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„Maternal nutritional supplementation affects offspring learning in foraging predatory mites"

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1. INTRODUCTION

1.1 Background

The ability to learn is an almost universal phenomenon, found in a wide range of animal taxa (e.g. Dukas, 1998; Alcock, 2009; Haselgrove, 2016) including plant-inhabiting predatory mites of the family Phytoseiidae (Acari, Mesostigmata) (Schausberger, 2007; Rahmani et al., 2009; Schausberger et al., 2010; Peralta Quesada and Schausberger, 2012; Christiansen et al., 2016; Seiter and Schausberger, 2016; Christiansen and Schausberger, 2017; Reichert et al., 2017). Many animals can learn throughout life. However, learning is especially important in sensitive phases early in life (Knudsen, 2004) and can have significant advantages in various contexts within fluctuating and/or unpredictable environments (Breed and Moore, 2016), especially when it comes to foraging. Phytoseiid mites that experience prey early in life can display shorter recognition times and decreased handling times of the same prey as adults (Schausberger et al., 2010; Christiansen et al., 2016; Seiter and Schausberger, 2016; Christiansen and Schausberger, 2017; Reichert et al., 2017; Schausberger and Peneder, 2017). Ultimately, this can improve net prey profitability (MacArthur and Pianka, 1966) and can generate various adaptive advantages throughout life (Papaj and Lewis, 1993; Dukas, 2008; Alcock, 2009), including enhanced survival (Rahmani et al., 2009).

The phenotypic expression of learning, however, depends on a number of immediate environmental circumstances (Mery, 2013), including diet (reviewed in Laus et al., 2011; Nyaradi et al., 2013), which can have ramifications on phenotypic expression, not only within individuals, but also across generations (Xia et al., 1997; Reichert et al., 2017). Instances in which a mother's environment influences the phenotypic expression of her offspring are known as maternal effects (Bernardo, 1996; Mousseau and Fox, 1998a,b; Wolf and Wade, 2009) and have recently been discovered to operate within the predatory mite *Amblyseius swirskii* with regard to maternal diet and offspring early learning ability in the context of foraging (Reichert et al., 2017). Offspring of pollen-reared mothers do not display learning ability in foraging contexts, but mothers reared on live spider mite prey produce offspring capable of learning. Maternal diet switches from one diet type to the other result in

either restoration or loss of learning ability in offspring from the respective lines (Reichert et al., 2017).

In recent decades, phytoseiid species have become increasingly important as acarine biocontrol agents of various pests in greenhouses (Gerson et al., 2003), especially *A. swirskii*, due to its ability to survive on a wide range of food types that include both animal- and plant-based foods (Ragusa and Swirski, 1975; Gerson and Weintraub, 2007; Calvo et al., 2015). Learning can ultimately be relevant to the field of biological control, as it may be used to increase the efficacy of predators (Hare and Morgan, 1997) and, therefore, diet-based differences in learning ability may be pertinent to the rearing processes of acarine predators used to combat various herbivorous pests.

1.2 Learning

Learning is a ubiquitous phenomenon throughout the animal kingdom, observed in taxa ranging from protozoans to humans (Applewhite et al., 1969; Dukas, 2008; Mery, 2013; Menzel and Benjamin, 2013; Haselgrove, 2016). In a broad sense, learning is defined as a change in behavior resulting from experience (Dukas, 1998; Breed and Moore, 2016). In neurological terms, it involves neuronal representations of new information, such as sensory or spatial information, motor patterns, or associations between novel stimuli and surroundings (Dukas, 2004; 2008) and is inherently tied to memory (Dukas, 1999). Learning can influence any of life's major endeavors, including foraging, mating, predator avoidance, and social interactions (Dukas, 1998; 2008) and may be highly variable depending on species, population, individual, and context (Dukas, 2013).

Though some animals learn throughout a lifetime, proverbs like "you can't teach an old dog new tricks" suggest the importance of learning early in life. In fact, early learning is crucial for a number of tasks within various taxa and can have long-lasting influences on behavior and/or adaptive advantages throughout life (Knudsen, 2004). These so-called learning sensitive phases usually arise shortly after birth or hatching, since neural systems are most plastic early on (Knudsen, 2004). Examples of learning during early sensitive periods are found across the animal kingdom, including song learning in songbirds (Leppelsack, 1986), filial imprinting in ducks and

geese (Lorenz, 1935; Ramsay and Hess, 1954), natal homing in salmon (Dittman et al., 1996), social behavior in ants (Champalbert and Lachaud, 1990), food imprinting in cuttlefish (Darmaillacq et al., 2006), and food learning in humans (Cashdan, 1994).

