estimated. Furthermore, at least two generations of **common garden experiment** rearing are needed to remove any population differences due to maternal effects.
- *Parent–offspring regression*: Each male is mated to a single female in the parental generation and the phenotypic trait of interest is measured for one (or both) parent(s). Offspring are reared under the same environmental conditions and the trait measured at the same age (or developmental stage) as their parents. The average of the trait in offspring for each family is then regressed against the parental value(s)—either one parent or the average of both—using linear regression. The slope of this regression line can be used to estimate the H^2 for the trait of interest. If the average phenotypic value of both parents is used in the regression, the slope equals H^2 and if the phenotypic value of only one parent is used, H^2 is twice the slope. While being one of the simpler breeding designs to execute, it is possible for estimates of H^2 to be biased if maternal and/or paternal or dominance effects are large, and if the environments experienced by parents and offspring are dramatically different.
- *Full-sibling analysis*: A full-sibling design is identical to the parent–offspring regression, with the exception that the trait of interest is *not* measured in the parents. Instead the among-family variance is estimated using a one-way analysis of variance (ANOVA). Just like the parent–offspring regression, estimates of H^2 can be biased by maternal and/or paternal effects and dominance variance, and because V_G is extracted from a 'family' term in the ANOVA, it does not allow the effects of mothers and fathers to be statistically separated. However, as the phenotypic trait of interest is not measured in both parents and offspring, the issue of differences in the environment experienced by parents and offspring is no longer a concern when estimating H^2.
- *Half-sibling analysis*: In this approach a series of males (sires) are each mated to a unique set of randomly chosen females (dams) and the trait of interest is measured for a number of offspring for each dam. Consequently, this design differs from the full-sibling design in that each sire is mated to multiple females, meaning that the design contains full-siblings (same mother and father), half-siblings (different mother, same father) and unrelated offspring (different mother and father). A nested ANOVA (with dams nested within sires) can then be used to determine the independent effects of males and females on offspring phenotype. This design is considered the 'gold standard' because V_A (and ultimately h^2) can be estimated through sires and, therefore, is free from maternal effects and dominance variance.
- *Inbred and iso-female lines*: Both of these approaches are based on the same principle—fixing a series of genotypes from the general population using inbreeding. In the case of iso- female lines, a series of gravid females are collected from the field, and their offspring isolated and subjected to brother–sister mating to provide individuals for subsequent generations. As females are collected from the field, it can be argued that the genotypes contained in these iso-female lines captures the genetic architecture present in the field, including **linkage disequilibrium**. In the case of inbred lines, male–female pairs are isolated from a laboratory population, mated to produce offspring, and brother–sister mating used to populate subsequent generations. After twenty generations, the inbred lines will be homozygous at 99.98 per cent of loci and will largely be identical by descent. The phenotypic trait of interest can then be measured in individuals from the

different lines and differences across lines assessed using ANOVA. Significant divergence in the trait across lines indicates a genetic variance for the trait, but is not possible to determine whether this genetic basis is due to additive and non-additive gene effects. Furthermore, as only certain genotypes in the population are likely to survive the inbreeding process, it has been argued that iso-female and inbred lines may upwardly bias genetic estimates (David et al. 2005).

- *Crosses*: There are various types of crossing designs available to estimate the various forms of genetic variance contained in Eqn [5]. For example, in a *diallel* cross, male and female parents (typically taken from iso-female or inbred lines) are crossed to produce hybrid offspring in which the trait of interest is measured. The crossing design is usually 'complete' (all possible parental combinations), but partial, reciprocal, or pooled reciprocal designs also exist. A two-factor ANOVA, including male and female genotype plus their interaction as terms in the model, can then be used to analyse the variation in offspring phenotype. The male term can be used to estimate V_A for the trait being examined and a significant interaction term indicates that non-additive genetic variance also contributes to the variation in this trait. More complex, cross-classified designs, such as the North Carolina Design III and the triple test cross, can be then be used to estimate V_D and V_I, respectively (Lynch and Walsh 1998). While crosses provide a powerful way to estimate additive and non-additive genetic variance for phenotypic traits, it is not possible for many sexually reproducing organisms where replicate individuals of a single genotype are not available.
- *Artificial selection*: The evolutionary response (R) of a given phenotypic trait to selection (S) can be predicted by the univariate breeder's equation as $R = h^2S$. Artificial selection experiments that enforce a known regime of selection on a trait and measure the evolutionary response across generations can therefore be used to estimate the h^2 of the trait by rearranging this equation to $h^2 = R/S$. Importantly, this estimate is referred to as a *realized* h^2 because it has been measured after an evolutionary response has already been observed.

2.2.3 Empirical evidence from quantitative genetics for the polygenic control of reproductive behaviors in insects

Studies using quantitative genetics have been instrumental in demonstrating the genetic contributions to differences in insect reproductive behavior (Ewing and Manning 1967; Krebs et al. 1993; Pianka 1999), and this is especially true for reproductive behaviors in insects (Thornhill and Alcock 1983; Arnholt and Mackay 2004; Markow and O'Grady 2005; Shuker and Simmons 2014). Table 2.1 provides a modest collection of quantitative genetic studies that have examined insect reproductive behavior using a variety of different breeding designs. Three clear patterns are apparent in these examples. First, there is a taxonomic bias in the quantitative genetics of reproductive behaviors in insects with the greater majority of studies being conducted on *Drosophila* and a number of field cricket species. This probably reflects the fact that these species are easier to breed in large numbers in the laboratory. Secondly, within these commonly used taxa, the quantitative genetics of reproductive behavior has been examined using a greater variety of breeding designs in *Drosophila*. The fast generation times in *Drosophila* and their suitability to inbreeding makes artificial selection and breeding designs based on inbred and iso-female lines possible. These approaches are much more difficult and time-consuming in other insect species. Finally, there does not appear to be any consistent differences in h^2 estimates across insect species, the sexes, the different reproductive behaviors examined, or the different breeding designs used. h^2 estimates vary from 0.03 to 0.90, although most are upwards of 0.20. This clearly shows that reproductive behavior has a strong genetic basis in insects and is likely to be under the control of many genes (polygenic).

2.2.4 Revealing specific gene effects through quantitative trait loci mapping and 'omics' approaches

A key development in the genetic analysis of quantitative traits has been the establishment of a large collection of molecular markers that have been used to construct genetic maps for a number of insect

Table 2.1 Some examples of quantitative genetic studies of reproductive behavior in insects.

Insect Order	Species name	Common name	Experimental design	Sex	Behavior examined	Heritability estimate	Reference
Coleoptera	*Callosobruchus maculatus*	Cowpea seed beetle	Parent–offspring regression	Female	Oviposition preference	0.35–0.88[A]	[1]
	Onthophagus taurus	Dung beetle	Half-sib	Female	Offspring provisioning	0.13 ± 0.09	[2]
Diptera	*Drosophila melanogaster*	Fruit fly	Inbred lines	Male	Courtship and mating behaviors	0.03–0.09[B]	[3]
			Iso-female lines	Female	Early life mating frequency	0.63 ± 0.18	[4]
			Artificial selection	Male	Courtship song structure	0.26 ± 0.03	[5]
				Female	Fecundity	0.28 ± 0.11	[6]
	Drosophila serrata	Fruit fly	Parent–offspring regression	Male	Cuticular hydrocarbons	0.06–0.73[C]	[7]
	Drosophila simulans	Fruit fly	Artificial selection	Female	Preference for ebony males	0.26 ± 0.11	[8]
Hymenoptera	*Nasonia vitripennis*	Parasitoid wasp	Half-sib	Female	Polyandry	0.03–0.82[D]	[9]
Lepidoptera	*Achroida grisella*	Pyramid moth	Half-sib	Female	Preference for male song	0.21 ± 0.13	[10]
	Euphydryas editha	Edith's checkerspot butterfly	Parent–offspring regression	Female	Oviposition preference	0.90	[11]
Mescoptera	*Panorpa vulgaris*	Scorpion fly	Half-sib	Male	Fighting ability[E]	1.07 ± 0.44	[12]
Orthoptera	*Gryllus bimaculatus*	Field cricket	Artificial selection	Male	Sperm length	0.52 ± 0.06	[13]
	Gryllodes sigillatus	Decorated cricket	Parent–offspring regression	Male	Spermatophylax investment	0.47 ± 0.21	[14]
	Gryllus firmus	Sand cricket	Half-sib	Male	Courtship song components	0.10–0.35[F]	[15]
		Sand cricket	Full-sib	Female	Oviposition behavior	0.17–0.45[G]	[16]
	Teleogryllus commodus	Black field cricket	Half-sib	Male	Advertisement call structure	0.17–0.72[H]	[17]
	Teleogryllus oceanicus	Polynesian field cricket	Half-sib	Male	Courtship call components	0.06–0.60[I]	[18]

References: [1] Fox (); [2] Hunt and Simmons (2002); [3] Gaertner et al. (2015); [4] Travers et al. (2016); [5] Ritchie and Kyriacou (1996); [6] Rose (1984); [7] Hine et al. (2004); [8] Sharma et al. (2010); [9] Shuker et al 2007; [10] Jang and Greenfield (2000); [11] Singer and Thomas (1988); [12] Thornhill and Sauer (1992); [13] Morrow and Gage (2001); [14] Sakaluk and Smith (1988); [15] Webb and Roff (1992); [16] Réale and Roff 2002; [17] Hunt et al. (2007); [18] Simmons et al. (2010).

