Innate immunity, developmental speed and their trade-offs in two hexapod models

Cheng, S.

Citation

Cheng, S. (2023, November 28). *Innate immunity, developmental speed and their trade-offs in two hexapod models*. Retrieved from https://hdl.handle.net/1887/3665319

Chapter 1

General introduction

The success of the insects

The earth's largest and most diverse group of organisms

Insects underwent unparalleled diversification and make up more than half of all animal species. Insects (Insecta or Hexapoda) are the largest class of the phylum Arthropoda, which is itself the largest of all animal phyla. According to the estimates from Grimaldi, 64 percent of described around 1.5 million species on earth are arthropods, of which more than 87 percent are insects (Grimaldi and Engel, 2005) (Figure 1-1). Globally, over one million species of insect have been described to date. The total amount of insect species is believed to vary from 2.5 to 5.5 million. It is also estimated that the number of living individual insects has reached 10 quintillion at this moment (Berenbaum, 1996; Stork, 2018). Insects have shown an incredible diversity, abundance and biomass over time. They occur in practically all habitats all over the earth (Price, 1997). Before providing some hypotheses for this spectacular success, I first introduce the taxonomy of insects in more detail, and discuss their importance for humans.

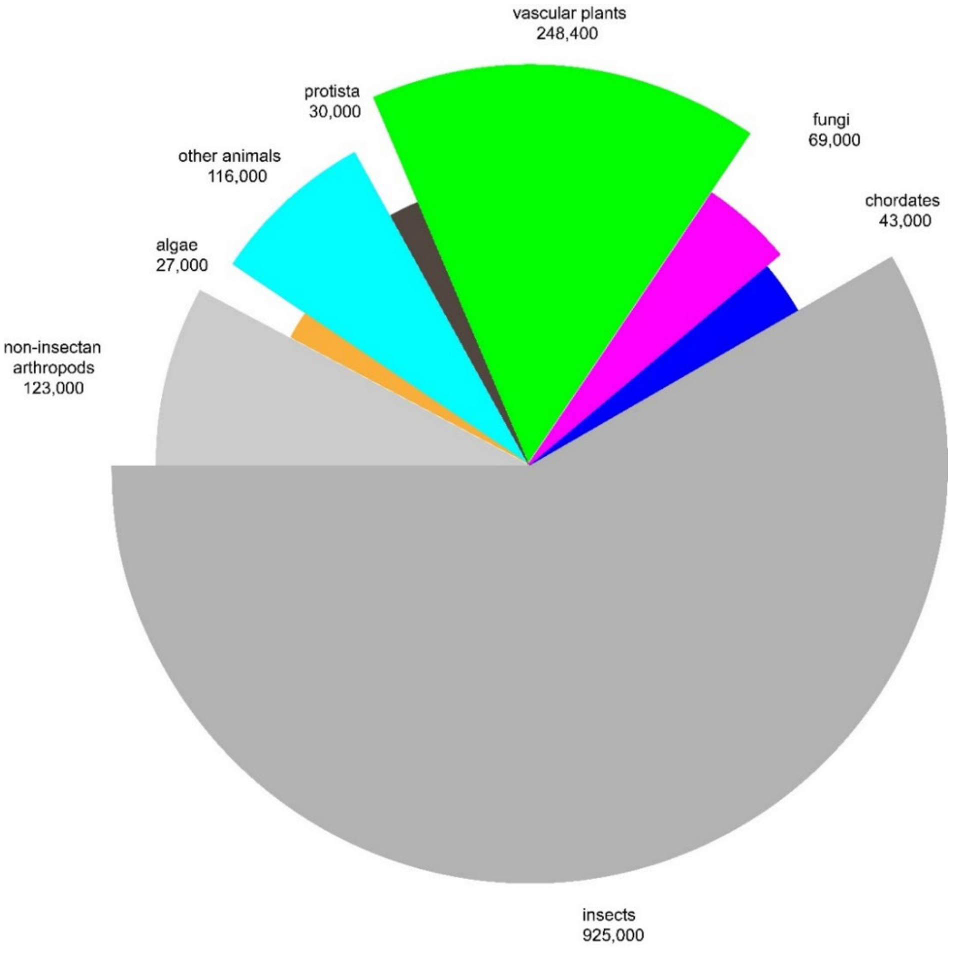

Figure 1-1. Relative proportions of described eukaryote taxa, with species numbers [summarized by (Grimaldi and Engel, 2005)].

Classification

The subphylum Hexapoda consists of four groups: Insecta, Collembola, Protura, and Diplura. The subphylum name refers to the most distinctive feature: the presence of six legs in the three consolidated segments of the thorax. The mouthparts of insects are ectognathous, while the other groups are endognathous. These non-insect hexapods are called the Entognatha and lack wings. In particular, Collembola (springtails) are extremely abundant in soil and are found in almost all terrestrial environments. I will study the immune competence of one springtail, *Orchesella cincta*, in Chapter 2. Collembola comprise more than 82 percent of all described entognathan species. Strikingly, insects comprise more than 99 percent of the Hexapod species and are thus the largest class (Zhang, 2011; Zhang, 2013) (Figure 1-2).

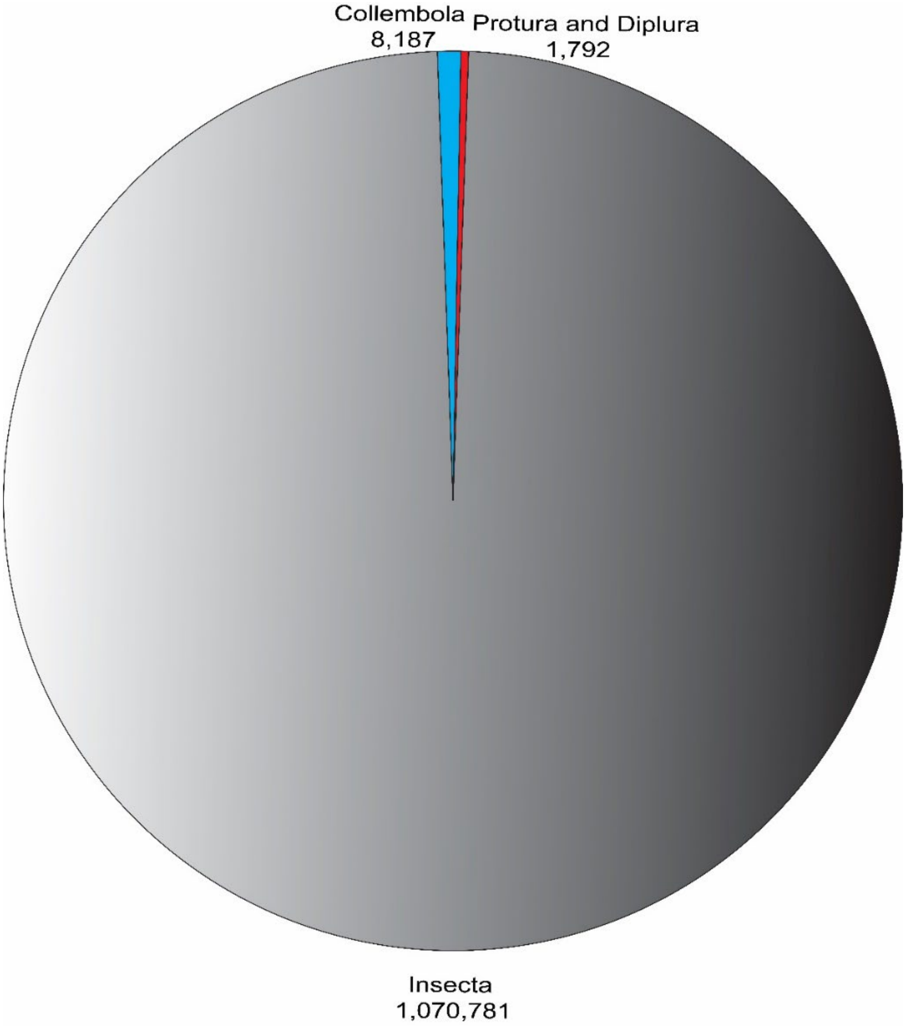

Figure 1-2. Relative proportions of described species in the subphylum Hexapoda, with species numbers [adopted from (Zhang, 2011; Zhang, 2013)].

Insects have an exoskeleton and three pairs of walking legs. The class Insecta is divided into two subclasses: Apterygota (primitively wingless insects) and Pterygota (winged or secondarily wingless insects). The Apterygota is a kind of small, agile insects. The Apterygota consist of the Archaeognatha (known as jumping bristletails) and the Zygentoma (known as silverfish and firebrats). There is no metamorphosis in apterygotes as their nymphs are similar to adults. The Pterygota include the majority of species in the class Insecta. Most of them have wings, while a few species lost their wings secondarily due to adaptation to the living environment. One typical characteristic of the Pterygota is the metamorphosis, namely the larva does not have wings. Some insects undergo several nymphal stages into the adult by a series of molts (incomplete metamorphosis), while others undergo complete metamorphosis through a pupal stage.

Incomplete metamorphosis includes three stages: egg, nymph, and adult in insect development. When these insects hatch from the egg, the nymph resembles the miniature adult. This type of insects are also called the hemimetabolous insects which contain orders of Hemiptera (true bugs), Orthoptera (grasshoppers, locusts and crickets), Mantodea (praying mantis), and so on. In contrast, holometabolous insects undergo complete metamorphosis. Their life cycle consists of four distinct stages: egg, larva, pupa, and adult. The pupa is a tough capsule (cocoon) formed by the hardening of the larval skin. Recent classifications showed that 39 insect orders exist in total, of which four orders are holometabolous insects but comprise over 80% of insect species. These orders are the Coleoptera, Diptera, Lepidoptera, and Hymenoptera (Zhang, 2011; Zhang, 2013) (Figure 1-3).