For learning to evolve, learning ability must be genetically-based, vary among individuals within a population, and be tied to individual fitness (Papaj and Prokopy, 1989; Dukas, 2008; Mery, 2013). One obvious fitness benefit associated with learning ability is the fact that learners can optimize their rate of resource acquisition, e.g. increase the units of food or mates encountered per unit time, translating to greater lifetime reproductive success (i.e. a proxy of evolutionary fitness). Like other adaptive traits, learning is ultimately governed by natural selection; it allows animals to adjust their behavioral repertoire to better cope with the selective pressures of their environments and quite often increases evolutionary fitness (Papaj and Lewis, 1993; Dukas, 2008; 2013; Alcock, 2009). According to Gould (1993), learning ability "exists to solve problems that innate information cannot satisfactorily deal with," (p. 27) and it is therefore generally assumed that learning is advantageous in fluctuating but moderately predictable environments (Stephens, 1987; reviewed in Papaj and Prokopy, 1989; Mery, 2013). For example, parasitoid wasps are able to learn cues for host selection in fluctuating environments (Vet et al., 1995), but learning ability is reduced in highly predictable habitats (Potting et al., 1997). Whereas predictable situations (e.g. constant resources) can be met with gene regulation or innate behavior, learning provides additional plasticity in situations unique in time and space (Dukas, 1998; 2013; Mery, 2013). Of course, completely random environments should not be conducive to learning either, since it should not be advantageous to learn cues that may quickly become unreliable in highly variable settings (Stephens, 1993).

Though it can bring many benefits, learning requires energy and binds resources (Laughlin et al., 1998) and, consequently, also incurs costs (Johnston, 1982; Mery and Kawecki, 2003; 2004; Snell-Rood et al., 2009; Christiansen et al., 2016). The costs of learning are usually classified as being either constitutive (i.e. the costs of developing brain structures and maintenance of learning ability) or operating (i.e. the costs of establishing neural connections and the storage/maintenance of information; Snell-Rood et al., 2009; Christiansen et al., 2016). Constitutive costs are paid by any individual with the potential to learn, regardless of whether or not learning actually

takes place (Burns et al., 2010), meaning that it is possible for an individual to pay these costs without ever reaping any of the benefits of learning. For example, *Drosophila* lines artificially selected for improved learning ability display poorer larval competitive ability than low-learning lines, presenting a constitutive cost of learning paid by learning-capable individuals, even without ever using said ability (Mery and Kawecki, 2003). The act of learning and memory formation in fruit flies can also incur operating costs, as is evident from shorter lifespans and reduced egg-laying rates in learning than in non-learning individuals (Mery and Kawecki, 2004).

Occasionally, learning brings about improvements in certain tasks at the cost of performance in others, known as trade-offs. In *A. swirskii*, for example, predators who experience the difficult-to-grasp prey western flower thrips (WFT) *Frankliniella occidentalis* as juveniles achieve faster attack times on thrips as adults at the cost of a decline in overall longevity (Schausberger and Peneder, 2017). Additionally, thrips-experienced *A. swirskii* display longer developmental times compared to thrips-naïve conspecifics that experienced two-spotted spider mites *Tetranychus urticae* (Christiansen et al., 2016). However, adult predators display higher fecundity rates when experiencing prey that matches juvenile prey experience, suggesting that developmental costs of learning do exist, but that they are often counterbalanced by advantages in other life history traits under certain circumstances (Christiansen et al., 2016). Based on their results, Christiansen et al. (2016) concluded that thrips-experienced predators carry the cost of initially using more energy for learning difficult prey early in life, resulting in prolonged development, but, as a result, require less energy in the recognition and handling of this prey later in life and can therefore invest more energy in egg production at that time. Because of such trade-offs, learning is assumed to be beneficial only as long as its benefits to an organism outweigh its fitness costs (Dunlap and Stephens, 2009). It is therefore important to recognize the possibility of fitness trade-offs when considering the adaptive significance of learning.