Notes:

[A] Heritabilites calculated as the regression of family average preference on parental preference in two different populations (Bay Area and Davis). Courtship and mating behaviors include movement patterns—orientating towards female, approaching female, wing vibration, genital licking, attempted copulation, and copulation.

[C] Measured a cocktail of four different CHC components. [D] Behaviors measured include courtship duration and copulation duration.

[F] Song components include pulses per chirp, pulse length, pulse rate, chirp length, and frequency. [G] Oviposition behaviors measured include digging depth, egg depth, egg distribution, and fecundity.

[H] Call components measured include chirp pulse number, chirp inter-pulse duration, trill number, inter-call duration, and dominant frequency.

[I] Call components measured include chirp length, chirp pulse interval, chirp pulse length, pulses per chirp, chirp-trill interval, trill length, trill pulse interval, trill pulse length, and pulses per trill.

species (e.g. Hill 2012). These markers are the foundation for **quantitative trait loci** (QTL) mapping approaches, which includes techniques such as single-marker, interval, and multiple trait mapping. QTL mapping allows for the statistical analysis of associations between phenotype and genotype, and the dissection of the regions of the genome that significantly contribute to the variation of quantitative traits (Hill 2012). It aims to open the 'black box' of quantitative genetics by locating and identifying the genomic regions responsible for quantitative genetic variation.

However, even when significant associations between quantitative traits and molecular markers are identified, studies have found that these genomic regions are often too large (and too expensive) to identify the specific genes that contribute to genetic variation (Doerge 2002; Hill 2012). Fortunately, the rapid development (and reduction in cost) of a high throughput 'omic' methods, such as **genome-wide association studies** (GWAS), has provided an opportunity to identify some of these genes. Genome sequences have the potential to provide a comprehensive list of genes in an organism and functional **genomics** approaches can then be used to generate information about gene functions, and about genetic interactions between gene complexes and the environment.

2.2.5 Identifying QTLs and candidate genes of interest for insect reproductive behavior

Initial QTL approaches were linkage-based analysis, which used related individuals (specifically F_1 individuals originating from inbred lines) to provide an observable number of loci to identify segregating genetic markers. These F_1 individuals were then crossed and the segregation of genetic markers and QTLs in the F_2 generation statistically modelled. However, further developments have accommodated the use of composite mapping, multiple loci and family analysis in random-mating populations (Doerge 2002; Mackay et al. 2009; Hill 2012). This has led to the use of a number of linkage-based methodologies to detect QTLs associated with a quantitative trait of interest. These include:

- *Single-marker analysis*: Single-marker tests using *t*-tests, ANOVA, or simple linear regression, assess the segregation of a phenotype with respect to a marker genotype. These tests ascertain which markers are associated with the quantitative trait of interest and suggest the existence of QTLs. Studies using single-marker analyses deal primarily with detecting individual markers, rather than genomic regions and are useful for screening a large population for specific traits (Hill 2012).
- *Genetic-linkage maps*: Single-marker analyses investigate individual genetic markers without any reference to their position, order on the chromosome, or relative distances between these genetic markers. Additional genetic information can be gained about the interactions between these markers by placing them in map order. A genetic-linkage map, therefore, provides a genetic representation of the chromosome on which the markers and QTL reside (Mackay et al. 2009; Hill 2012).
- *Interval mapping*: Uses an estimated genetic map as the framework for determining the location of QTLs. Interval mapping statistically tests for a single QTL at each increment across the ordered genetic markers in the genome (Lander and Botstein 1989).
- *Multiple QTL*: Statistical approaches for locating multiple QTLs are more powerful than locating single QTLs because they can potentially differentiate between linked and/or interacting QTLs. However, locating multiple QTLs is a more complicated approach due to the large number of potential QTLs and their interactions. A number of methods have been developed to test for multiple QTLs. A simple technique is to first identify a single QTL, then to build a statistical model with these QTLs and their interactions, and then search in one dimension for significant interactions. However, one-dimensional searches can be challenged by the multiplicity of the effects of QTL interactions. An alternative approach is to split the search for interactions between QTLs into two parts—the relationship between QTLs and the quantitative trait, and the location of the QTLs (Mackay et al. 2009).

Linkage-based analyses have proved highly successful in identifying QTLs associated with variation in quantitative traits. However, limitations such as the inability to do finer scale mapping has seen linkage-based analyses being replaced with 'association

Table 2.2 Some examples of QTL-based studies showing the location and number of genes for reproductive behavior in insects.

Insect Order	Species name	Common name	Sex	Behavior examined	Location of genes/loci controlling behavior	Number of genes/loci controlling behavior	Reference
Diptera	*Drosophila elegans/gunungcola*	Fruit fly	Male	Wing spot	Ch 3 and X	4 QTL[A]	[1]
				Courtship wing display			
	Drosophila melanogaster	Fruit fly	Male	Courtship song	Ch 2, 3, 4, 5, and X	21 QTL[B]	[2] [3]
			Male and female	Cuticular hydrocarbon production	Ch 2, 3, and X	15–25 QTL	[4]
			Male	Courtship and copulation occurrence and latency	Ch 2, 3, and X	4 QTL[C]	[5]
			Male and female	Aggression	Ch 2 and 3	5 QTL[D]	[6]
			Male and female	Aggressive behavior	—	10 genes identified[E]	[7]
	Drosophila simulans/sechellia	Fruit fly	Male	Courtship song	Ch 2, 3, and 4	1–20 QTL[F]	[8]
	Drosophila virilise	Fruit fly	Male	Courtship song	Ch 2, 3, 4, and X	8–13 QTL[G]	[9]
Hymenoptera	*Nasonia giraulti/oneida*	Jewel wasp	Male and female	Male pheromone production	Ch 1, 2, 3, and 4	1–3 QTL[H]	[10]
				Male courtship behavior			
				Female mate discrimination			
Lepidoptera	*Achroia grisella*	Lesser wax moth	Male and female	Male courtship song	-	20–25 QTL[I]	[11]
				Female preference			
Orthoptera	*Laupala paranigra/kohalensis*	Hawaiian cricket	Male	Courtship song	Ch 1, 3, 4, 5, and X	5 QTL	[12]

References: [1] Yeh et al. (2006); [2] Etges et al. (2006); [3] Etges et al. (2007); [4] Foley et al. (2007); [5] Moehring and Mackay (2004); [6] Edwards and Mackay (2009); [7] Zwarts et al. (2011); [8] Gleason and Ritchie (2004); [9] Huttunen et al. (2004); [10] Diao et al. (2016); [11] Limousin et al. (2012); [12] Shaw et al. (2007).

Notes:

[A] Two pairs of loci, *y* and *Moe*,on the X chromosome, and *e* and *TfIIA-L* on the third chromosome right arm.

[B] Six loci (2_2868a, 2_6540c, 2010, 2030, 2_1603a, 2200) on chromosome 2, three loci (3030, 3101, 3100) on chromosome 3, five loci (4010, 4050, 4300, 4301, 4302) on chromosome 4, three (5_1232a, 5100, 5200b) on chromosome 5 and four loci (X010, X030, X090, X110) on X chromosome.

[C] One on chromosome 2, two on chromosome 3 and one on X chromosomes. [D] Two on chromosome 2 and three on chromosome 3.

[E] Genes encompassed many biological and molecular processes—a transcription factor (*mbl*), protein kinases (*Doa*), a guanine exchange factor (*siz*), an NMDA receptor subunit (*Nmdar1*), a UDP-glucose transferase (*sgl*), an extracellular matrix protein (*LanA*), a cell adhesion molecule (*ed*), two Notch signalling regulation genes (*neur* and *Gp150*).

[F] Eight found on X chromosome, sixteen found on chromosome 2, twenty found on chromosome 3, one found on chromosome 4.

[G] Eight significant loci markers affecting variation in pulse train length (one on chromosome 2, six on chromosome 3 and one on chromosome 3) and thirteen significant loci markers affecting variation in pulse train (four on chromosome 2, six on chromosome 3 and two on chromosome 4).

[H] Three found for male pheromone quantity (one on chromosome 1, one on chromosome 4, one on chromosome 5), one found on chromosome 4 for male courtship behavior and one loci on chromosome 3 for female mate discrimination. Ten candidate genes on chromosome 1 were associated with copulation success and five associated with copulation success, six candidate genes on chromosome 3 were associated with copulation success, five candidate genes on chromosome 4 were associated with pheromone quantity and three candidate genes on chromosome 5 were associated with pheromone quantity.