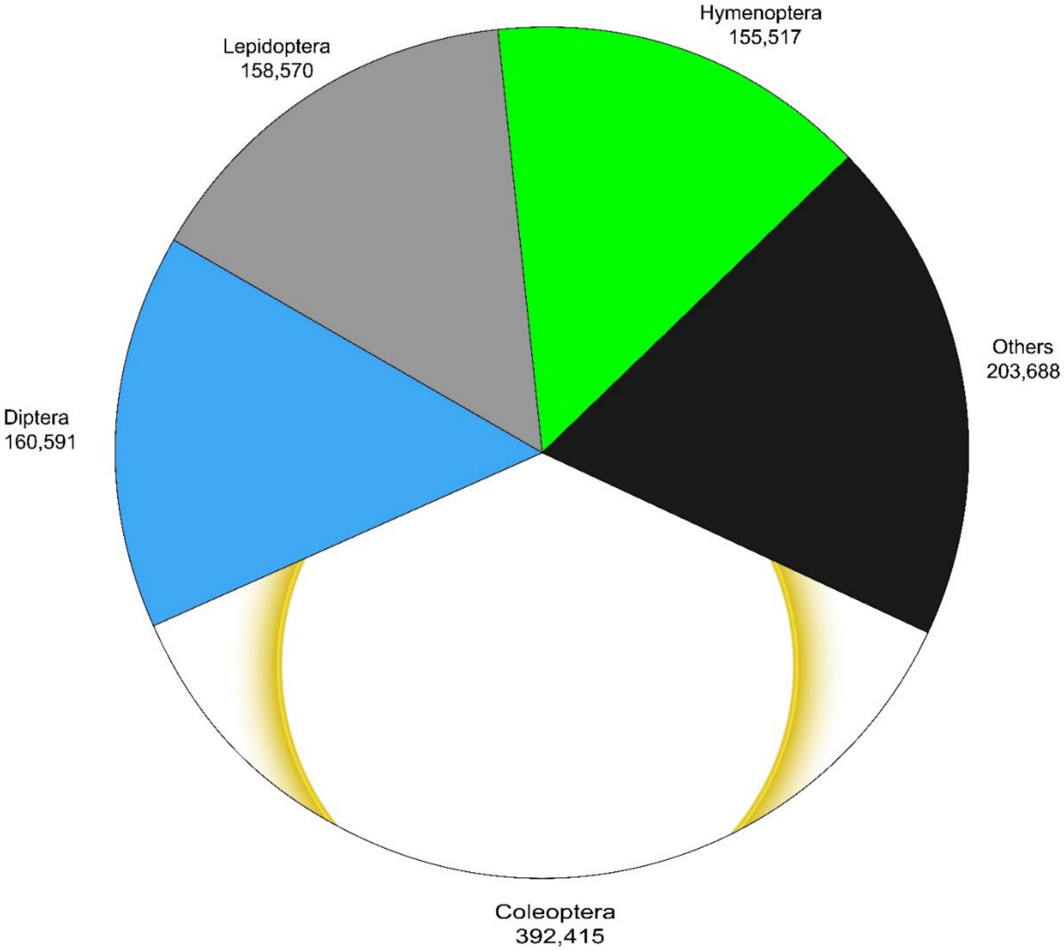

Figure 1-3. Relative proportions of named species in the orders in the Insecta, with species numbers [adopted from (Zhang, 2011; Zhang, 2013)].

Diptera, normally known as true flies only use a single pair of wings to fly and their hindwings have evolved into halters that act as assistance for balance during flight. The fruit fly *Drosophila melanogaster*, one of the most thoroughly studied organisms by humans, belongs to this insect order. The knowledge on development and genetics is mostly based on experiments done on *D. melanogaster* (Dubrovsky, 2005).

Coleoptera, commonly known as beetles, are the largest insect order and account for approximately 37% of insect species. The most characteristic and diagnostic feature of beetles is the hardening of the forewings into elytra (Crowson, 2013). The red flour beetle *Tribolium castaneum* belongs to this order. In the last decades, *T. castaneum* has emerged as a powerful model system for studying evolutionary and developmental biology. The development of *T. castaneum* is evolutionary more primitive than that of *D. melanogaster* even though they are both holometabolous. For example, *Drosophila* undergoes long-germ embryogenesis. This means that it patterns the future segments (head, thorax and abdomen) simultaneously at the blastoderm stage, before gastrulation. *Tribolium* and most other insects undergo short-germ embryogenesis, patterning only the anterior segments (head and thorax) at the blastoderm stage, while the remaining segments develop from a posterior growth zone after gastrulation (Brown et al., 2009; Rosenberg et al., 2009).

Lepidoptera consist of butterflies and moths. The most obvious features of them are the scales which cover the body and wings and are the source of various color patterns, and a proboscis (Powell, 2009).

Hymenoptera are one of the most species-rich insects orders, only behind Coleoptera and Lepidoptera. This order includes all wasps, bees and ants, of which many are eusocial. A series of hooks act as a key feature of this order to connect the hind wings and the fore wings (Quicke, 2009). In the updated classification, the order Hymenoptera is consisted of two suborders (Symphyta and Apocrita) (Aguiar et al., 2013).

In addition to the above mentioned 4 holometabolous orders, there are 35 orders in the Insecta. Insects adapted to every land and freshwater habitat. Whether we like them or not, they interact with other species as well as humans in countless ways.

Importance

Insects perform crucial ecological functions in all terrestrial ecosystems. For instance, butterflies and moths play a crucial role in the natural ecosystem as pollinators and herbivores (Ahmed et al., 2015). Hymenoptera play vital roles as pollinators as well (Peters et al., 2017) and bees are regarded as the most known and understood insect pollinators (Gill et al., 2016; Sanchez-Bayo and Goka, 2014). Insects are also crucial in the nutrient cycle by eating dead animals, plants, fungi or decaying organic matter. Thus, insects act as primary or secondary decomposers in nature. At the same time, insects are very important food sources for many amphibians, reptiles, birds, and mammals (Pough, 1991; Rytkönen et al., 2019).

From a human perspective, insects can be beneficial or harmful. Harmful insects include blood-feeding insects like mosquitoes, because they can be vectors of disease. For example, human malaria is a vector-borne disease caused by the infection with the protist *Plasmodium* (Chulay et al., 1986; Kaaya, 1989). The World Health Organization (WHO) estimates that there are hundreds of millions of malaria cases and more than half a million deaths each year (Emerson et al., 2022; Organization, 2022). Additionally, some insects can cause allergic or adverse reactions through their bite or sting. These insects include Anoplura (lice), Siphonaptera (fleas), and Hemiptera (bed bugs) (Sarwar, 2015).

Harmful insects also include agricultural pests such as locusts. The impact of locusts dates back millennia and locusts continue a major economic pest of agricultural crops today throughout the world (Le Gall et al., 2019). Zhang et al. reported that locusts and grasshoppers (Orthoptera: Acridoidea) are among the most dangerous polyphagous pests of agricultural crops (Zhang et al., 2019). Insects consume up to 20% of global crops (Oerke et al., 2012). As insects perform better at higher temperature, a global mean surface temperature increase of 2-degree Celsius is predicted to rise crop losses to 46% for wheat, 31% for corn and 19% for rice (Deutsch et al., 2018). So, insect pest management and control are essential sociological challenges associated with our economics and politics (Shelton et al., 2002).

But in general, only 0.1% of insect species constitute pests and produce damage, disease, or even death to humans, livestock, crops, and manufactured goods. About 1% species of insects interact with humans directly or indirectly (Gillott, 2005). Most of them are beneficial.

For agricultural crops, for instance, insects are essential pollinators. Thus, insect pollinators play a crucial role in affecting human food supplies. Insects also produce commercially valuable products, as do honey bees, silkworms, lac insects, and pela wax scale insects etc. (Gillott, 2005). Honey has served humans for many thousands of years and is nevertheless still an irreplaceable product. Silk production greatly affected human history, during which the Silk Road increased the connections among the world. Lac is a product from lac scale insects mainly made in India and a component of shellac. Beeswax is a naturally occurring wax produced by worker honey bees of the genus *Apis*, and used for constructing the honeycombs where to store honey and pollen as well as house their larvae (Buchwald et al., 2006). In the past decades, the total production of beeswax exceeded 60 kilo tons worldwide per year (see in the Food and Agriculture Organization Corporate Statistical (FAOSTAT) database (http://faostat.fao.org/), which is more than doubled in 1960s (Phiri et al., 2022). Owing to its higher melting point than any other wax (about 63-65 ºC), beeswax was used for candles in the past, which therefore remain upright in hot season. At present, beeswax has many uses in cosmetics and body products, in the food industry, in pharmacy, in medicine, and in research exploiting its antimicrobial activity (Fratini et al., 2016; Tulloch, 1980).

Insects themselves are also important biological control agents of agricultural pests. Hymenoptera are widely used as parasites of pests (Dongol et al., 2014). It is worth noting that Hymenopteran parasitoids make up nearly 78% of the estimated insect parasitoid species and often cause host death (Abram et al., 2019; Feener Jr and Brown, 1997). There are a range of control methods available to pest insects, including chemical, biological, microbial, genetic, and physical control etc. Each of them has its advantages and disadvantages in use in a certain situation. The use of chemicals is the oldest approach of pest control either to kill or to repel harmful insect pests. However, today, insecticide resistance is an increasing problem both in many insect vectors of disease and agricultural pests, threatening effective insect pest management and control (Gillott, 2005; Hemingway and Ranson, 2000; Roush and Tabashnik, 2012). It is worth recognizing the role of insects in developing ecofriendly approaches for the control of pests in future.

Another potential use of insects is the feeding of livestock. This is because insects are rich in proteins, essential amino acids, fat and active substances (such as polyunsaturated fatty acids, antimicrobial peptides) (Józefiak et al., 2016). Until now, one of the most popular nutrient insects, the yellow mealworm *Tenebrio molitor*, is a valuable food source for fish, poultry, and human pets (Khosravi et al., 2018; Peng et al., 2019).From another perspective, a variety of insects, including butterflies, are sometimes used for decoration.

Finally, insects (e.g. the fruit fly *Drosophila melanogaster*) are also important in research, and are used to study for instance human pathogens such as human immunodeficiency virus (HIV) or diseases such as cancer (Bairagi, 2019; Mirzoyan et al., 2019). Common characteristics of many insects, including short generation time and high fecundity, make them widely reared globally under laboratory conditions for both teaching and research. Studies of the fruit fly *Drosophila melanogaster* have provided crucial data to genetics, and developmental biology and behavioral biology (Mirzoyan et al., 2019; Ugur et al., 2016). The red flour beetle *T. castaneum* is the second most important model insect after *Drosophila*, and has provided a lot of information for fundamental and applied research (Campbell et al., 2021). Techniques such as RNA interference (RNAi) and CRISPR/Cas are well-established in *Tribolium* (Grau et al., 2017). In addition to *Tribolium*, non-model insects like the cowpea seed beetle *Callosobruchus maculatus*, the yellow mealworm *T. molitor*, the pea aphid *Acyrthosiphon pisum*, the milkweed bug *Oncopeltus fasciatus*, the tobacco hornworm *Manduca sexta* etc., are also proving to be valuable research models (Beck and Blumer, 2020).

In conclusion, without insects, many of plants including crops would not survive, and ecosystems will collapse. However, a 27 years long study in Germany showed a seasonal decline of 76% in flying insect biomass, and worse in mid-summer (82%) (Hallmann et al., 2017). A recent study showed that terrestrial insect abundance declined by 9% on average per decade, while freshwater insect abundance increased by 15%, which has raised concerns among scientists (Dornelas and Daskalova, 2020; van Klink et al., 2020). There is no doubt that the attention of the public should continuously be drawn to insects.

Traits may explain the extraordinary success of insects

Over the last century, many hypotheses have been formulated to explain the success of insects. Several authors had suggested that the small body size of insects and their co-evolution with plants contributed (Gillott, 2005; Grimaldi and Engel, 2005; Novotny et al., 2006). Four key evolutionary innovations and their corresponding taxa have commonly been recognized as major contributors to this great diversification of hexapods (Figure 1-4): (i) the Insecta possesses the insect body ground plan or *Bauplan*, (ii) the Pterygota possesses wings, (iii) the Neoptera possesses wing folding, and (iv) the Holometabola possesses complete metamorphosis (Mayhew, 2002; Mayhew, 2007; Nicholson et al., 2014; Waegele and Bartolomaeus, 2014). The insect *Bauplan* (i) includes specialized legs, external mouthparts, an ovipositor and an eleven-segmented abdomen that allow accessibility to diverse ecological niches. Fight (ii) provides great conveniences for insects to forage, avoid enemies and expand their distribution range. Wing folding (iii) allows neopterous insects to exploit more concealed places such as holes, without damaging their wings; and complete metamorphosis (iv) contributes to the utilization of different food sources for insects at different developmental stages (Rolff et al., 2019). There is no doubt that these characters have contributed a lot to the success of insect. Fossil evidence also confirmed the importance of the origin of wings and complete metamorphosis for insect diversity (Nicholson et al., 2014).