It is also important to consider an animal's ecology when interpreting its learning abilities, as these can vary depending on context (Johnston, 1982). An organism's learning ability may be contingent not only upon the degree of environmental predictability (Stephens, 1993), but also on the amount and kinds of foraging decisions made over a lifetime (Roitberg et al., 1993), since learning can improve

foraging and food acquisition for many animals (reviewed in Gould, 1993; Dukas, 2013). For generalist predators, prey occurrence and availability may vary within environments or throughout a lifetime (Schoener, 1971), making learning among different diet options especially beneficial in cases where value, profitability, or abundance varies among prey types (Stephens, 1987; Dukas, 1998). Assuming that different prey types each require distinct actions to lead to a successful predation event, then a predator's ability to associate specific prey cues with the corresponding behavior from its repertoire should significantly shorten searching, recognition, and/or acceptance times, in turn increasing net profitability of prey (MacArthur and Pianka, 1966) and positively affecting life history traits (Schoener, 1971). Moreover, for omnivorous predators (among the definitions of diet generalists), diet preference may change throughout a lifetime (e.g. seasonal or habitat effects, food suitability within specific developmental stages, life history omnivory; Coll and Guershon, 2002), which contributes to the idea that generalist feeders might profit especially from learning how to acquire different food types (reviewed in Gould, 1993). Whereas specialists often use more fixed or innate behaviors in the acquisition of relatively uniform and/or consistent diets, generalists may benefit from a larger degree of plasticity in order to adjust behavior to available food sources. Learning may also help in distinguishing edible from inedible foods in changing environments (reviewed in Gould, 1993); for example, generalist bat species are able to learn to avoid foods associated with toxicosis, whereas specialist vampire bats (obligate feeders on mammal blood) are not capable of the same aversive learning (Ratcliffe et al., 2003), likely due to the fact that they are unlikely to ever encounter toxic foods in nature (Dukas, 1998). Although specialist feeders can of course also benefit from learning within the context of foraging (Dukas, 1998), sole and heavy reliance on innate abilities for diet acquisition is lacking in generalist feeders, thus making learning particularly advantageous when encountered food types are likely to vary.

In recent decades, members of the predatory mite family Phytoseiidae, which includes both diet generalists and specialists, have been the subjects of a wide range of studies investigating learning ability in various contexts. Phytoseiid mites are important biological control agents of pests in greenhouses worldwide (see http://www.koppert.com). Learning in the context of foraging has been investigated in a number of effective predators, including the generalist predatory mite *A. swirskii*, the focal animal of this thesis. *Amblyseius swirskii* can survive and reproduce on both

animal- and plant-based food sources (Ragusa and Swirski, 1975; Gerson and Weintraub, 2007; Calvo et al., 2015) and shows variation in the expression of learning ability based on rearing diet, with multigenerational effects (Christiansen et al., 2016; Seiter and Schausberger, 2016; Reichert et al., 2017), making it an ideal test subject for studies investigating maternal effects and diet-based variation in learning ability.

1.2.1 Arthropod Learning

Literature from the middle to end of the twentieth century sometimes claims that animals with small body size or short life span should not display much ability to learn (e.g. Staddon, 1983), however, more recent evidence strongly supports the contrary (reviewed in Dukas, 2008; Burns et al., 2011; Mery, 2013). Many studies show that learning in arthropods is a widespread and significant phenomenon although contexts and processes vary greatly within and among taxa (for insects, see Table 1 in Hollis and Guillette, 2015; for crustaceans: reviewed in Krasne, 1973; for arachnids: e.g. Egas et al., 2003; dos Santos et al., 2013). Among insects, parasitoid wasps (Vet et al., 1995; Potting et al., 1997), orthopterans (Dukas and Bernays, 2000; Raubenheimer and Tucker, 1997), and fruit flies (Xia et al., 1997; Mery and Kawecki, 2002; 2003; 2004) are widely used model taxa for investigating learning ability, but the most well-studied group by far are the social hymenopterans (reviewed in Gould, 1993; Menzel and Benjamin, 2013). The waggle-dance of honey bees (Menzel and Müller, 1996) and tandem-running in ants (Franklin and Franks, 2012) provide notable examples of insect learning, the latter also being one of the few cases of teaching in a nonhuman animal (Franks and Richardson, 2006). Within the Acari, phytoseiid mites provide much evidence for the importance of learning (more specifically, learning occurring in early sensitive phases) within the contexts of kin recognition (Schausberger, 2007; Christiansen and Schausberger, 2017) and foraging (Schausberger et al., 2010; Christiansen et al., 2016; Seiter and Schausberger, 2016; Reichert et al., 2017). Like in many vertebrates, learning by mites may even occur prior to birth. In the first study on prenatal learning in an arthropod, Peralta Quesada and Schausberger (2012) showed that embryonic chemosensory experience affects prey choice in the predatory mite *Neoseiulus californicus.*