[I] Between two brood groups twenty QTLs found in brood Xt7 and 25 QTLs in Xt19. Most QTLs were distributed among thirty linkage groups in the *A.grisella* genome, but the authors did not find any obvious cluster of QTLs in certain chromosomes.

mapping', which uses individuals from natural populations that experience linkage disequilibrium (Mackay et al. 2009; Ott et al. 2011). Linkage disequilibrium is a sensitive indicator of the population genetic forces that structure the genome (Falconer and Mackay 1996; Lynch and Walsh 1998) and the resultant strong association between markers and QTLs it generates allows for much finer mapping and potentially uncovers the specific genes or mutations that are responsible for quantitative genetic variation (Ott et al. 2011; Hill 2012).

Association studies are routinely conducted across the entire genome using GWAS. GWAS are performed by genotyping many thousands of **single-nucleotide polymorphisms** (SNPs), which are single base-pair changes occurring at a high frequency in a DNA sequence and are used as genetic markers in GWAS (Ott et al. 2011; Bush and Moore 2012). GWAS utilize many of the same basic methodologies as linkage-based QTL studies and have become highly successful in identifying QTLs for quantitative traits of interest in a variety of species (e.g. Stranger et al. 2011; Bush and Moore 2012). Furthermore, because many GWAS use samples from the entire population, they potentially reflect natural genetic variation in quantitative traits, allowing for more accurate predictions about the underlying genetic architecture (Mackay et al. 2009; Ott et al. 2011). However, there are limitations to GWAS approaches, namely the low number of insect species that have a library of fully sequenced genomic data available, which can affect the availability of SNP markers and make detecting QTLs with strong environmental effects harder to detect (Hill 2012). Furthermore, methods for incorporating GEIs in GWAS studies are currently lacking.

2.2.6 Empirical evidence from QTL-based studies examining the polygenic control of reproductive behaviors in insects

Table 2.2 provides some examples of empirical studies utilizing QTL analyses to examine the polygenic control of reproductive behaviors in a number of insect species. Similar to the examples provided in Table 2.1, there is a strong taxonomic bias in studies using QTL-based approaches to locate the position and number of genes (or QTL regions) responsible for insect reproductive behavior. By far the greatest numbers of studies have used *Drosophila* (especially *D. melanogaster*) as a model and this probably reflects the availability of a fully mapped genome for a number of *Drosophila* species, which makes identifying genes of interest much more efficient and easier than in other insect species (Markow and O'Grady 2005). The examples provided show a simple pattern. In most cases, multiple QTLs have been identified, providing further support for the polygenic control of reproductive behaviors in insects. Furthermore, in those instances where the location of QTLs was identified, they appear to be spread across the entire genome. Perhaps not surprisingly, a large number of these studies (six of the nine studies where the location of QTLs was identified) have shown that QTLs for insect reproductive behavior occur on the X chromosome. This supports the more widespread view that the X chromosome is a 'hot spot' for genomic evolution (e.g. Bailey et al. 2004). Finally, other than the increased occurrence of QTLs on the X chromosome, there does not appear to be any consistent patterns in the location or number of QTLs across the different reproductive behaviors. For example, the production of a courtship song is influenced by a large number of QTLs in *Drosophila* (typically more than ten QTLs), but is only influenced by five QTLs in *Laupala*. Whether this represents a more widespread taxonomic difference between Diptera and Orthoptera, however, will require more empirical testing.

2.3 Genes that have a major effect on insect reproductive behavior: the exception to the polygenic rule

In Section 2.2, we make the argument that insect reproductive behavior is polygenic and provides empirical support from quantitative genetic and QTL-based studies to support this argument. We would be negligent, however, if we did not mention the obvious exception to this argument—when single genes (or a small number of genes) have a major effect on reproductive behavior. Genes of major effect have been shown to be important for a range of non-reproductive behaviors in insects, including stinging behavior in the honey bee (*Apis mellifera*; Hunt et al. 1998) and feeding behavior in the pea

aphid (*Acyrthosiphon pisum*; Caillaud and Via 2012). There is also evidence to suggest that genes that have a major effect on non-reproductive behavior in insects may also have a conserved function in other taxonomic groups. For example, the *for* gene and its associated orthologs have been found to be responsible for variation in foraging behavior in a number of insect species, including *Drosophila melanogaster* (e.g. Allen et al. 2017), honey bees (Ben-Shahar et al. 2002) and ants (e.g. Malé et al. 2017).

Unfortunately, there are only a limited number of studies that have examined genes of major effect on insect reproductive behavior. Table 2.3 provides an overview of existing empirical studies that have used a variety of QTL mapping and GWAS analysis to locate genes that have a major effect. The majority of studies have identified genes of major effect for reproductive behaviors in *Drosophila*. Work on this genus has identified genes having a major effect for male courtship behaviors, especially elements of the courtship song (Table 2.3). For example, both the *fruitless* (*fru*) and *doublesex* (*dsx*) sex determining genes in *D. melanogaster* are located in close proximity on the right arm of the third chromosome and play a key role in courtship song production (Rideout et al. 2007). Similarly, genes having a major effect on male courtship song have also been documented in the brown planthopper (*Nilaparvata lugens*, Butlin 1996) and the Australian field cricket (*Teleogryllus oceanicus*, Tinghitella 2008), although in the latter species this relationship is driven by a wing mutation (*flatwing*) at a single loci, which results in males lacking the wing apparatus needed to produce a courtship song. Finally, genes having a major effect on **pheromone** production have been identified in females of two lepidopteran species (*Heliothis subflexa* and *Ostrinia nubilalis*; Lassance et al. 2010; Groot et al. 2013). Both studies have been facilitated by the availability of a well-annotated reference genome for the moth, *Bombyx mori*.

Table 2.3 Examples of genes of major effect on reproductive behavior in insects.

Insect Order	Species name	Common name	Sex	Reproductive behavior examined	Number of genes involved	Reference
Diptera	*Drosophila melanogaster*	Fruit Fly	Male	Courtship behavior	Fruitless '*fru*' gene[A]	[1]
			Male	Courtship Song	Doublesex '*dsx*' gene[A], and '*fru*' gene	[2]
			Male	Interpulse interval courtship song	3 loci	[3]
	Drosophila virilis/*littoralis*		Male	Courtship song	2-6 loci	[4]
	Drosophila elegans/*gunungcola*		Male	Wing pigmentation and display	'Few' loci	[5]
Homoptera	*Nilaparvata lugens*	Brown planthopper	Male and female	Courtship song and female response	1.5–5 loci	[6]
Lepidoptera	*Heliothis subflexa*	Noctuid moth	Female	Pheromone production	KAIKOGA052256 BGIBMGA013924 BGIBMGA013740	[7]
	Ostrinia nubilalis	European corn borer	Female	Pheromone production	pgFAR	[8]
Orthoptera	*Teleogryllus oceanicus*	Polynesian field cricket	Male	Presence/absence of courtship song	1 loci	[9]

References: [1] Ryner et al. (1996); [2] Rideout et al. (2007); [3] Gleason et al. (2002); [4] Hoikkala et al. (2000); [5] Yeh et al. (2006); [6] Butlin (1996); [7] Groot et al. (2013); [8] Lassance et al. (2010); [9] Tinghitella (2008).

Notes:

[A] Both the '*fru*' gene and '*dsx*' gene are located on the right arm of chromosome 3.

2.4 Genes for reproductive behavior are often linked to other traits

We have so far limited our discussion to individual insect reproductive behaviors. Organisms, however, are not simply collections of independent phenotypic traits, but rather these traits are often interconnected at the genetic level due to shared functional, developmental, and/or physiological pathways (Falconer and Mackay 1996; Conner and Hartl 2004). This genetic association means that phenotypic traits are seldom free to evolve independently in the population, with a change in one trait influencing the expression of any other traits genetically associated with it. Quantitative genetic theory posits that the strength of the genetic association between two traits can be quantified through the sign and magnitude of the **genetic correlation** (Falconer and Mackay 1996; Lynch and Walsh 1998). As with phenotypic correlations, values for genetic correlations range from –1 to +1. The closer the genetic correlation is to these limits, the stronger the association is between the genes for the two traits,whereas the sign indicates whether the genes that increase one trait are linked to genes that increase (a positive correlation) or decrease (negative correlation) the second trait.

A genetic correlation between two traits can be generated in two ways: pleiotropy and linkage disequilibrium (Falconer and Mackay 1996). **Pleiotropy** generates a genetic correlation between two traits when a locus has a casual effect on both traits. In contrast, linkage disequilibrium will generate a genetic correlation between two traits when the alleles at two or more loci for the traits are associated with a higher or lower degree than would be expected through random association (Falconer and Mackay 1996). Genetic correlations generated through pleiotropy are expected to evolve, either through adaptation or by genetic drift, and are produced by common functional mechanism(s) that underlie the production of these correlated traits. Correlations through pleiotropy are not expected to break down in the population through neutral processes, such as random genetic drift. In contrast, genetic correlations generated by linkage disequilibrium are expected to be temporary, contributing very little to evolutionary change, and are expected to be eroded over time through recombination (Falconer and Mackay 1996; Lynch and Walsh 1998; Saltz et al. 2017).