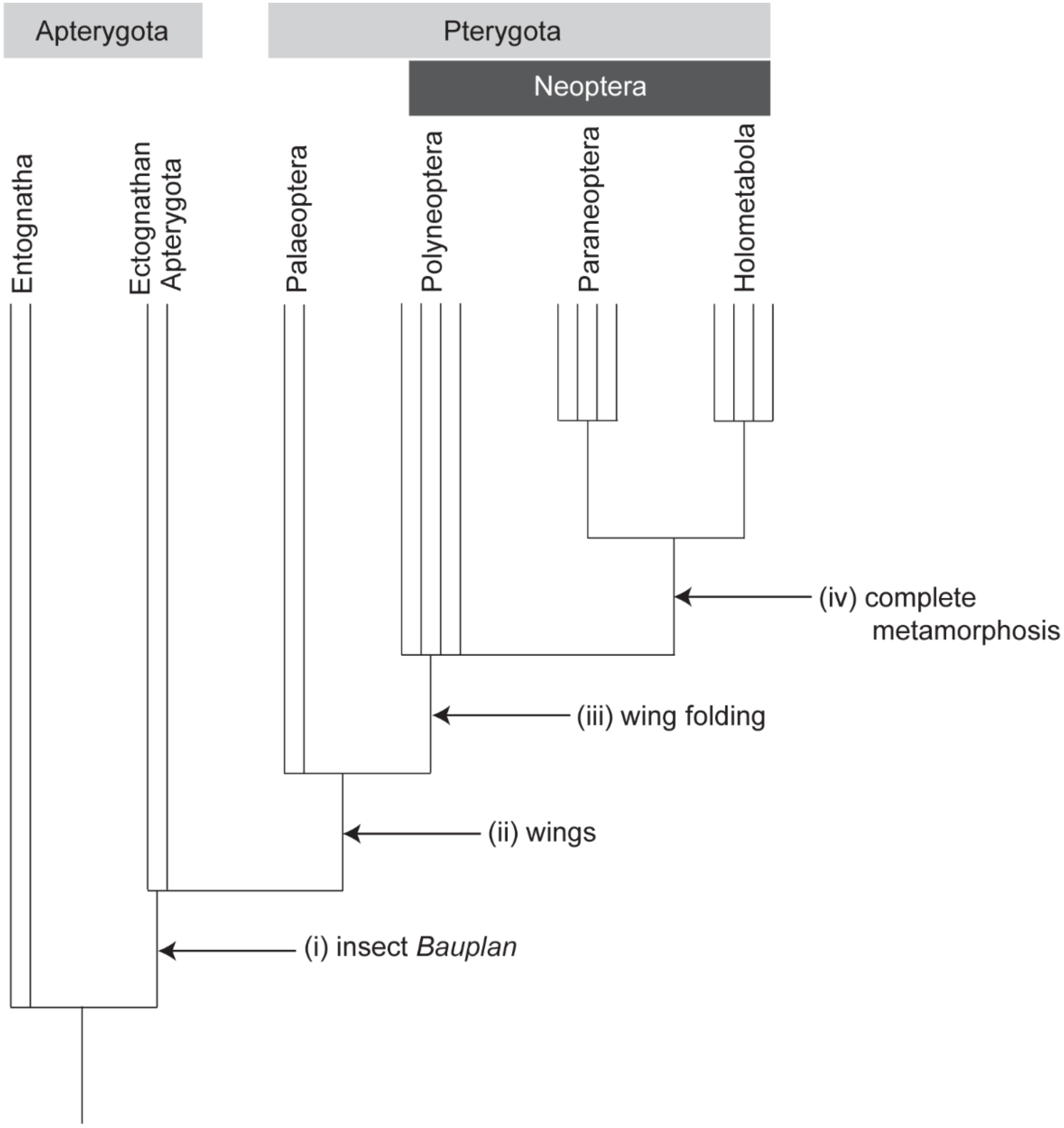

Figure 1-4. Four key evolutionary innovations and their corresponding taxa. Holometabola is also referred to as Endopterygota. Adopted from (Nicholson et al., 2014).

However, all these traits are based on traits of adult insects. In contrast to most other arthropods, there is no parental care in the majority of insects (Rezende et al., 2016). Crustaceans, for instance, usually carry their eggs with them (Sastry et al., 1983). Therefore, if insects eggs would not be adapted to the diverse habitats, insects would never have been so successful. Zeh et al. (1989) proposed that attributes of the egg stage must not be overlooked for the unparalleled diversification of insects. Egg-stage characters include maternal effects such as the ovipositor and architecture of the eggshell; and zygotic characters such as the amnion and serosa. These characters were proposed to have enabled the expansion of insects into previously inaccessible niches, and to allow rapid and extensive divergence. Additionally, the self-sufficient eggs may be associated with the low incidence of postzygotic parental egg care among insects compared to other terrestrial arthropods (Zeh et al., 1989). Thus, egg-stage characters may have made significant contributions to the extraordinary success of the insects.

Development of insect eggs

Structure of insect eggs

The size of insect eggs varies greatly among species. For instance, the eggs of the desert locust (*Schistocerca gregaria*) are around 7-8 mm in length while the eggs of the egg parasitoid *Trichogramma dendrolimi* are around 0.2-0.3 mm long (Maeno et al., 2020; Zhou et al., 2020). The shapes of insect eggs are also diverse. The most common shape is oval (Figure 1-5). The egg is covered by a protective egg shell, called the chorion, which is secreted by exocrine glands before oviposition. The chorion layer has a complex structure and is the interface between the egg and the environment. The other layer, the vitelline envelope, is a relatively simple proteinaceous layer outside the oocyte (Jagadeeshan and Singh, 2007; Margaritis et al., 1980). Beneath these eggshells, the serosal cuticle is secreted by the extraembryonic serosa and becomes the innermost eggshell layer, which serves to protect insect eggs from desiccation, physical insult and bacterial infection (Jacobs et al., 2013; Jacobs et al., 2014a). The extraembryonic epithelium, the serosa, enfolds the embryo and yolk in almost all insect eggs. At the same time, the other extraembryonic membrane, the amnion, covers the embryo ventrally. Thus, the serosa and the amnion form a bilayer to protect normal development of the embryo. Strikingly, both of them rupture and withdraw actively in late embryogenesis (Hilbrant et al., 2016; Rezende et al., 2008).

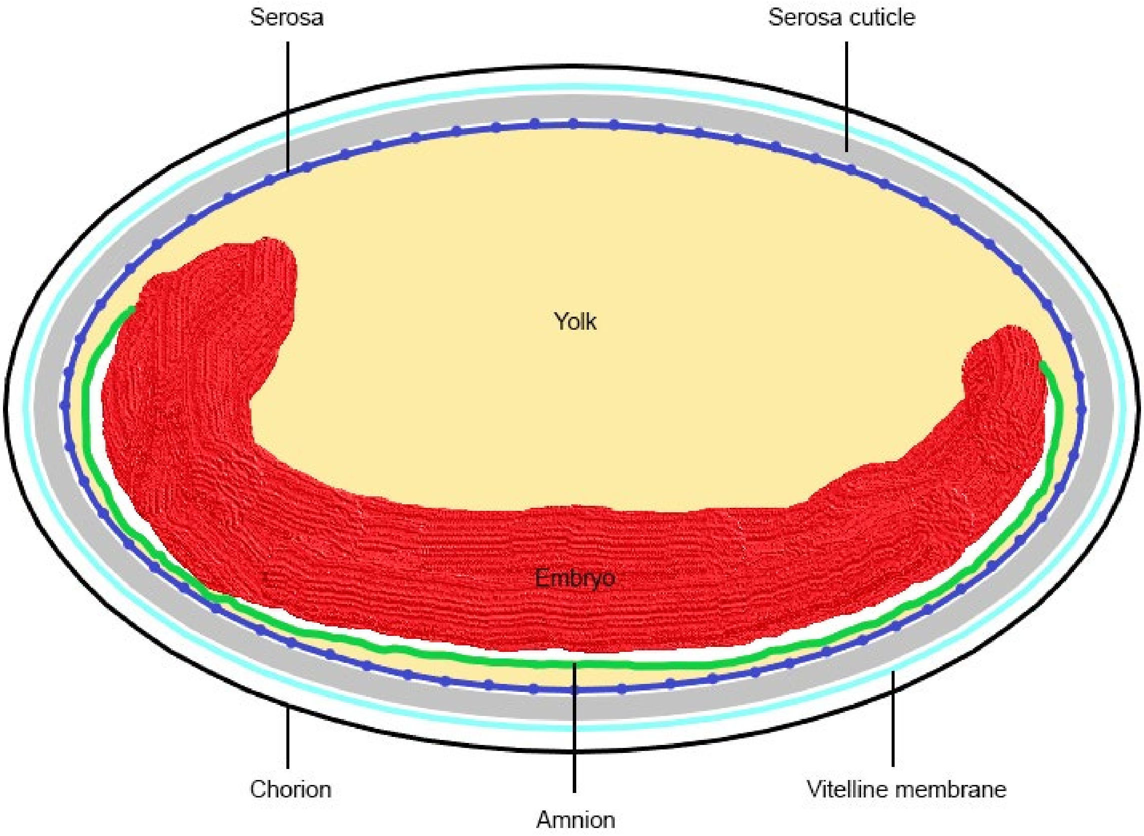

Figure 1-5. Schematic structure of holometabolous insect egg. Adopted from (Jacobs et al., 2015).

Early embryonic development of insect eggs

Although parthenogenesis occurs in quite some insects (e.g. aphids, ants and bees), embryonic development of insects generally begins after the eggs are fertilized. Within the fertilized egg, the single nucleus divides hundreds of times into many cells in the yolk. After this, these nuclei move to the egg periphery of the yolk, become enclosed by cell membranes and form a single cell layer surrounding the yolk, called the blastoderm. Subsequently, the cells begin to differentiate, with a subset of cells giving rise to the embryo or germ and the remaining cells giving rise to the serosa. Exceptionally, *Drosophila* eggs only develop a small dorsal region as an extraembryonic tissue called the amnioserosa (Benton, 2018; Schmidt-Ott et al., 2010).

Next follows gastrulation, a process that is accompanied by a considerable longitudinal stretching of the germ Anlage. Before gastrulation, a strip of cells along the ventral midline is specified as the presumptive mesoderm. Gastrulation then begins with cellular invagination to form two germ layers. The outer layer is the ectoderm, and the inner germ layer is the mesoderm, which arises from the internalization of mesodermal cells. Subsequently, the serosal cells migrate over, and then surround the embryo and yolk, forming a complete, final barrier to protect the embryo from injury (Figure 1-6A) (Roth, 2004). At the same time, the amnion forms a fluid-filled cavity (amniotic cavity) to protect the embryo.