It is well established in quantitative genetic theory that selection rarely targets single phenotypic traits in isolation and that traits are often genetically correlated (Lande 1979; Lande and Arnold 1983). Furthermore, it has been known for well over three decades that this pattern of complex selection and the genetic variance in and covariance between traits can be used to predict the phenotypic evolution of traits across generations with the multivariate breeder's equation:

$$\Delta\bar{z} = \beta\mathbf{G} \qquad [2.6]$$

where $\Delta\bar{z}$ is the vector of phenotypic responses of traits across generations, β is the vector of linear selection gradients targeting those traits and $\mathbf{G}$ is a matrix of genetic variances in, and covariances between, these traits (Lande 1979). The key outcome of this equation is that the evolution of a given phenotypic trait is not only due to selection *directly* targeting the genetic variance in the trait, but also *indirectly* due to selection targeting other, genetically correlated traits. Consequently, to understand how a reproductive behavior evolves, it is necessary to know both the genetic variance in this behavior and how this behavior is genetically correlated with other important traits under selection.

2.4.1 Estimating genetic correlations between traits using quantitative genetics

The quantitative genetic breeding designs outlined in Section 2.2 can be used to estimate the genetic correlation between different traits (Falconer and Mackay 1996; Lynch and Walsh 1998). The key difference is that because the genetic relationship between two traits is now being examined, it is necessary to estimate the genetic variance in both traits, as well as the genetic covariance between the traits. As was discussed for heritability estimates in Section 2.2, however, genetic correlations have different meaning when estimated from these different breeding designs. That is, genetic correlations can be derived from the additive genetic (co)variance between traits (r_A) and represent a narrow-sense estimate or from the total genetic (co)variance between traits (r_G) and represent a broad-sense estimate (i.e. includes

variance due to dominance and/or epistasis). As with heritability estimates, r_A provides a better estimate than r_G in how the genetic association between different traits directs phenotypic evolution. The following equations outline how r_A and r_G can be estimated from the breeding designs outlined in Section 2.2.

Using a parent–offspring regression, r_G can be calculated by dividing the covariances between different traits X and Y (cov_{XY}) in parents and offspring with the square root product of the covariances between the same traits ($cov_{XX}$and cov_{YY}, respectively) in parents and offspring (Falconer and Mackay 1996; Lynch and Walsh 1998):

$$r_G = \frac{cov_{XY}}{\sqrt{cov_{XX}cov_{YY}}} \qquad [2.7]$$

As there are two possible products of cov_{XY}, there are two estimates of r_G (r_{G1} and r_{G2}) and the arithmetic mean of both estimates is generally provided. When using a full-sibling analysis, r_G is simply calculated as the covariance between mean of the two traits across full-sibling families using a regression (Falconer and Mackay 1996; Lynch and Walsh 1998). Likewise, this approach can also be used to calculate r_G when using inbred or iso-females lines, with the exception that line means for the two traits are used.

Using a half-sibling design, the additive genetic covariance between the two traits can be calculated at the sire level (and, thus, should largely be free from the effects of dominance and epistasis) using a nested analysis of covariance. r_A can then simply be calculated by dividing the additive genetic covariance between the two traits (cov_{XY}) by the square root product of the additive genetic variance in each trait (var_X and var_Y, respectively; Falconer and Mackay 1996; Lynch and Walsh 1998):

$$r_A = \frac{cov_{XY}}{\sqrt{var_X var_Y}}[6] \qquad [2.8]$$

Finally, r_G can be measured in two ways when using artificial selection. First, r_G can be measured indirectly through the correlated response to selection. That is, if a given trait (X) is subject to artificial selection and shows an evolutionary response across generations, then a second trait (Y) can also be measured in the terminal generation. As Y has not been selected directly, any response to the selection regimes would indicate that that X and Y are genetically correlated. This commonly used approach, however, does not measure the strength of r_G, only the sign. If X and Y respond in the same direction, r_G is positive; if X and Y respond in opposite directions, r_G is negative. Secondly, a *double* selection experiment (where X is selected in one line and Y in another) can be used to measure both the direct (R_X and R_Y) and correlated responses (CR_X and CR_Y) of both traits. A joint estimate of r_G can then be obtained as (Falconer and Mackay 1996):

$$r_G = \frac{CR_X}{R_X}\frac{CR_Y}{R_Y}[7] \qquad [2.9]$$

This approach is not often used, however, given that it is twice the work of normal artificial selection experiment.

2.4.2 Empirical examples of genetic correlations between reproductive behavior and other traits in insects

In Table 2.4 we provide some examples of genetic correlations between reproductive behavior and other important phenotypic traits in insects. These examples clearly illustrate that insect reproductive behavior is genetically correlated with a range of other important **life-history traits**. Most available data has examined two important genetic correlations—between lifespan and reproductive behavior, and between immunity and reproductive behavior—that are often collectively viewed as 'costs of reproduction'. For females in the majority of species examined, there is a negative genetic correlation between lifespan and reproductive behavior (most commonly fecundity) and this appears to be independent of the particular quantitative genetic design used. The notable exception to this is the study by Khazaeli and Curtsinger (2010) that found a strong and positive genetic correlation between these traits in female *Drosophila melanogaster* when using inbred lines. The genetic correlation between lifespan and reproductive behavior is less clear in males, being negative in some species (Hunt et al. 2006; Brown et al. 2009) and positive in others (Brandt and Greenfield 2004). Studies on this relationship in males has largely been restricted to

Table 2.4 Examples of empirical studies showing that genes for reproductive behavior in insects are associated with genes for other important phenotypic traits. Standard errors or 95 per cent confidence intervals (in brackets) are provided for estimates of r_A or r_G. In studies using artificial selection, the sign (+ve or –ve) of the genetic correlation is provided.

Insect Order	Species name	Common name	Experimental design	Sex	Reproductive behavior examined	Linked trait	r_A or r_G	Reference
Coleoptera	*Gnatocerus cornutus*	Broad horned flour beetle	Artificial selection	Male	Fighting behavior	Mandible length	+ve	[1]
	Nicrophorus vespilloides	Burying beetle	Artificial selection	Male	Mating rate	Genital shape	+ve	[2]
	Callosobruchus maculatus	Seed beetle	Half-sib	Male	Copulation duration	Lifespan	–0.16 ± 0.20	[3]
	Callosobruchus chinensis	Azuki bean weevil	Half-sib	Female	Fecundity	Lifespan	–0.89 ± 0.12	[4]
Diptera	*Drosophila melanogaster*	Fruit fly	Half-sib	Female	Fecundity	Lifespan	–0.71	[5]
			Inbred lines	Female	Fecundity	Lifespan	0.75	[6]
	Drosophila nigrospiracula		Artificial selection	Female	Fecundity	Immunity to ectoparasitic mite	–ve	[7]
	Bactrocera cucurbitae	Melon fly	Artificial selection	Female	Fecundity	Lifespan	-ve	[8]
Hemiptera	*Bactericera cockerelli*	Potato psyllid	Inbred lines	Female	Fecundity	Lso infection[A]	–0.40	[9]
Hymenoptera	*Apis melifera*	Honey bee	Artificial selection	Male	Worker reproduction	Age at foraging	–ve	[10]
Lepidoptera	*Achroia grisella*	Acoustic moth	Half-sib	Male	Attractiveness	Lifespan	0.64 ± 0.09	[11]
	Pieris napi	White butterfly	Half-sib	Male	Spermatophore weight	Body size	0.35 ± 0.30	[12]
Orthoptera	*Allonemobius socius*	Ground cricket	Full-sib	Female	Fecundity	Presence of wings	–0.53 ± 0.15	[13]
	Gryllodes sigillatus	Decorated cricket	Inbred line	Male	Spermatophylax weight	Encapsulation ability	0.76 ± 0.03	[14]
				Male	Early-life calling effort	Rate of ageing	0.44 ± 0.17	[15]
				Female	Early-life fecundity	Rate of ageing	0.97 ± 0.06	[15]
	Gryllus firmus	Sand cricket	Artificial selection	Female	Fecundity	Proportion of winged morph	–ve	[16]
			Half-sib	Female	Fecundity	Wing morph	–0.86 ± 0.17	[17]
	Teleogryllus oceanicus	Polynesian field cricket	Half-sib	Male	Amount of trill in the courtship song	Encapsulation ability	–0.47 (–0.49, –0.45)	[18]

(Continued)

Table 2.4 Continued

Insect Order	Species name	Common name	Experimental design	Sex	Reproductive behavior examined	Linked trait	r_A or r_G	Reference
					Amount of trill in the courtship song	Haemocyte load	–0.48 (–0.50, –0.46)	[18]
	Teleogryllus commodus	Black field cricket	Artificial selection	Male	Time spent calling	Lifespan	–ve	[19]
			Half-sib	Female	Fecundity	Lifespan	–0.63 ± 0.27	[20]

References: [1] Okada and Miyatake (2009); [2] Hopwood et al. (2016); [3] Brown et al. (2009); [4] Nomura and Yonezawa (1990); [5] Rose and Charlesworth (1980); [6] Khazaeli and Curtsinger (2010); [7] Luong and Polak (2007); [8] Miyatake (1998); [9] Nachappa et al. (2014); [10] Oldroyd and Beekman (2008); [11] Brandt and Greenfield (2004); [12] Wedell (2006); [13] Roff and Bradford (1996); [14] Gershman et al (2010), [15] Archer et al. (2012); [16] Roff et al. (1999); [17] Roff et al. (1997); [18] Simmons et al. (2010); [19] Hunt et al. (2006); [20] Zajitschek et al. (2007).

Notes:

[A]Also infection refers to infection by the bacterium *Candidatus Liberibacter solanacearum*.

insect species where males produce an acoustic signal (field crickets and an acoustic moth), as this provides a much easier way to assess reproductive effort (Hunt et al. 2006) and attractiveness (Brandt and Greenfield 2004) than in species lacking this form of **communication**. In the decorated cricket (*Gryllodes sigillatus*) both sexes show a strong positive genetic correlation between reproductive behaviors early-in-life and the rate of ageing, which further supports the view that reproduction is costly in insects and also demonstrates that reproductive behavior in the sexes (especially females) has important implications for the evolution of lifespan and ageing (Archer et al. 2012).

The examples presented in Table 2.4 also show that a negative genetic correlation between reproductive behavior and immune function is common in insects. Again, this genetic relationship appears to be largely consistent in both males (e.g. Gershman et al. 2010; Simmons et al. 2010) and females (e.g. Luong and Polak 2007; Nachappa et al. 2014) and also appears to be independent of the specific measure of immunity used (i.e. encapsulation ability, haemocyte load, immunity to bacterial or ectoparasite challenge) and the type of breeding design used. In females, fecundity was the most commonly studied reproductive behavior, whereas a much broader range of reproductive behaviors were examined in males, including the production of a large **nuptial gift** (spermatophylax, Gershman et al. 2010) and a more elaborate courtship song (Simmons et al. 2010). In both examples, the negative genetic correlation between immunity and these aspects of reproductive behavior is likely to have important implications for the operation of **sexual selection** in these species, challenging the view that females gain 'good genes' for immune function by mating with males with more elaborate sexual traits.

Reproductive behavior also appears to be genetically correlated with a range of important morphological traits in insects. For example, artificial selection has been used to show that there is a positive genetic correlation between fighting behavior and mandible length in the broad-horned flour beetle (*Gnatocerus cornutus*, Okada and Miyatake 2009), and between mating rate and genital shape in the burying beetle (*Nicrophorus vespilloides*, Hopwood et al. 2016). Furthermore, a positive genetic correlation between spermatophore weight and body size was also shown in the white butterfly (*Pieris napi*, Wedell 2006) using a half-sibling design. Mandible length, genital shape, and body size are all known to be important determinants of male reproductive success in these, as well as other species of insects. Perhaps one of the best known examples of genetic correlations between reproductive behavior and morphology occurs in females of a number of cricket species—the negative genetic correlation between fecundity and the development of long wings (known as macroptery). This relationship has been documented using both artificial selection and sibling designs in two different cricket species, *Allonemobius socius* (Roff and Bradford 1996) and *Gryllus firmus* (Roff et al. 1997, 1999), although considerably more work has been done in the latter species. Although macroptery is an important determinant of flight capability and, therefore, the capacity for **dispersal**, these studies clearly show that the genes for this trait have a negative effect on those for reproduction; a finding that is also supported in male *G. firmus*, where a negative genetic correlation between testis mass and macroptery has also been documented (Saglam et al. 2008).

In the examples provided in Table 2.4, we only examine the genetic correlation between reproductive behavior and other important phenotypic traits using breeding designs. It is also possible to use molecular approaches to quantify the genetic association between reproductive behavior and other traits. For example, Kronforst et al. (2006) used QTL genetic linkage mapping to show an association between male mate preference and female forewing colour in two species of *Heliconius* (*H. cydo* and *H. pachinus*) butterflies. More specifically, mapping places the preference locus in the same genomic region as the locus determining forewing colour, which itself is linked to the wing patterning candidate gene, *wingless*. This suggests that wing colour and colour preference are either controlled by loci that are located in an inversion or result from the pleiotropic effect of a single locus (Kronforst et al. 2006). This tight genetic association between preference and wing colour patterns is likely to have played a key role in the high degree of speciation in the *Heliconius* genus.

2.5 Genes can have non-additive effects on reproductive behavior

As discussed in Section 2.2, additive genetic effects are the most important type of gene action in sexually reproducing species because they are directly transmitted across the generations and, therefore, contribute to evolution in a relatively straightforward manner. However, as noted in Eqn [2.5], non-additive gene effects also contribute to an individual's phenotype. Despite this, the majority of studies that investigate the genetic basis of reproductive behavior (and animal behavior more generally) tend to ignore dominance and epistasis, either because of the difficultly in estimating their effects or because their effects are considered to be unimportant (Meffert et al. 2002; Roff and Emerson 2006). This is an unfortunate trend given that the effects of dominance and epistasis on important phenotypic traits appear to be large, especially for traits that are more closely related to fitness (Roff and Emerson 2006). For example, a review of additive and non-additive genetic effects on morphological and life-history traits found that epistatic effects were detected more often in life-history than in morphological traits (79 versus 67 per cent, respectively), whereas dominance effects were reported for 95 per cent of traits, irrespective of trait type (Roff and Emerson 2006). Furthermore, for both dominance and epistasis, the ratio of non-additive to additive effects in life-history traits is approximately twice as large as for morphological traits (Roff and Emerson 2006). Given the close link to fitness, it is likely that non-additive genetic effects will also be important for insect reproductive behavior.

2.5.1 Estimating the effects of dominance and epistasis on phenotype using quantitative genetics

In Section 2.2, we discuss how a significant interaction between male and female genotypes in a *diallel* breeding design indicates that non-additive genetic effects have an important influence on the phenotypic trait being examined. A number of additional approaches have also been used to show the importance of non-additive genetic effects. The first approach is to examine the difference in the estimates of V_G from a parent-son and a parent–daughter regression. If genes are additive (and autosomal) in effect, half should be inherited from each parent and these regression coefficients should be the same. Any deviance from this (especially when the parent–daughter coefficient exceeds the parent–son coefficient) has been taken as evidence for non-additive genetic effects. The second approach is to compare the genetic variance explained by sires and dams in a nested half-sibling design. Again, any asymmetry in these variance estimates (especially if the dam variance exceeds the sire variance) is often taken as evidence of non-additive genetic effects. The main issue with this approach, however, is a bias in the dam variance, which can be caused by non-genetic maternal effects. It is important to note that none of the above approaches allow dominance or epistatic variance to be directly estimated, just that one or both is likely to be important in determining phenotypic variation.

A large number of different breeding designs are available to directly estimate the contribution of dominance and epistasis to any observed non-additive genetic effects. We do not attempt to cover all of these designs here, but instead provide two commonly used breeding designs to estimate dominance and epistatic variance in phenotypic traits. The first is a line-cross technique (known as the *North Carolina Design III)* that can be used to estimate the degree of dominance (Lynch and Walsh 1998). This approach crosses two parental genotypes (inbred lines, different populations or species are commonly used) to produce an F_1 generation that is then subject to random breeding to generate an F_2 generation. Random members of the F_2 generation are then backcrossed to each of the parental lines and the phenotype of these backcrossed families is measured. If $\bar{z}_1$ and z_2 denote the mean phenotypes of progeny derived from the F_2 individuals backcrossed to parental line 1 and 2, respectively, and the sum of families is $S = \bar{z}_1 + \bar{z}_2$ and the family differences is $\Delta = \bar{z}_1 - \bar{z}_2$, then a one-way ANOVA can be used to estimate the variances of the family sums $[\sigma^2(S)]$ and differences $[\sigma^2(\Delta)]$. In the absence of epistasis and gametic phase disequilibrium, $\sigma^2(S)$ is equivalent to the V_A in the F_2 backcrossed population, while $\sigma^2(\Delta)$ is equivalent to twice the V_D. This approach will lead to inflated estimates of V_D,

however, when gene frequencies are not equal, but when this assumption applies, the benefit of this approach is that it estimates V_A and V_D with nearly equal precision (Lynch and Walsh 1998).