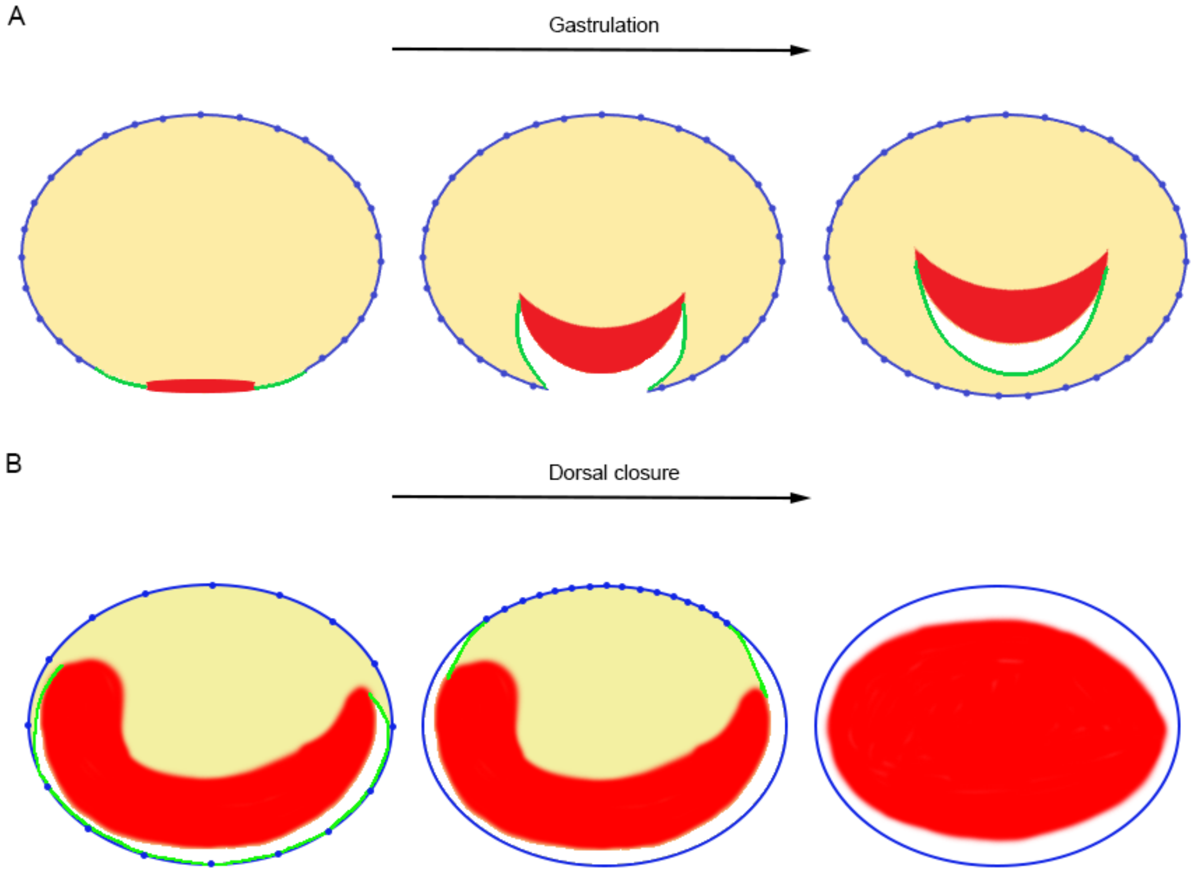

Figure 1-6. A, Formation of the complete serosa during gastrulation. B, Fate of the serosa during dorsal closure. Key: Yolk = light yellow; germ band/embryo = red; amnion = green; serosal cells = blue dots; serosal cuticle = blue ovals.

After gastrulation, segments are added one by one from a posterior growth zone, and appendages form on some of the segments. Strikingly, the serosa always surrounds the embryo and yolk prior to dorsal closure (Benton et al., 2013; Münster et al., 2019; Panfilio, 2008). Just before dorsal closure, however, the amnion and serosa actively rupture and withdraw into the body (Figure 1-6B). Then, two edges of the germ band meet and fuse along the dorsal midline, internalizing the yolk (Hilbrant et al., 2016; Sander, 1976).

Function of the serosa

An evolutionary novelty in insect eggs

It has been reported that there is a correlation between the capacity of arthropod eggs to develop in terrestrial environments, and the presence of a serosa which completely envelops the embryo and yolk (Jacobs et al., 2013). The higher flies (Diptera), including *Drosophila*, are unique among insects in that they have secondarily lost the serosa and formed a single dorsal extraembryonic epithelium called the amnioserosa (Jacobs et al., 2013; Rafiqi et al., 2008). This might explain why eggs of these flies cannot survive easily outside humid environments. Most chelicerates, myriapods and crustaceans do not have the serosa at all. They have a single extraembryonic tissue which covers the dorsal yolk but that never enfolds the embryo (Figure 1-7). Thus, the serosa is considered to be an evolutionary novelty in insect eggs.

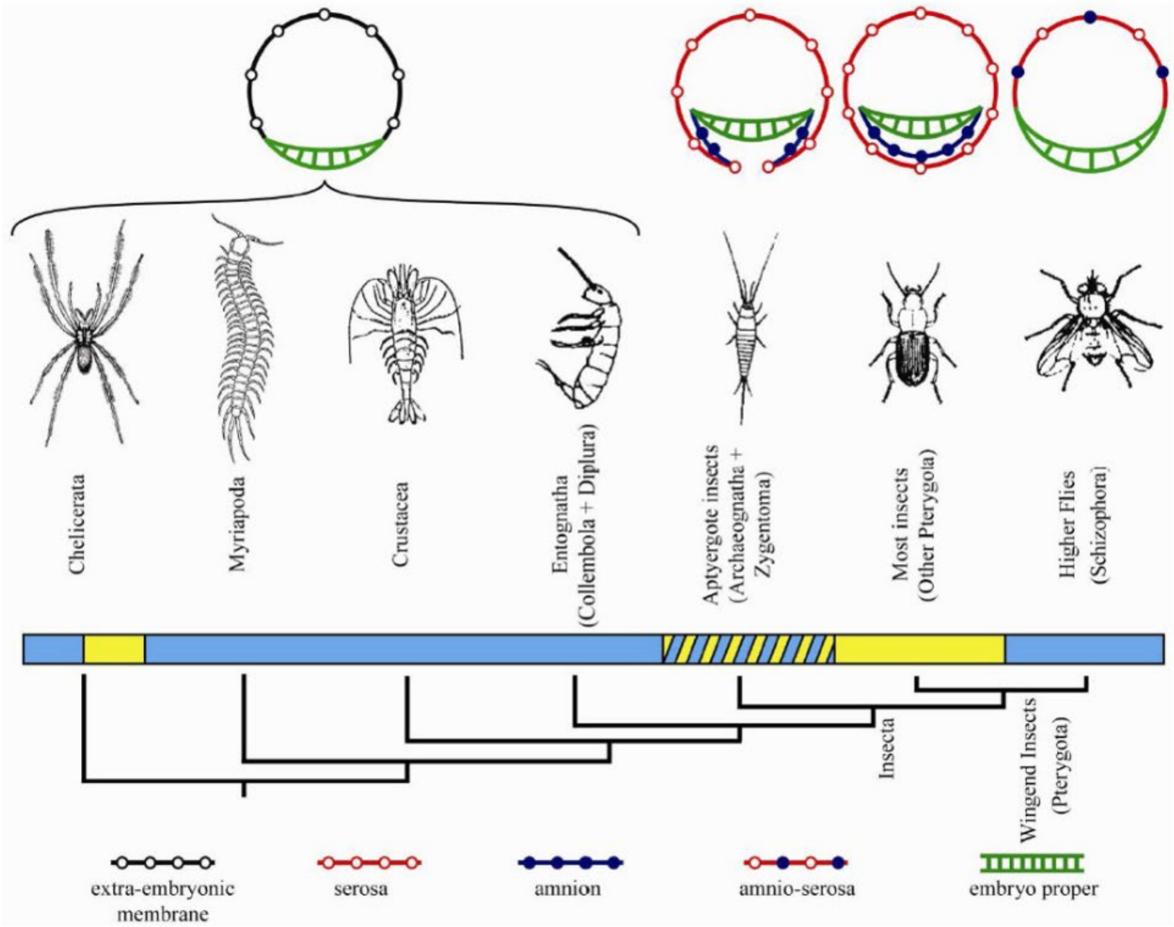

Figure 1-7. Phylogeny of all major arthropod groups. The bar under the groups shows the egg lives in aquatic (blue), terrestrial (yellow) or humid environments (striped). The top row shows a schematic overview of the egg, focusing on the serosa/amnion and embryo (from (Jacobs et al., 2013)).

Function of the serosa

It is impossible to assess function of the serosa in *Drosophila* because it is absent. However, the serosa is present in the second most important insect model species *T. castaneum*. In 2005, van der Zee *et al*. developed a technique using parental RNAi with the gene *Zerknüllt1* (*Tc-zen1*) to remove the serosa from *Tribolium* eggs, without damaging the maternal eggshell layers. As a consequence, in parental *Tc-zen1* RNAi eggs, the amnion only covers the yolk at the dorsal side (van der Zee et al., 2005). These serosa-less eggs are prone to desiccation and infection (Jacobs et al., 2013; Jacobs et al., 2014a). It is particularly the secreted serosal cuticle that protects *Tribolium* eggs from desiccation (Jacobs et al., 2013). In the egg of mosquitoes, desiccation resistance also coincides with the formation of the serosal chitinized cuticle (Goltsev et al., 2009; Rezende et al., 2008). At the same time, the serosa promotes correct dorsal closure by pulling the amnion and embryo dorsally (Jacobs et al., 2013; van der Zee et al., 2005).

Another important function of the serosa in insect eggs is as barrier to infection. The NF-κB homologue Dorsal, a transcription factor entering the nucleus upon Toll signaling, is present in the serosa during early *Tribolium* development (Chen et al., 2000). Also, antimicrobial protein mRNAs and lysozyme are expressed only in extraembryonic tissues of immune-challenged the tobacco hornworm *M. sexta* eggs (Gorman et al., 2004).

In response to infections, the serosa mounts a full-range immune response protecting the embryo in the red flour beetle (*T. castaneum*) (Jacobs et al., 2013; Jacobs et al., 2014a)*,* the tobacco hornworm (*Manduca sexta*) (Gorman et al., 2004), the milkweed bug (*O. fasciatus*) and the migratory locust (*Locusta migratoria*) (Jacobs et al., 2022). Interestingly the carrion beetle *Nicrophorus vespilloides* has a serosa, but it does not mount an immune response (Jacobs et al., 2014b). The eggs of *T. castaneum, O. fasciatus* and *L. migratoria* possess the extraembryonic serosa. *Drosophila* eggs have lost the serosa, desiccation resistance and immune competence altogether (Jacobs et al., 2013; Rafiqi et al., 2008). In chapter 2, I will investigate if the serosa is a prerequisite for an inducible innate immune response in the springtail *O. cincta*.