The second approach, known as the *triple test cross*, is specifically designed to test the importance of epistatic variance (Lynch and Walsh 1998). This approach is very similar to the *North Carolina Design III*, with the major exception that F_2 individuals are backcrossed to both the parental lines and the F_1 population (not just the parentals). The logic behind this test is that F_1 individuals produce recombinant gametes, whose average gene expression will deviate from that of the mean of the parental line gametes if epistatic interactions are significant (Lynch and Walsh 1998). If $\bar{z}_3$ represents the mean phenotype of progeny from a backcross between F_1 and F_2 individuals, then $\bar{z}_1 + \bar{z}_2 - 2\bar{z}_3$ will have an expectation of zero in the absence of epistasis. A one-way ANOVA can again be used to test the significance of epistatic variance by evaluating whether then variance among the observed family values of $\bar{z}_1 + \bar{z}_2 - 2\bar{z}_3$ is greater than expected from sampling error. More complex analyses can also be used to estimate additive and dominance effects, as well as partitioning the different forms of epistatic variance (additive × additive epistasis, additive × dominance epistasis, dominance × dominance epistasis; Lynch and Walsh 1998). Furthermore, if reciprocal crosses and backcrosses are included in this design, additive genetic maternal variance, dominance genetic maternal variance, cytoplasmic variance, and Y chromosome variance can also be estimated.

2.5.2 Empirical examples of non-additive genetic effects for insect reproductive behavior

Unfortunately, there are only a handful of empirical studies that have investigated the role of non-additive genetic effects for insect reproductive behavior and we provide an overview of these studies in Table 2.5. The most compelling evidence

Table 2.5 Examples of empirical studies documenting non-additive genetic effects (dominance and/or epistasis) for reproductive behavior in insects.

Insect Order	Species name	Common name	Experimental design	Sex	Behavior examined	Reference
Coleoptera	*Callosobruchus maculatus*	Seed beetle	Reciprocal backcrosses	Female	Egg dispersion behavior[A]	[1]
			Reciprocal backcrosses	Female	Egg dispersion behavior[A]	[2]
	Acanthos celides obtectus	Seed beetle	Reciprocal backcrosses	Female	Oviposition site preference[B]	[3]
Diptera	*Drosophila tripunctata*	Fruit fly	Reciprocal backcrosses	Female	Oviposition-site preference[B]	[4]
	Musca domestica	Housefly	P–O regression	Male and female	Courtship behavior[C]	[5]
			Reciprocal backcrosses	Male and female	Courtship behavior[C]	[5]
	Eurosta soligaginis	Tephritid fly	Reciprocal backcrosses		Oviposition-site preference[D]	[6]
Hymenoptera	*Nasonia vitripennis*	Parasitoid wasp	Half-sib	Female	Polyandry[E]	[7]

References: [1] Fox et al. (2004); [2] Fox et al. (2009); [3] Tucić and Šešlija (2007); [4] Jaenike (1987); [5] Meffert et al. (2002); [6] Craig et al. (2001); [7] Shuker et al. (2007).

Notes:

[A]Egg dispersion behavior describes how uniformly females disperse eggs across seeds.

[B]Oviposition site preference was measured as the number of eggs laid on each host.

[C]A total of eight courtship behaviors were measured in males (mount, close, creep, touch, buzz, lunge, hold, and lift) and two courtship behaviors in females (female and wing out).

[D]Oviposition site preference was measured as the amount of ovipunctures on each host plant.

[E]Polyandry was measured as the product of four female behaviors (receptivity at first courtship (R_1), receptivity after 10 minutes (R_{10}), courtship duration, and copulation duration).

for non-additive effects on reproductive behavior is for egg dispersion behavior and oviposition-site preference in female insects. Egg dispersion behavior has been studied exclusively in female seed beetles (*Callosobruchus maculatus*) and describes how uniformly females disperse their eggs across seeds (Fox et al. 2004, 2009). Using a series of reciprocal backcrosses, Fox et al. (2004) showed that dominance, additive × additive epistasis, and dominance × dominance epistasis all significantly influenced egg dispersion behavior when females were reared on cowpea seeds, but that only the latter two forms of epistatic variance influenced this behavior when females were reared on mung bean seeds. A subsequent study, however, found that additive genetic, dominance, and additive–additive epistasis all influenced female egg dispersion in this species, irrespective of whether females were reared on cowpea or mung bean seeds (Fox et al. 2009).

Oviposition site preference describes the behavior that females exhibit when deciding which host to lay their eggs on when given the choice and the genetics of this behavior has been examined in a more taxonomically diverse range of insect species. In the seed beetle *Acanthos celides obtectus*, additive × additive epistasis, dominance × dominance epistasis and additive × dominance epistasis all influence oviposition site preference when females are reared on bean seeds, whereas additive genetic, dominance, and additive × additive and dominance × dominance epistasis influence this behavior when females are reared on chickpea seeds (Tucic and Seslija 2007). This contrasts work on the seed beetle *C. maculatus* where oviposition site preference in females was best described by an additive model, irrespective of whether females were reared on mung bean or cowpea seeds (Fox et al. 2004). Oviposition site preference has also been examined in a number of dipteran species. In *Drosophila tripunctata*, both dominance and epistasis were shown to be important sources of variation in female oviposition site preference for mushrooms or tomatoes, although the more explicit forms of the epistasis were not examined (Jaenike 1987). In the tephritid fly (*Eurosta soligaginis*), however, dominance, but not epistasis appears to regulate oviposition-site preference in females for two species of goldenrod (*Solidaginis gigantea* and *S. altissima*; Craig et al. 2001).

The importance of non-additive genetic variation to courtship and mating behavior in insects has also been examined. In the housefly (*Musca domesticus*), average heritability estimates for a range of courtship behaviors in males and females were significantly higher from parent–daughter analysis than parent–son analysis suggesting that non-additive genetic variance is likely to contribute to these reproductive behaviors (Meffert et al. 2002). A more detailed analysis using reciprocal backcrosses verified that both dominance and epistasis have important effects on courtship behavior in male and female houseflies but that the exact nature of these genetic effects varied for the different behaviors (Meffert et al. 2002). For example, only additive genetic effects were present for the 'buzz' courtship behavior in females, whereas dominance, dominance × additive epistasis, and dominance × dominance epistasis were important for the 'lunge', 'hold', 'lift wing', and 'wing out' courtship behaviors (Meffert et al. 2002). Furthermore, Shuker et al. (2007) used a nested half-sibling design to show that dam heritability estimates were, on average, seven times greater than sire heritability estimates for four reproductive behaviors linked to **polyandry** (receptivity at first courtship (R_1), receptivity after 10 minutes (R_{10}), courtship duration, and copulation duration) in female **parasitoid** wasps (*Nasonia vitripennis*). While this was taken as evidence that non-additive genetic effects were important for these behaviors, it is important to note that this asymmetry in sire and dam variances could also be driven by non-genetic maternal effects. Thus, further experimentation is needed to verify the contribution of non-additive genetic effects to these reproductive behaviors in *N. vitripennus*.

2.6 Genes for reproductive behavior frequently interact with the environment

Genotype-by-environment interactions (GEIs) exist whenever genotypes respond differently to environmental variation and are often illustrated using reactions norms where the phenotypic value of a trait in each environment is plotted separately for different genotypes (see Chapter 5). Most empirical studies investigating GEIs have focused on the influence of abiotic environments (Hunt and

Hosken 2014). This is especially true for insects, where many studies have documented GEIs involving a wide range of abiotic factors (e.g. Rodríguez and Greenfield 2003; Danielson-Francois et al. 2005; Weddle et al. 2012).

A number of studies have also started to consider GEIs that include the biotic environment, especially the presence of conspecifics or competitors (e.g. Saltz 2013; Pascoal et al. 2016b). Collectively, the environment provided by others in the population is referred to as the 'social' environment and as we outline in Section 2.2, this represents an important source of phenotypic variation referred to as *special environmental variance* (V_{Es}). V_{Es} differs from sources of general environmental variance (V_{Eg}), such as diet and temperature, because the social environment is provided by other individuals in the population. This means that genotype-by-social environment interactions (GSEIs) can have very different effects on the evolutionary dynamics of phenotypic traits compared with GEIs that involve the abiotic environment (Wolf et al. 2014). For example, traits that are influenced by GSEIs are expected to be much more labile and more evolutionarily dynamic than those subject to GEIs, especially in viscous populations with little re-assortment of individuals between environments (Wolf et al. 2014). This is due to the fact that both the focal trait and the social environments evolve simultaneously, making it easier to build and lose genotype-environment combinations compared with GEIs (Wolf et al. 2014).