Like the *Drosophila* embryo, the *Nicrophorus* egg exhibits a remarkably faster embryonic development compared to other insect taxa (Fernández-Moreno et al., 2007). *Drosophila* eggs develop extremely quickly and hatch within 24 hours at 25 °C. *Nicrophorus* eggs hatch in two days at 25 °C which is also much more rapid than in other insect taxa. On the contrary, *Tribolium, Manduca, Oncopeltus* and *Locusta* eggs have a slow embryonic development. Embryonic development in *Tribolium*, *Oncopeltus* and *Manduca* is approximately 3–6 times slower than *Nicrophorus* (Chipman, 2017; Howe, 1956; Kingsolver and Nagle, 2007). Embryogenesis of *L. migratoria* takes 14–15 days at 30 °C (Tanaka, 2017). Thus, immune competence seems to trade-off with developmental speed. This trade-off may have driven the loss of the serosa in higher flies. In chapter 3, I will investigate whether immune competence trades off with developmental speed in lines of *Tribolium castaneum* that have been selected for fast or slow development.

Insect immunity

Insects rely completely on the innate immune system when infected by pathogens (Charles and Killian, 2015). The two components of the innate immune system are cellular and humoral elements (Tsakas and Marmaras, 2010). For cellular immunity, pathogenic microorganisms are eliminated by phagocytes, such as the hemocytes of *Drosophila* (Kim and Kim, 2005) and mosquito (Hillyer and Strand, 2014). For humoral immunity, microbes are eliminated mainly by antimicrobial peptides (AMPs) that are secreted into the haemolymph. The systemic cooperation of the cellular and humoral immune components contributes to protection against pathogenic microorganisms (Tsakas and Marmaras, 2010; Yang et al., 2021). For instance, pro-phenoloxidase (PPO), the inactive zymogen form of phenoloxidase (PO), is synthesized and stored in hemocytes (cellular). Upon infection, however, the activation of PPO to the active PO is executed by humoral immune reactions (González-Santoyo and Córdoba-Aguilar, 2012; Yang et al., 2021).

The innate immune system is activated by pattern-recognition receptors (PRRs) that recognize specific pathogen-associated molecular patterns (PAMPs). These PAMPS are mainly peptidoglycans, that are present on both pathogenic and non-pathogenic bacteria (Janeway Jr and Medzhitov, 2002). Diaminopimelic (DAP) type peptidoglycans of Gram negative bacteria mainly activate the IMD pathway, while Lysine (Lys) type peptidoglycans of Gram positive bacteria mainly activate the Toll pathway. Through a cytoplasmic signaling cascade, this leads to the nuclear localization of NF-κB that inducing the expression of effectors such as antimicrobial peptides, propheonoloxidases, or other genes involved in the oxidative response such as the Dual oxidase (DUOX) (Davis and Engström, 2012; Ferrandon, 2013; Lemaitre and Hoffmann, 2007; Ligoxygakis, 2013).

In insects, a potent immune response has been identified in post-embryonic development in many species. In Table 1-1, I present a selection of immune studies in larvae and adults of the most used insect models. In eggs, however, innate immunity has only been experimentally demonstrated in a few insect species to date, including the tobacco hornworm *M. sexta* (Gorman et al., 2004), *T. castaneum* (Jacobs et al., 2014a), *T. molitor* (Jacobs et al., 2017), the milkweed bug *O. fasciatus* and the migratory locust *L. migratoria* (Jacobs et al., 2022). These eggs do have a serosa as described under 'Function of the serosa'. Thus, the serosa seems to be a prerequisite for an inducible innate immune response. In chapter 2, I will test this hypothesis by investigating the immune response in *Orchesella cincta*, a species outside of the insects that does not have a serosa.

Table 1-1. A few examples of studies describing the immune response of insects during post-embryonic development.

Common name	Scientific name	References
Fruit fly	*Drosophila melanogaster*	(Ghosh et al., 2015; Hoffmann, 2003; Jacobs and van der Zee, 2013)
Red flour beetle	*Tribolium castaneum*	(Altincicek et al., 2008; Behrens et al., 2014; Bi et al., 2019; Choi et al., 2022; Contreras et al., 2013; Jacobs et al., 2014a; Jacobs and van der Zee, 2013; Milutinović et al., 2013; Zou et al., 2007)
Yellow mealworm	*Tenebrio molitor*	(Johnston et al., 2014)
Monarch butterfly	*Danaus plexippus*	(Lindsey and Altizer, 2009)
Tobacco budworm	*Heliothis virescens*	(Barthel et al., 2015)
Honey bees	*Apis mellifera*	(Barribeau et al., 2015; Evans et al., 2006)
Red imported fire ant	*Solenopsis invicta*	(Holmes and Johnston, 2023; Schluns and Crozier, 2009; Wei et al., 2021)
Kissing bugs	*Rhodnius prolixus* and *Triatoma infestans*	(Lobo et al., 2015; Salcedo-Porras and Lowenberger, 2019; Vieira et al., 2015)
Migratory locust	*Locusta migratoria*	(Mullen and Goldsworthy, 2003; Zhang et al., 2015)

Evolutionary trade-offs

All living organisms are subject to finite resources. This means that life-history traits (or fitness components) are often negatively related to each other, resulting in trade-offs. At its core, a trade-off is a process of optimization and occurs when a beneficial change in one life-history trait is linked to a detrimental change in another (Stearns, 1989). So, trade-offs are typically seen as the result of tactical or strategic choices over limited resources of organisms. In other words, these trade-offs are the manifestation of the resource allocation of organisms. For instance, investment in immune defense must reduce investment in traits. Thus, trade-offs are used to optimize the competing requirements of life-history traits, such as growth, maintenance and reproduction, preventing sole allocation of limited resources to a single trait (Adamo et al., 2001; Bolund, 2020; Schmid-Hempel, 2005) (Figure 1-8). Trade-offs have played a key role in the development of life-history theory and interpretation (Zera and Harshman, 2001).

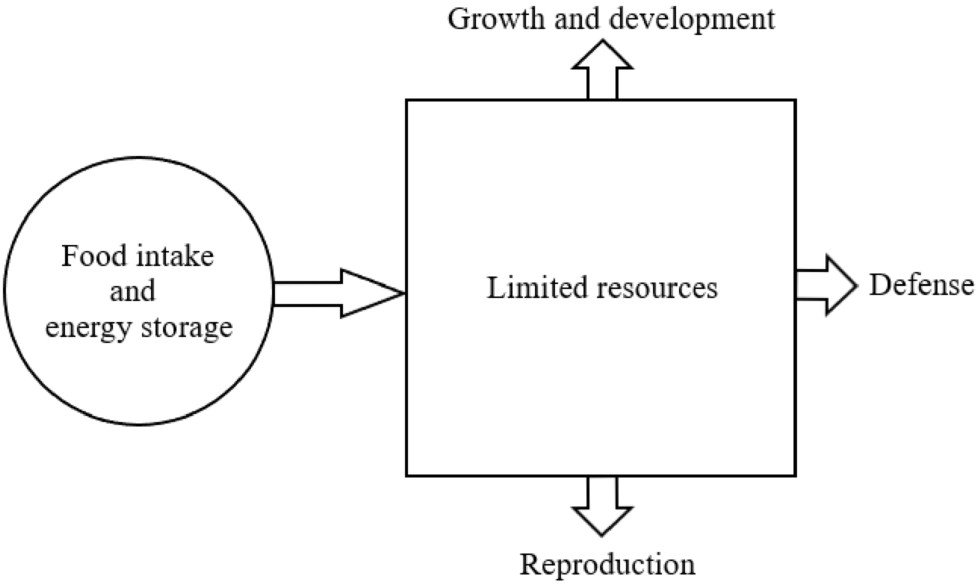

Figure 1-8. A conceptual diagram of trade-offs associated with the allocating of limited resources to competing physiological functions. Adopted from (Züst and Agrawal, 2017).

In the absence of trade-offs, an organism could maximize all its fitness components simultaneously and therefore become a 'Darwinian Demon' that reproduces directly after being born, lives forever and produces infinitely many offspring (Bonsall et al., 2004; Stearns, 1992). However, such an organism would not exist in nature. All living organisms undergo birth, development, growth, reproduction and death. The evolutionary trade-offs are ubiquitous and of fundamental importance in the evolution of life-history traits. Insects need to perform multiple tasks during their lifetime, such as growth, maintenance and defense. Such functional trade-offs must exist in insects. Evolutionary trade-offs have been widely investigated in a diverse range of insect species, such as the tobacco hornworm *M. sexta* (Diamond and Kingsolver, 2011; Mira and Bernays, 2002), parasitoid wasps (e.g. Aphaereta minuta) (Jervis et al., 2008; Schwenke et al., 2016), and the fruit fly *D. melanogaster* (Ahmed et al., 2020; Chippindale et al., 1996). For instance, shorter developmental time is predicted to lead to reduced adult weight and fecundity.

In many organisms, the most prominent trade-off between life-history traits is associated with the cost of reproduction which has two major parts: costs paid for survival, such as parental survival; and costs paid for reproduction, such as fecundity (Stearns, 1989). A classic example of this kind of trade-off is that long-lived flies have decreased early fecundity in the fruit fly *D. melanogaster* (Djawdan et al., 2004; Leroi et al., 1994; Zwaan et al., 1995). The trade-off between reproduction and immune responses has been observed in a diversity of insects, probably owing to the alternative allocation of resources (Schwenke et al., 2016). In recent years, it has been demonstrated that reproduction trades off with many other life-history traits in insects, such as survival, maintenance, migration, flight capability (or dispersal), longevity and body structures (Ellers, 1996; Hanski et al., 2006; Langellotto et al., 2000; Lin et al., 2016; Rodrigues and Flatt, 2016; Tanaka and Suzuki, 1998; Wilson et al., 2020).

However, what remains unclear is whether there are any proximate mechanisms underlying these trade-offs. These proximate mechanisms could be shared across insect taxa (Harshman and Zera, 2007; Schwenke et al., 2016). In insects, most decisions on physiological, developmental, and behavioral events are mediated by hormones (Gade et al., 1997; Ketterson and Nolan, 1999; Lösel and Wehling, 2003; Stearns, 1989). There are two major classes of hormone in insects: (i) the true hormones produced by conventional glandular tissues comprising the prothoracic glands for ecdysteroids and the corpora allata for juvenile hormones; (ii) the neuropeptide hormones produced by the neurosecretory cells that reside in the central nervous system. Molting and metamorphosis are the best studied hormonal-stimulated events in the life cycle of insects, of which ecdysone executes these events, and juvenile hormone modulates ecdysteroid action. By contrast, most insect hormones are neurosecretory products. They play a crucial role in physiological, developmental, and behavioral processes controlled by the neurosecretory cells in the central nervous system (Gade et al., 1997; Nijhout, 1998; Rolff et al., 2019). However, it is still unknown how so many hormones cooperate to mechanistically regulate the trade-off in a molecular network. In chapter 4, I will study the genetic basis of different developmental speed caused by altered ecdysone pulses.

Aim and outline of this thesis

The general aim of this work is to investigate:

1) the relation between the presence of a serosa and the innate immune response in the eggs of hexapoda;
2) the trade-offs between developmental speed and other life history traits including immune competence, pupal weight and fecundity;
3) the genetic basis of this trade-off in insects.

In **Chapter 2**, I ask whether focus on the evolution of the immune competence in eggs of a very basal non-insect springtail (*Orchesella cincta*), which belongs to the closely related hexapod subclass Collembola (Figure 1-7) that do not have a serosa. We do not find any evidence for maternal provision of antimicrobials to *Orchesella* eggs in zone-of-inhibition assays of egg extracts. However, we find that *Orchesella* eggs can upregulate immune genes after bacterial challenge in the absence of a serosa. Thus, I conclude that the serosa is not an absolute prerequisite for an innate immune response in insect eggs.