2.6.1 Estimating GEIs and GSEIs for phenotypic traits using quantitative genetics

Most of the breeding designs outlined in Section 2.2 can be easily modified to estimate the variance in phenotype explained by GEIs or GSEIs. In all cases, this modification involves splitting each genotype across alternate environments, an approach known as a 'split brood' design (Lynch and Walsh 1998). However, the breeding designs in Section 2.2 will differ in the strength of support they provide for a GEI (or GSEI). In the case of common garden and artificial selection experiments, populations and selection lines can be split across environments, and any significant interactions between population or selection line, and the environment taken as evidence of a GEI. These approaches, however, should be interpreted with caution as both populations and selection lines will have a range of different genotypes, rather than a single, fixed genotype. Furthermore, these approaches do not allow the direct estimation of V_{GEI} and, therefore, should only be taken as evidence that GEIs are likely to exist (not as definitive evidence that they do).

In the case of inbred and iso-female lines, as well as full- and half-sibling designs, V_{GEI} can be estimated directly when discrete genotypes are split across environments, although it is only in the latter design that additive × environment interactions can be estimated. In each design, the statistical models outlined in Section 2.2 can easily be extended to include an 'environment' term as a fixed effect, and V_{GEI} estimated using an ANOVA-based approach (Lynch and Walsh 1998). A variety of statistical approaches also exist where the environment can be included as a continuous variable and also where the genetic basis of the **reaction norm** across environments can be estimated (Lynch and Walsh 1998). Parent–offspring analysis can also be conducted in different environments and any difference in the slope of the regression lines in the different environments assessed using analysis of covariance (ANCOVA), and used to estimate V_{GEI}, although this approach is rarely used.

2.6.2 Empirical examples of GEIs for insect reproductive behavior

Table 2.6 provides some empirical examples of GEIs and GSEIs for insect reproductive behavior. Although this list is not exhaustive, a number of clear patterns exist. First, inbred and iso-female lines are the most commonly used designs to empirically measure GEIs and GSEIs for reproductive behavior. This is not surprising, given that GEIs and GSEIs are far simpler to estimate using these designs. The use of inbred or iso-female lines, however, is restricted to those insect species where the effects of inbreeding depression are minor, including numerous *Drosophila* species, the lesser wax moth (*Achroia grisella*) and the decorated cricket (*Gryllodes sigillatus*). For species where inbred or iso-female lines are not feasible, full- and half-sibling designs have been used to quantify both GEIs (Rodríguez and Greenfield 2003; Lewis

Table 2.6 Some examples of empirical studies documenting genotype-by-environment (GEI) and genotype-by-social environment effects on reproductive traits in insects.

Insect Order	Species name	Common name	Experimental design	Environment	Sex	Reproductive behavior	Reference
Coleoptera	*Tribolium castaneum*	Flour beetle	Half-sib	Diet	Male	Mating rate	[1]
Diptera	*Drosophila melanogaster*	Fruit fly	Iso-female lines	Cold stress[A]	Female	Mate choice[B]	[2]
		Fruit fly	Iso- female lines	Social environment[C]	Male	Courtship display	[3]
					Female	Mating frequency	[3]
		Fruit fly	Inbred lines	Social environment[D]	Male	Aggression	[4]
	Drosophila simulans	Fruit fly	Iso-female lines	Diet	Male	Cuticular hydrocarbon expression	[5]
				Temperature	Male	Cuticular hydrocarbon expression	[5,6]
			Iso-female lines	Temperature	Female	Mate choice[E]	[6]
Hemiptera	*Enchenopa binotata*	Treehopper	Full-sib	Social environment[F]	Female	Mate choice[G]	[7]
			Full-sib	Host plant species	Male	Courtship song	[8]
Lepidoptera	*Achroia grisella*	Lesser wax moth	Full-sib	Temperature	Female	Mate choice[H]	[9]
			Inbred lines	Diet	Male	Courtship song	[10]
			Inbred lines	Social environment[I]	Male	Courtship song	[11]
Orthoptera	*Gryllodes sigillatus*	Decorated cricket	Inbred lines	Diet	Male	Cuticular hydrocarbon expression	[12]
	Teleogryllus oceanicus	Polynesian field cricket	Common garden[J]	Social environment[K]	Male	Cuticular hydrocarbons expression	[13]
					Male	Advertisement song	[14]
	Teleogryllus commodus	Australian field crickets	Common garden[L]	Social environment[M]	Female	Mate choice[N]	[15]

References: [1] Lewis et al. (2011); [2] Narraway et al. (2010); [3] Higgins et al. (2005); [4] Saltz (2013); [5] Ingleby et al. (2013a); [6] Ingleby et al. (2013b); [7] Rebar and Rodriguez (2013); [8] Rodriguez and Al-Watchiqui (2012); [9] Rodríguez and Greenfield (2003); [10] Danielson-Francois et al. (2005); [11] Danielson-Francois et al. (2009); [12] Weddle et al. (2012); [13] Pascoal et al. (2016b); [14] Pascoal et al. (2017); [15] Bailey and Macleod (2014).

Notes

[A]Cold stress was measured as cold shock (4°C for 15 minutes every day for 10 days versus non-stress (maintained at 25°C constantly).

[B]Female preference was measured in two ways—mate acceptance and mating latency. [C]Social behavior was examined in small populations consisting of five male and five female competitors.

[D]The social environment consisted of a focal individual, plus two other males that were always the same genotype. [E]Mate choice was measured as female preference and choosiness.

[F]Social environment was manipulated by placing different full-sib families together on the same host plant. [G]Mate choice was measured as female preference (with preference functions generated).

[H]Mate choice was measured as the time taken to respond to a song playback. [I]Social environment was manipulated by rearing different larvae of known genotype together.

[J]A total of seven different populations were examined after common garden rearing.

[K]The social environment was manipulated by either playing or not playing acoustic signals to focal individuals from each population during development. [L]Two different populations were examined after common garden rearing.

[M]Social environment was manipulated by either playing the song of other males (from *T. commodus* or *T. oceanicus*) or not during development.

[N]Mate choice was measured as female responsiveness and preference for songs produced by males from the same species or from *T. oceanicus* males

et al. 2011, Rodríguez and Al-Wathiqui 2012) and GSEIs (Rebar and Rodríguez 2013), and a common garden approach has been used to demonstrate the potential for GSEIs in the field crickets, *Teleogryllus oceanicus* (Pascoal et al. 2016b, 2017) and *T. commodus* (Bailey and Macleod 2014).

Secondly, the most common abiotic environments examined in GEI studies of insect reproductive behavior are diet and temperature. This reflects the biological importance of these environmental factors to the life-history and fitness of most insect species. Importantly, most GEI studies have treated diet and temperature as discrete (Table 2.6), rather than as continuous variables. While sufficient to demonstrate the existence of GEIs, this approach focuses exclusively on linear reaction norms and, therefore, is likely to seriously underestimate the complexity of how different genotypes respond to the abiotic environment.

Thirdly, there has been a recent increase in the number of empirical studies examining GSEIs for insect reproductive behavior. These studies have focused on a diverse range of reproductive behaviors that are fundamental to sexual interactions in many insect species (Table 2.6). This range, however, appears far greater in males than females, where reproductive behaviors examined include courtship displays (Higgins et al. 2005) and signals (Danielson-François et al. 2009), aggressive behavior (Saltz 2013), and the expression of sexual traits that are known to be socially flexible and enhance mating success (Pascoal et al. 2016b, 2017). In contrast, GSEIs for reproductive behavior in female insects have focused on mating frequency (Higgins et al. 2005) and mate choice, with support for the latter being demonstrated in treehoppers (Rebar and Rodríguez 2013), wax moths (Rodríguez and Greenfield 2003), and field crickets (Bailey and Macleod 2014). The way that the social environment has been manipulated in these studies also varies, taking two major forms. The first approach alters the social environment by rearing or housing focal individuals of known genotype in different social groups (Higgins et al. 2005; Danielson-François et al. 2009; Rebar and Rodríguez 2013; Saltz 2013). The second uses social cues to manipulate a focal individual's **perception** of the social environment (Pascoal et al. 2016b, 2017). This latter approach, however, requires that important social cues are known and can be manipulated in a reliable manner. Consequently, this approach may not be possible for many insect species.

Finally, we note that all of the empirical examples provided in Table 2.6 are based on quantitative genetic data, which constitutes most of the support for GEIs and GSEIs. It is, however, possible to use genomic approaches to study GEIs and GSEIs, and this approach is becoming increasingly common. For example, Etges et al. (2007) used QTL analysis on two divergent populations of *Drosophila mojavensis* to show significant GEIs for mating success and a number of different courtship song parameters (especially inter-burst interval, number of bursts), when flies were reared on two different host cacti species. Interestingly, four QTLs showing GEIs were located for mating success, and two each for inter-burst interval and the number of bursts, making GEI effects as common as main effects, and likely to play a key role in incipient speciation in *D. mojavensis*. We expect that genomic studies of this nature will become even more common as the price of sequencing continues to decrease.