In **Chapter 3**, I ask what trade-offs are associated with increased developmental speed. We set up an evolutionary experiment comparing fast vs. slow embryonic development in the model organism *T. castaneum*. We do not find a trade-off between immune defense and embryonic development time in the selection populations. We do demonstrate a strong negative correlation between fast embryonic development and early fecundity. These results broaden our understanding of trade-offs between fitness components in life-history theory.

In **Chapter 4**, I analyze the genetic underpinning of fast embryonic development in *T. castaneum*. I identify two genomic regions under the selection by pooled resequencing. Using qPCR and a small-scale RNAi screen, we identify two alleles: a deletion upstream of the ecdysone-degrading enzyme *cytochrome18a1*, and a region upstream of *melted*, a gene involved in insulin signaling (Baker and Thummel, 2007; Teleman et al., 2005). The first allele is associated with a peak of ecdysone that induces dorsal closure during embryonic development. This is a major finding, as ecdysone is mainly known from larval and pupal development of insects (Dubrovsky, 2005; Moeller et al., 2017; Robbins et al., 1968). Finally, I confirm the functionality of the deletion upstream of *cytochrome18a1* using Crispr/cas9 technology. This deletion advances ecdysone peaks and also mediates the trade-off with pupal weight and fecundity.

In **Chapter 5**, I discuss the results in an evolutionary perspective.

References

Abram, P. K., Brodeur, J., Urbaneja, A. and Tena, A. (2019). Nonreproductive effects of insect parasitoids on their hosts. *Annual Review of Entomology*, 259-276.

Adamo, S. A., Jensen, M. and Younger, M. (2001). Changes in lifetime immunocompetence in male and female Gryllus texensis (formerly G. integer): trade-offs between immunity and reproduction. *Animal Behaviour* **62**, 417-425.

Aguiar, A. P., Deans, A. R., Engel, M. S., Forshage, M., Huber, J. T., Jennings, J. T., Johnson, N. F., Lelej, A. S., Longino, J. T. and Lohrmann, V. (2013). Order Hymenoptera. In: Zhang, Z.-Q.(Ed.) Animal Biodiversity: An Outline of Higher-level Classification and Survey of Taxonomic Richness (Addenda 2013). *Zootaxa* **3703**, 51-62.

Ahmed, M. Z., Araujo-Jnr, E. V., Welch, J. J. and Kawahara, A. Y. (2015). Wolbachia in butterflies and moths: geographic structure in infection frequency. *Frontiers in Zoology* **12**, 1-9.

Ahmed, S. M. H., Maldera, J. A., Krunic, D., Paiva-Silva, G. O., Pénalva, C., Teleman, A. A. and Edgar, B. A. (2020). Fitness trade-offs incurred by ovary-to-gut steroid signalling in Drosophila. *Nature* **584**, 415-419.

Altincicek, B., Knorr, E. and Vilcinskas, A. (2008). Beetle immunity: Identification of immune-inducible genes from the model insect Tribolium castaneum. *Developmental & Comparative Immunology* **32**, 585-595.

Bairagi, S. H. (2019). Insects with Potential Medicinal Significance: A Review. *Biomedical Journal of Scientific & Technical Research* **16**, 12024-12027.

Baker, K. D. and Thummel, C. S. (2007). Diabetic larvae and obese flies—emerging studies of metabolism in Drosophila. *Cell metabolism* **6**, 257-266.

Barribeau, S. M., Sadd, B. M., du Plessis, L., Brown, M. J., Buechel, S. D., Cappelle, K., Carolan, J. C., Christiaens, O., Colgan, T. J. and Erler, S. (2015). A depauperate immune repertoire precedes evolution of sociality in bees. *Genome biology* **16**, 1-21.

Barthel, A., Staudacher, H., Schmaltz, A., Heckel, D. G. and Groot, A. T. (2015). Sex-specific consequences of an induced immune response on reproduction in a moth. *BMC evolutionary biology* **15**, 1-12.

Beck, C. W. and Blumer, L. S. (2020). Advancing Undergraduate Laboratory Education Using Non-Model Insect Species. *Annual Review of Entomology* **66**.

Behrens, S., Peuß, R., Milutinović, B., Eggert, H., Esser, D., Rosenstiel, P., Schulenburg, H., Bornberg-Bauer, E. and Kurtz, J. (2014). Infection routes matter in population-specific responses of the red flour beetle to the entomopathogen Bacillus thuringiensis. *BMC genomics* **15**, 1-17.

Benton, M. A. (2018). A revised understanding of Tribolium morphogenesis further reconciles short and long germ development. *PLoS biology* **16**, e2005093.

Benton, M. A., Akam, M. and Pavlopoulos, A. (2013). Cell and tissue dynamics during Tribolium embryogenesis revealed by versatile fluorescence labeling approaches. *Development* **140**, 3210-3220.

Berenbaum, M. R. (1996). *Bugs in the system: insects and their impact on human affairs*: Basic Books.

Bi, J., Feng, F., Li, J., Mao, J., Ning, M., Song, X., Xie, J., Tang, J. and Li, B. (2019). AC-type lectin with a single carbohydrate-recognition domain involved in the innate immune response of Tribolium castaneum. *Insect molecular biology* **28**, 649-661.

Bolund, E. (2020). The challenge of measuring trade-offs in human life history research. *Evolution and Human Behavior* **41**, 502-512.

Bonsall, M. B., Jansen, V. A. and Hassell, M. P. (2004). Life history trade-offs assemble ecological guilds. *Science* **306**, 111-114.

Brown, S. J., Shippy, T. D., Miller, S., Bolognesi, R., Beeman, R. W., Lorenzen, M. D., Bucher, G., Wimmer, E. A. and Klingler, M. (2009). The red flour beetle, Tribolium castaneum (Coleoptera): a model for studies of development and pest biology. *Cold Spring Harbor Protocols* **2009**, pdb. emo126.

Buchwald, R., Breed, M. D., Greenberg, A. R. and Otis, G. (2006). Interspecific variation in beeswax as a biological construction material. *Journal of experimental biology* **209**, 3984-3989.

Campbell, J. F., Athanassiou, C. G., Hagstrum, D. W. and Zhu, K. Y. (2021). Tribolium castaneum: A Model Insect for Fundamental and Applied Research. *Annual review of entomology* **67**.

Charles, H. M. and Killian, K. A. (2015). Response of the insect immune system to three different immune challenges. *Journal of insect physiology* **81**, 97-108.

Chen, G., Handel, K. and Roth, S. (2000). The maternal NF-kappaB/dorsal gradient of Tribolium castaneum: dynamics of early dorsoventral patterning in a short-germ beetle. *Development* **127**, 5145-5156.

Chipman, A. D. (2017). Oncopeltus fasciatus as an evo-devo research organism. *genesis* **55**, e23020.

Chippindale, A. K., Chu, T. J. and Rose, M. R. (1996). Complex trade-offs and the evolution of starvation resistance in Drosophila melanogaster. *Evolution* **50**, 753-766.

Choi, B., Park, W.-R., Kim, Y.-J., Mun, S., Park, S.-J., Jeong, J.-H., Choi, H.-S. and Kim, D.-K. (2022). Nuclear receptor estrogen-related receptor modulates antimicrobial peptide expression for host innate immunity in Tribolium castaneum. *Insect Biochemistry and Molecular Biology*, 103816.

Chulay, J. D., Schneider, I., Cosgriff, T. M., Hoffman, S. L., Ballou, W. R., Quakyi, I. A., Carter, R., Trosper, J. H. and Hockmeyer, W. T. (1986). Malaria transmitted to humans by mosquitoes infected from cultured Plasmodium falciparum. *The American journal of tropical medicine and hygiene* **35**, 66-68.

Contreras, E., Rausell, C. and Real, M. D. (2013). Proteome response of Tribolium castaneum larvae to Bacillus thuringiensis toxin producing strains. *PloS one* **8**, e55330.

Crowson, R. A. (2013). *The biology of the Coleoptera*: Academic press.

Davis, M. M. and Engström, Y. (2012). Immune response in the barrier epithelia: lessons from the fruit fly Drosophila melanogaster. *Journal of innate immunity* **4**, 273-283.

Deutsch, C. A., Tewksbury, J. J., Tigchelaar, M., Battisti, D. S., Merrill, S. C., Huey, R. B. and Naylor, R. L. (2018). Increase in crop losses to insect pests in a warming climate. *Science* **361**, 916-919.

Diamond, S. E. and Kingsolver, J. G. (2011). Host plant quality, selection history and trade-offs shape the immune responses of Manduca sexta. *Proceedings of the Royal Society B: Biological Sciences* **278**, 289-297.

Djawdan, M., Sugiyama, T. T., Schlaeger, L. K., Bradley, T. J. and Rose, M. R. (2004). Metabolic aspects of the trade-off between fecundity and longevity in Drosophila melanogaster. In *Methuselah flies: A case study in the evolution of aging*, pp. 145-164: World Scientific.

Dongol, T., Dhananjaya, B., Shrestha, R. K. and Aryal, G. (2014). Pharmacological and immunological properties of wasp venom. *Pharmacology and Therapeutics*, 47-81.

Dornelas, M. and Daskalova, G. N. (2020). Nuanced changes in insect abundance. *Science* **368**, 368-369.

Dubrovsky, E. B. (2005). Hormonal cross talk in insect development. *Trends in Endocrinology & Metabolism* **16**, 6-11.

Ellers, J. (1996). Fat and eggs: an alternative method to measure the trade-off between survival and reproduction in insect parasitoids. *Netherlands Journal of Zoology* **46**, 227-235.

Emerson, C., Meline, J., Linn, A., Wallace, J., Kapella, B. K., Venkatesan, M. and Steketee, R. (2022). End Malaria Faster: Taking Lifesaving Tools Beyond "Access" to "Reach" All People in Need. *Global Health: Science and Practice* **10**.

Evans, J., Aronstein, K., Chen, Y. P., Hetru, C., Imler, J. L., Jiang, H., Kanost, M., Thompson, G., Zou, Z. and Hultmark, D. (2006). Immune pathways and defence mechanisms in honey bees Apis mellifera. *Insect molecular biology* **15**, 645-656.

Feener Jr, D. H. and Brown, B. V. (1997). Diptera as parasitoids. *Annual review of entomology* **42**, 73-97.

Fernández-Moreno, M. A., Farr, C. L., Kaguni, L. S. and Garesse, R. (2007). Drosophila melanogaster as a model system to study mitochondrial biology. In *Mitochondria*, pp. 33-49: Springer.

Ferrandon, D. (2013). The complementary facets of epithelial host defenses in the genetic model organism Drosophila melanogaster: from resistance to resilience. *Current opinion in immunology* **25**, 59-70.

Fratini, F., Cilia, G., Turchi, B. and Felicioli, A. (2016). Beeswax: A minireview of its antimicrobial activity and its application in medicine. *Asian Pacific Journal of Tropical Medicine* **9**, 839-843.

Gade, G., Hoffmann, K.-H. and Spring, J. H. (1997). Hormonal regulation in insects: facts, gaps, and future directions. *Physiological reviews* **77**, 963-1032.

Ghosh, S., Singh, A., Mandal, S. and Mandal, L. (2015). Active hematopoietic hubs in Drosophila adults generate hemocytes and contribute to immune response. *Developmental cell* **33**, 478-488.

Gill, R. J., Baldock, K. C., Brown, M. J., Cresswell, J. E., Dicks, L. V., Fountain, M. T., Garratt, M. P., Gough, L. A., Heard, M. S. and Holland, J. M. (2016). Protecting an ecosystem service: approaches to understanding and mitigating threats to wild insect pollinators. In *Advances in ecological research*, pp. 135-206: Elsevier.

Gillott, C. (2005). *Entomology*: Springer Science & Business Media.

Goltsev, Y., Rezende, G. L., Vranizan, K., Lanzaro, G., Valle, D. and Levine, M. (2009). Developmental and evolutionary basis for drought tolerance of the Anopheles gambiae embryo. *Developmental biology* **330**, 462-470.

González-Santoyo, I. and Córdoba-Aguilar, A. (2012). Phenoloxidase: a key component of the insect immune system. *Entomologia Experimentalis et Applicata* **142**, 1-16.

Gorman, M., Kankanala, P. and Kanost, M. (2004). Bacterial challenge stimulates innate immune responses in extra-embryonic tissues of tobacco hornworm eggs. *Insect molecular biology* **13**, 19-24.

Grau, T., Vilcinskas, A. and Joop, G. (2017). Probiotic Enterococcus mundtii isolate protects the model insect Tribolium castaneum against Bacillus thuringiensis. *Frontiers in microbiology* **8**, 1261.

Grimaldi, D. and Engel, M. S. (2005). *Evolution of the Insects*: Cambridge University Press.

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H. and Hörren, T. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PloS one* **12**, e0185809.

Hanski, I., Saastamoinen, M. and Ovaskainen, O. (2006). Dispersal-related life-history trade-offs in a butterfly metapopulation. *Journal of animal Ecology* **75**, 91-100.

Harshman, L. G. and Zera, A. J. (2007). The cost of reproduction: the devil in the details. *Trends in ecology & evolution* **22**, 80-86.

Hemingway, J. and Ranson, H. (2000). Insecticide resistance in insect vectors of human disease. *Annual review of entomology* **45**, 371-391.

Hilbrant, M., Horn, T., Koelzer, S. and Panfilio, K. A. (2016). The beetle amnion and serosa functionally interact as apposed epithelia. *Elife* **5**, e13834.

Hillyer, J. F. and Strand, M. R. (2014). Mosquito hemocyte-mediated immune responses. *Current opinion in insect science* **3**, 14-21.

Hoffmann, J. A. (2003). The immune response of Drosophila. *Nature* **426**, 33-38.

Holmes, V. R. and Johnston, J. S. (2023). Differential Gene Expression of Innate Immune Response Genes Consequent to Solenopsis invicta Virus-3 Infection. *Genes* **14**, 188.

Howe, R. (1956). The effect of temperature and humidity on the rate of development and mortality of Tribolium castaneum (Herbst)(Coleoptera, Tenebrionidae). *Annals of Applied Biology* **44**, 356-368.

Jacobs, C. G., Braak, N., Lamers, G. E. and van der Zee, M. (2015). Elucidation of the serosal cuticle machinery in the beetle Tribolium by RNA sequencing and functional analysis of Knickkopf1, Retroactive and Laccase2. *Insect biochemistry and molecular biology* **60**, 7-12.

Jacobs, C. G., Gallagher, J. D., Evison, S. E., Heckel, D. G., Vilcinskas, A. and Vogel, H. (2017). Endogenous egg immune defenses in the yellow mealworm beetle (Tenebrio molitor). *Developmental & Comparative Immunology* **70**, 1-8.

Jacobs, C. G., Rezende, G. L., Lamers, G. E. and van der Zee, M. (2013). The extraembryonic serosa protects the insect egg against desiccation. *Proceedings of the Royal Society B: Biological Sciences* **280**, 20131082.

Jacobs, C. G., Spaink, H. P. and van der Zee, M. (2014a). The extraembryonic serosa is a frontier epithelium providing the insect egg with a full-range innate immune response. *elife* **3**, e04111.

Jacobs, C. G., van der Hulst, R., Chen, Y.-T., Williamson, R. P., Roth, S. and van der Zee, M. (2022). Immune function of the serosa in hemimetabolous insect eggs. *Philosophical Transactions of the Royal Society B* **377**, 20210266.

Jacobs, C. G. and van der Zee, M. (2013). Immune competence in insect eggs depends on the extraembryonic serosa. *Developmental & Comparative Immunology* **41**, 263-269.

Jacobs, C. G., Wang, Y., Vogel, H., Vilcinskas, A., van Der Zee, M. and Rozen, D. E. (2014b). Egg survival is reduced by grave-soil microbes in the carrion beetle, Nicrophorus vespilloides. *BMC Evolutionary Biology* **14**, 1-8.

Jagadeeshan, S. and Singh, R. S. (2007). Rapid evolution of outer egg membrane proteins in the Drosophila melanogaster subgroup: a case of ecologically driven evolution of female reproductive traits. *Molecular biology and evolution* **24**, 929-938.

Janeway Jr, C. A. and Medzhitov, R. (2002). Innate immune recognition. *Annual review of immunology* **20**, 197-216.

Jervis, M. A., Ellers, J. and Harvey, J. A. (2008). Resource acquisition, allocation, and utilization in parasitoid reproductive strategies. *Annu. Rev. Entomol.* **53**, 361-385.

Johnston, P. R., Makarova, O. and Rolff, J. (2014). Inducible defenses stay up late: temporal patterns of immune gene expression in Tenebrio molitor. *G3: Genes, Genomes, Genetics* **4**, 947-955.

Józefiak, D., Józefiak, A., Kierończyk, B., Rawski, M., Świątkiewicz, S., Długosz, J. and Engberg, R. M. (2016). 1. Insects–a natural nutrient source for poultry–a review. *Annals of Animal Science* **16**, 297-313.

Kaaya, G. P. (1989). A review of the progress made in recent years on research and understanding of immunity in insect vectors of human and animal diseases. *International Journal of Tropical Insect Science* **10**, 751-769.

Ketterson, E. D. and Nolan, J., Val (1999). Adaptation, exaptation, and constraint: a hormonal perspective. *the american naturalist* **154**, S4-S25.

Khosravi, S., Kim, E., Lee, Y. S. and Lee, S. M. (2018). Dietary inclusion of mealworm (Tenebrio molitor) meal as an alternative protein source in practical diets for juvenile rockfish (Sebastes schlegeli). *Entomological Research* **48**, 214-221.

Kim, T. and Kim, Y. (2005). Overview of innate immunity in Drosophila. *Journal of biochemistry and molecular biology* **38**, 121.

Kingsolver, J. G. and Nagle, A. (2007). Evolutionary divergence in thermal sensitivity and diapause of field and laboratory populations of Manduca sexta. *Physiological and Biochemical Zoology* **80**, 473-479.

Langellotto, G. A., Denno, R. F. and Ott, J. R. (2000). A trade-off between flight capability and reproduction in males of a wing-dimorphic insect. *Ecology* **81**, 865-875.

Le Gall, M., Overson, R. and Cease, A. J. (2019). A global review on locusts (Orthoptera: Acrididae) and their interactions with livestock grazing practices. *Frontiers in Ecology and Evolution* **7**, 263.

Lemaitre, B. and Hoffmann, J. (2007). The host defense of Drosophila melanogaster. *Annu. Rev. Immunol.* **25**, 697-743.

Leroi, A. M., Chen, W. R. and Rose, M. R. (1994). Long-term laboratory evolution of a genetic life-history trade-off in Drosophila melanogaster. 2. Stability of genetic correlations. *Evolution* **48**, 1258-1268.

Ligoxygakis, P. (2013). Genetics of immune recognition and response in Drosophila host defense. *Advances in Genetics* **83**, 71-97.

Lin, X., Yao, Y., Wang, B., Emlen, D. J. and Lavine, L. C. (2016). Ecological trade-offs between migration and reproduction are mediated by the nutrition-sensitive insulin-signaling pathway. *International journal of biological sciences* **12**, 607.

Lindsey, E. and Altizer, S. (2009). Sex differences in immune defenses and response to parasitism in monarch butterflies. *Evolutionary Ecology* **23**, 607-620.

Lobo, L. S., Luz, C., Fernandes, E. K., Juárez, M. P. and Pedrini, N. (2015). Assessing gene expression during pathogenesis: Use of qRT-PCR to follow toxin production in the entomopathogenic fungus Beauveria bassiana during infection and immune response of the insect host Triatoma infestans. *Journal of invertebrate pathology* **128**, 14-21.

Lösel, R. and Wehling, M. (2003). Nongenomic actions of steroid hormones. *Nature reviews Molecular cell biology* **4**, 46-55.

Maeno, K. O., Piou, C. and Ghaout, S. (2020). The desert locust, Schistocerca gregaria, plastically manipulates egg size by regulating both egg numbers and production rate according to population density. *Journal of Insect Physiology* **122**, 104020.

Margaritis, L., Kafatos, F. and Petri, W. (1980). The eggshell of Drosophila melanogaster. I. Fine structure of the layers and regions of the wild-type eggshell. *Journal of cell science* **43**, 1-35.

Mayhew, P. J. (2002). Shifts in hexapod diversification and what Haldane could have said. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **269**, 969-974.

---- (2007). Why are there so many insect species? Perspectives from fossils and phylogenies. *Biological Reviews* **82**, 425-454.

Milutinović, B., Stolpe, C., Peuβ, R., Armitage, S. A. and Kurtz, J. (2013). The red flour beetle as a model for bacterial oral infections. *PloS one* **8**, e64638.

Mira, A. and Bernays, E. A. (2002). Trade-offs in host use by Manduca sexta: Plant characters vs natural enemies. *Oikos* **97**, 387-397.

Mirzoyan, Z., Sollazzo, M., Allocca, M., Valenza, A. M., Grifoni, D. and Bellosta, P. (2019). Drosophila melanogaster: A model organism to study cancer. *Frontiers in Genetics* **10**, 51.

Moeller, M. E., Nagy, S., Gerlach, S. U., Soegaard, K. C., Danielsen, E. T., Texada, M. J. and Rewitz, K. F. (2017). Warts signaling controls organ and body growth through regulation of ecdysone. *Current Biology* **27**, 1652-1659. e1654.

Mullen, L. and Goldsworthy, G. (2003). Changes in lipophorins are related to the activation of phenoloxidase in the haemolymph of Locusta migratoria in response to injection of immunogens. *Insect biochemistry and molecular biology* **33**, 661-670.

Münster, S., Jain, A., Mietke, A., Pavlopoulos, A., Grill, S. W. and Tomancak, P. (2019). Attachment of the blastoderm to the vitelline envelope affects gastrulation of insects. *Nature* **568**, 395-399.

Nicholson, D. B., Ross, A. J. and Mayhew, P. J. (2014). Fossil evidence for key innovations in the evolution of insect diversity. *Proceedings of the Royal Society B: Biological Sciences* **281**, 20141823.

Nijhout, H. F. (1998). *Insect hormones*: Princeton University Press.