2.7 Wider evolutionary implications and areas for future research on the genetic architecture of insect reproductive behavior

This chapter has covered what are considered to be key topics on the genetics of insect reproductive behavior. It shows that many (if not most) insect reproductive behaviors are polygenic, with genes each having a small effect (although we acknowledge that genes having a major effect do exist) and that reproductive behavior is often genetically correlated with other important traits. Furthermore, it has been shown that non-additive genetic effects and interactions between genes, and the biotic (GEIs) and social (GSEIs) environments are likely to make important contributions to insect reproductive behaviors. While this is undoubtedly important information to have, it can be argued that more interesting questions arise when considering how this complex genetic architecture influences the evolutionary process. Simply put, a shift is needed

away from studies that focus exclusively on characterizing the genetic architecture of insect reproductive behavior, towards those that also examine the wider evolutionary implications of this genetic architecture.

The evolutionary response of a given reproductive behavior is the product of selection acting on this behavior and the amount of additive genetic variance regulating this behavior: this is the core of the univariate breeder's equation ($R = h^2S$). Since many reproductive behaviors are known to be under strong selection in insects (e.g. Brooks et al. 2005; Bentsen et al. 2006; Steiger et al. 2013) and the examples presented in Tables 2.1 and 2.2 suggest a likely abundance of additive genetic variation, it appears that reproductive behaviors have the core ingredients necessary for rapid evolution. However, phenotypic evolution is far more complex than this because many reproductive behaviors consist of multiple components, which are genetically correlated and often targeted differentially by selection. Consequently, the mean response ($\Delta\bar{z}$) of a complex behavior is determined by the genetic architecture (characterized by the **additive genetic variance-covariance matrix** or **G**) and the pattern of selection targeting these specific components (characterized by the vector of linear selection gradients or β), and can be predicted by the multivariate breeder's equation ($\Delta\bar{z} = \beta\mathbf{G}$; Lande 1979). Exactly how **G** is aligned with β will determine whether **G** facilitates or constrains the evolution of reproductive behavior, and it has been argued that this alignment has been fundamental in population divergence and possibly even speciation (Schluter 2000). Few studies, however, have examined how **G** interacts with β to influence these processes, especially for reproductive behaviors in insects. The notable exception to this is work on cuticular hydrocarbons (CHCs) expression in the fruit fly *Drosophila serrata*. Like most insect species, CHCs in this species play a key role in desiccation resistance and are also the target of female mate choice. *D. serrata* shows a pronounced **latitudinal cline** in CHC expression along the east coast of Australia, which correlates with temperature and moisture differences across populations (Frentiu and Chenoweth 2010). However, differences in female mate choice for CHCs across populations only weakly predicted the observed divergence in male CHCs. This relationship was greatly improved when population estimates of **G** were included in the statistical models (Chenoweth et al. 2010), demonstrating that **G** has biased the evolutionary trajectories of CHCs in these populations (Chenoweth et al. 2010).

The examples presented in Table 2.4 show that insect reproductive behaviors are often genetically correlated with other important morphological and life-history traits. This suggests that the potential for reproductive behavior to also constrain or facilitate the evolution of such traits. Unfortunately, there are currently few clear empirical examples documenting this process, although numerous studies manipulating the degree of polyandry in experimental populations of insects have shown that evolved changes in this reproductive behavior are associated with changes in a range of non-reproductive traits, such as lifespan (e.g. Martin and Hosken 2003) and immunity (e.g. McNamara et al. 2013). An obvious exception to this is recent work in the rapid evolution of a 'flatwing' mutation in the field cricket *Teleogryllus oceanicus* (Zuk et al. 2006). *T. oceanicus* has a wide geographic distribution spanning northern Australia, Polynesia, and three Hawaiian islands (Oahu, Hawaii, and Kauai), where there is overlap with the acoustically orientating parasitoid fly, *Ormia ochracea* (Zuk et al. 2006). This fly finds its host using the calling song and the fly larvae burrow into the male cricket and develop inside, killing the host on emergence. Due to this intense selection, more than 90 per cent of male crickets on Kauai island have a wing mutation (flatwing) where the normal stridulatory apparatus required for sound production (the file and scraper) is missing, rendering them silent (Zuk et al. 2006). Crosses of laboratory populations have shown that the flatwing phenotype is inherited as a sex-linked single gene (Tinghitella 2008), and RNA-seq analysis has shown that most differentially expressed transcripts in flatwing versus wild-type males were down-regulated (625 up versus 1716 down), with differences between morphs not restricted to a single pathway (Pascoal et al. 2016a). Genomic analysis (using RAD-seq) of the genetic divergence of Oahu compared with Kauai populations has shown that of the 7226 flatwing-associated SNP markers, only 0.30 per cent were shared between the two islands (Pascoal et al. 2014), which

is consistent with independent mutational events. This demonstrates the powerful effects that reproductive behavior can have on morphology and how rapidly convergent evolution can occur.

Although not covered in this chapter, it is also likely that many shared reproductive behaviors will be positively genetically correlated between the sexes because males and females share most of their genome. Under contrasting selection on shared behaviors, intralocus **sexual conflict** (ISC) will exist and prevent the independent evolution of shared reproductive behaviors (Bonduriansky and Chenoweth 2009). Ultimately, ISC should oppose the evolution of sexual dimorphism in any shared reproductive behaviors, yet this phenomenon remains pervasive in nature (Bonduriansky and Chenoweth 2009). For example, CHC expression in *D. serrata* is sexually dimorphic, the magnitude of which is known to vary across populations in eastern Australia (Chenoweth and Blows 2008), despite opposing selection on CHCs between the sexes (Chenoweth and Blows 2004), and strong positive genetic correlations between CHC components in the sexes (Chenoweth and Blows 2008). Various mechanisms are known to help resolve ISC, and in the case of *D. serrata* many CHC components are X-linked, which reduces the intersexual genetic correlations (Chenoweth and Blows 2008). However, we still know very little about the operation of other mechanisms (such as genomic imprinting and gene duplication) that help resolve ISC for insect reproductive behaviors and more genomic studies are desperately needed.

Non-additive genetic effects occur due to interactions between alleles, either at the same locus (dominance) or different loci (epistasis). In general, there are far fewer studies documenting the contribution of non-additive genetic effects to phenotypic traits compared with additive gene effects, particularly for reproductive behaviors. This is surprising, given the important role that non-additive genetic effects are predicted to have for the evolution of phenotypic traits. Theoretical models show that dominance and epistatic variance can be converted into additive genetic variance by a number of different processes (Hansen and Wagner 2001) and can influence the response to selection through the build-up of linkage disequilibrium, as parents not only transmit half of the additive effects to offspring, but also a quarter of pairwise epistatic effects and smaller fractions of high-order interactions (Lynch and Walsh 1998). This suggests that some of the linkage disequilibrium built by gene interactions can be converted into response to selection and that non-additive genetic effects are likely to have important long-term evolutionary consequences. While the limited examples in Table 2.5 demonstrate the importance of non-additive genetic effects on insect reproductive behaviors, there are currently no studies available that have examined the wider consequences of non-additive genetic effects to the evolution of these behaviors.

The empirical examples we provide in Table 2.6 suggest that GEIs are important contributors to the genetic architecture of reproductive behavior. Most GEI studies, however, have focused on interactions involving diet and temperature, and have taken a dichotomous approach (e.g. high versus low temperature). Clearly, this approach does not encompass the full complexity of environments experienced by most insect species, especially in nature, and therefore limits biological interpretation. Consequently, more studies are needed that cover both a broader range of environmental factors and more levels within a given environmental factor. As outlined in Section 6, the latter will enable the genetic basis of reaction norms to be estimated using random regression-based approaches. Theoretically, GEIs are predicted to have a number of important evolutionary consequences, including the maintenance of genetic variation in a population, facilitating the evolutionary response to a changing environment and promoting population divergence (e.g. Via and Lande 1985, 1987). Unfortunately, there are no empirical studies that have examined how GEIs for reproductive behaviors influence the above processes, making this a priority for future research.

The examples in Table 2.6 also show that GSEIs represent an important source of variation in insect reproductive behavior. As GSEIs involve the social environment they are predicted to be more labile and evolutionary dynamic compared with GEIs involving the abiotic environment (Wolf et al. 2014). It is also possible for the social environment to have a genetic basis, although **indirect genetic effects** (IGEs), meaning that the social environment can

itself evolve (Wolf et al. 1998). Like GSEIs, IGEs are expected to have important effects on the evolution of phenotypic traits, including altering the rate and direction of evolution, promoting evolutionary time-lags and permitting traits to evolve that lack a genetic basis (Wolf et al. 1998). IGEs have been shown for a range of reproductive behaviors in insects, including parental care (Agrawal et al. 2001; Hunt and Simmons 2002; Head et al. 2012), CHC production (Petfield et al. 2005), and female mate choice (Rebar and Rodríguez 2013). Unfortunately, there are currently no empirical tests of the long-term evolutionary consequences of either GSEIs or IGEs for reproductive behaviors in insects. This should be a priority for future research, especially since studies on livestock and poultry have shown that IGEs can significantly alter the evolutionary response of traits in artificial selection experiments spanning multiple generations (e.g. Muir 2005, Camerlink et al. 2015).

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