Novotny, V., Drozd, P., Miller, S. E., Kulfan, M., Janda, M., Basset, Y. and Weiblen, G. D. (2006). Why are there so many species of herbivorous insects in tropical rainforests? *science* **313**, 1115-1118.

Oerke, E.-C., Dehne, H.-W., Schönbeck, F. and Weber, A. (2012). *Crop production and crop protection: estimated losses in major food and cash crops*: Elsevier.

Organization, W. H. (2022). World malaria report 2021. 2021. *Reference Source.*

Panfilio, K. A. (2008). Extraembryonic development in insects and the acrobatics of blastokinesis. *Developmental biology* **313**, 471-491.

Peng, B.-Y., Su, Y., Chen, Z., Chen, J., Zhou, X., Benbow, M. E., Criddle, C. S., Wu, W.-M. and Zhang, Y. (2019). Biodegradation of polystyrene by dark (Tenebrio obscurus) and yellow (Tenebrio molitor) mealworms (Coleoptera: Tenebrionidae). *Environmental science & technology* **53**, 5256-5265.

Peters, R. S., Krogmann, L., Mayer, C., Donath, A., Gunkel, S., Meusemann, K., Kozlov, A., Podsiadlowski, L., Petersen, M. and Lanfear, R. (2017). Evolutionary history of the Hymenoptera. *Current Biology* **27**, 1013-1018.

Phiri, B. J., Fèvre, D. and Hidano, A. (2022). Uptrend in global managed honey bee colonies and production based on a six-decade viewpoint, 1961–2017. *Scientific Reports* **12**, 21298.

Pough, F. H. (1991). Recommendations for the care of amphibians and reptiles in academic institutions. *ILAR Journal* **33**, S1-S21.

Powell, J. A. (2009). Lepidoptera: moths, butterflies. In *Encyclopedia of insects*, pp. 559-587: Elsevier.

Price, P. W. (1997). *Insect ecology*: John Wiley & Sons.

Quicke, D. L. (2009). Hymenoptera: Ants, Bees, Wasps. In *Encyclopedia of Insects*, pp. 473-484: Elsevier.

Rafiqi, A. M., Lemke, S., Ferguson, S., Stauber, M. and Schmidt-Ott, U. (2008). Evolutionary origin of the amnioserosa in cyclorrhaphan flies correlates with spatial and temporal expression changes of zen. *Proceedings of the National Academy of Sciences* **105**, 234-239.

Rezende, G. L., Martins, A. J., Gentile, C., Farnesi, L. C., Pelajo-Machado, M., Peixoto, A. A. and Valle, D. (2008). Embryonic desiccation resistance in Aedes aegypti: presumptive role of the chitinized serosal cuticle. *BMC developmental biology* **8**, 1-14.

Rezende, G. L., Vargas, H. C. M., Moussian, B. and Cohen, E. (2016). Composite eggshell matrices: Chorionic layers and sub-chorionic cuticular envelopes. In *Extracellular composite matrices in arthropods*, pp. 325-366: Springer.

Robbins, W., Kaplanis, J., Thompson, M., Shortino, T., Cohen, C. and Joyner, S. (1968). Ecdysones and analogs: effects on development and reproduction of insects. *Science* **161**, 1158-1160.

Rodrigues, M. A. and Flatt, T. (2016). Endocrine uncoupling of the trade-off between reproduction and somatic maintenance in eusocial insects. *Current opinion in insect science* **16**, 1-8.

Rolff, J., Johnston, P. R. and Reynolds, S. (2019). Complete metamorphosis of insects. *Philosophical Transactions of the Royal Society B* **374**, 20190063.

Rosenberg, M. I., Lynch, J. A. and Desplan, C. (2009). Heads and tails: evolution of antero-posterior patterning in insects. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms* **1789**, 333-342.

Roth, S. (2004). Gastrulation in other insects. *Gastrulation: from cells to embryos* **105**, 121.

Roush, R. and Tabashnik, B. E. (2012). *Pesticide resistance in arthropods*: Springer Science & Business Media.

Rytkönen, S., Vesterinen, E. J., Westerduin, C., Leviäkangas, T., Vatka, E., Mutanen, M., Välimäki, P., Hukkanen, M., Suokas, M. and Orell, M. (2019). From feces to data: A metabarcoding method for analyzing consumed and available prey in a bird-insect food web. *Ecology and evolution* **9**, 631-639.

Salcedo-Porras, N. and Lowenberger, C. (2019). The innate immune system of kissing bugs, vectors of chagas disease. *Developmental & Comparative Immunology* **98**, 119-128.

Sanchez-Bayo, F. and Goka, K. (2014). Pesticide residues and bees–a risk assessment. *PloS one* **9**, e94482.

Sander, K. (1976). Morphogenetic movements in insect embryogenesis. In *Symposia of the Royal Entomological Society of London*.

Sarwar, M. (2015). Direct Possessions of Insect Arthropods on Humans Owing to Allergen, Bloodsucking, Biting, Envenomation and Stinging Side By Side Case Diagnosis and Treating. *International Journal of Bioinformatics and Biomedical Engineering* **1**, 331-337.

Sastry, A., Vernberg, F. and Vernberg, W. (1983). Ecological aspects of reproduction. *The biology of Crustacea* **8**, 179-270.

Schluns, H. and Crozier, R. H. (2009). Molecular and chemical immune defenses in ants (Hymenoptera: Formicidae). *Myrmecological News* **12**, 237-249.

Schmid-Hempel, P. (2005). Evolutionary ecology of insect immune defenses. *Annu. Rev. Entomol.* **50**, 529-551.

Schmidt-Ott, U., Rafiqi, A. M. and Lemke, S. (2010). Hox3/zen and the evolution of extraembryonic epithelia in insects. *Hox Genes*, 133-144.

Schwenke, R. A., Lazzaro, B. P. and Wolfner, M. F. (2016). Reproduction–immunity trade-offs in insects. *Annual review of entomology* **61**, 239-256.

Shelton, A. M., Zhao, J.-Z. and Roush, R. T. (2002). Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. *Annual review of entomology* **47**, 845-881.

Stearns, S. C. (1989). Trade-offs in life-history evolution. *Functional ecology* **3**, 259-268.

---- (1992). *The evolution of life histories*: Oxford university press Oxford.

Stork, N. E. (2018). How many species of insects and other terrestrial arthropods are there on Earth? *Annual review of entomology* **63**, 31-45.

Tanaka, S. (2017). Locusta migratoria (Orthoptera: Acrididae) embryos monitor neighboring eggs for hatching synchrony. *Journal of orthoptera research*, 103-115.

Tanaka, S. and Suzuki, Y. (1998). Physiological trade-offs between reproduction, flight capability and longevity in a wing-dimorphic cricket, Modicogryllus confirmatus. *Journal of Insect Physiology* **44**, 121-129.

Teleman, A. A., Chen, Y.-W. and Cohen, S. M. (2005). Drosophila Melted modulates FOXO and TOR activity. *Developmental cell* **9**, 271-281.

Tsakas, S. and Marmaras, V. (2010). Insect immunity and its signalling: an overview. *Invertebrate Survival Journal* **7**, 228-238.

Tulloch, A. P. (1980). Beeswax—composition and analysis. *Bee world* **61**, 47-62.

Ugur, B., Chen, K. and Bellen, H. J. (2016). Drosophila tools and assays for the study of human diseases. *Disease models & mechanisms* **9**, 235-244.

van der Zee, M., Berns, N. and Roth, S. (2005). Distinct functions of the Tribolium zerknu llt genes in serosa specification and dorsal closure. *Current Biology* **15**, 624-636.

van Klink, R., Bowler, D. E., Gongalsky, K. B., Swengel, A. B., Gentile, A. and Chase, J. M. (2020). Meta-analysis reveals declines in terrestrial but increases in freshwater insect abundances. *Science* **368**, 417-420.

Vieira, C. S., Mattos, D. P., Waniek, P. J., Santangelo, J. M., Figueiredo, M. B., Gumiel, M., Da Mota, F. F., Castro, D. P., Garcia, E. S. and Azambuja, P. (2015). Rhodnius prolixus interaction with Trypanosoma rangeli: modulation of the immune system and microbiota population. *Parasites & vectors* **8**, 1-13.

Waegele, J. W. and Bartolomaeus, T. (2014). *Deep Metazoan Phylogeny: the Backbone of the Tree of Life: new insights from analyses of molecules, morphology, and theory of data analysis*: Walter de Gruyter.

Wei, Z., Ortiz-Urquiza, A. and Keyhani, N. O. (2021). Altered expression of chemosensory and odorant binding proteins in response to fungal infection in the red imported fire ant, Solenopsis invicta. *Frontiers in Physiology* **12**, 596571.

Wilson, J. K., Ruiz, L. and Davidowitz, G. (2020). Within-host competition drives energy allocation trade-offs in an insect parasitoid. *PeerJ* **8**, e8810.

Yang, L., Qiu, L. M., Fang, Q., Stanley, D. W. and Ye, G. Y. (2021). Cellular and humoral immune interactions between Drosophila and its parasitoids. *Insect Science* **28**, 1208-1227.

Zeh, D. W., Zeh, J. A. and Smith, R. L. (1989). Ovipositors, amnions and eggshell architecture in the diversification of terrestrial arthropods. *The Quarterly Review of Biology* **64**, 147-168.

Zera, A. J. and Harshman, L. G. (2001). The physiology of life history trade-offs in animals. *Annual review of Ecology and Systematics* **32**, 95-126.

Zhang, L., Lecoq, M., Latchininsky, A. and Hunter, D. (2019). Locust and grasshopper management. *Annual review of entomology* **64**, 15-34.

Zhang, W., Chen, J., Keyhani, N. O., Zhang, Z., Li, S. and Xia, Y. (2015). Comparative transcriptomic analysis of immune responses of the migratory locust, Locusta migratoria, to challenge by the fungal insect pathogen, Metarhizium acridum. *BMC genomics* **16**, 1-21.

Zhang, Z.-Q. (2011). *Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness*: Magnolia press.

---- (2013). Phylum Arthropoda. In: Zhang, Z.-Q.(Ed.) Animal Biodiversity: An Outline of Higher-level Classification and Survey of Taxonomic Richness (Addenda 2013). *Zootaxa* **3703**, 17–26-17–26.

Zhou, J. C., Liu, Q. Q., Wang, Q. R., Ning, S. F., Che, W. N. and Dong, H. (2020). Optimal clutch size for quality control of bisexual and Wolbachia-infected thelytokous lines of Trichogramma dendrolimi Matsumura (Hymenoptera: Trichogrammatidae) mass reared on eggs of a substitutive host, Antheraea pernyi Guérin-Méneville (Lepidoptera: Saturniidae). *Pest management science* **76**, 2635-2644.

Zou, Z., Evans, J. D., Lu, Z., Zhao, P., Williams, M., Sumathipala, N., Hetru, C., Hultmark, D. and Jiang, H. (2007). Comparative genomic analysis of the Tribolium immune system. *Genome biology* **8**, 1-16.

Züst, T. and Agrawal, A. A. (2017). Trade-offs between plant growth and defense against insect herbivory: an emerging mechanistic synthesis. *Annual review of plant biology* **68**, 513-534.

Zwaan, B., Bijlsma, R. and Hoekstra, R. (1995). Direct selection on life span in Drosophila melanogaster. *Evolution* **49**, 649-659.