1.2.2 Learning in the Context of Foraging

Two overarching categories of learning types can be distinguished, non-associative and associative learning, both of which have been found to operate in arthropods in various contexts (for reviews: Papaj and Prokopy, 1989; Papaj and Lewis, 1993; Menzel and Benjamin, 2013; Nargeot and Bédécarrats, 2017; Yu and Rankin, 2017). Non-associative learning involves behavioral changes in response to a single stimulus without any reinforcement and includes the phenomena habituation and sensitization. Associative learning, on the other hand, elicits behavioral changes in response to two previously unrelated stimuli (or a behavior and its consequence) after association through reinforcement. Associative learning often involves conditioning, where a temporal coupling of two previously unrelated events elicits a specific response (i.e. classical conditioning), or a behavior becomes associated with a consequential reward or punishment (i.e. operant conditioning; Breed and Moore, 2016; Nargeot and Bédécarrats, 2017), which has provided experimental evidence of learning in many studies. For example, cockroaches can be classically conditioned to salivate in response to odors associated with sucrose solution (Watanabe and Mizunami, 2007) and honey bees can be operantly trained to exhibit a novel behavior when paired with a food reward (Abramson et al., 2016).

Within foraging contexts, associative learning helps animals increase foraging efficiency by allowing them to repeat behaviors that have previously been advantageous in the search for and acquisition of food. Arthropod species can learn to associate environmental cues not only with the presence of food, but also with food nutritional quality (including aversion learning to nutritionally deficient foods; reviewed in Bernays, 1993). For example, locusts can learn to associate visual color cues with diet quality (Raubenheimer and Tucker, 1997). After first a training and then a deprivation period, during which test groups received one of two diets (each lacking different nutrients but together providing a balanced diet), experimental individuals spent significantly more time at the color that was originally associated with the opposite diet, i.e. the animals learned where to seek out the diet they needed in order to regain deprived nutrients (Raubenheimer and Tucker, 1997). In a study on grasshoppers, Dukas and Bernays (2000) showed that associative learning for diet quality can have adaptive advantages. One group of grasshoppers received artificial diets (one balanced, but the other lacking carbohydrates) in association with

distinct environmental cues, whereas the other group received cues randomly. Due to their ability to recognize the sites containing nutritiously balanced diets, learned individuals were able to spend the majority of time consuming the more nutritious diet, which resulted in a 20% higher growth rate within individuals of that group. Without any association of environmental cues with diet quality, non-learners visited both food types at equal rates and often broke off meals at the nutritionally deficient sites, hence spending overall less time feeding at nutritious sites. In natural settings, an increase in travel time between food patches not only decreases time that could be spent feeding, but also increases energy output and the possibility of encountering dangers while traveling. Dukas and Bernays' (2000) study, therefore, provides a prime example for some of the advantages of associative learning in foraging contexts.

Non-associative learning has been shown in arthropods within the context of foraging, albeit the majority of studies tend to focus on habituation or sensitization processes brought about in response to tactile, mechanical, or electrical stimulation, rather than a food source (reviewed in Yu and Rankin, 2017). Nonetheless, praying mantis females, for example, display habituation responses by reducing attack behavior after repeated exposure to, but restraint from catching, a prey item (Maldonado, 1972). In a study on the predatory mite *N. californicus*, Schausberger and Peneder (2017) argued that the non-associative learning process goes hand in hand with associative learning. The authors found that both contact with prey cues alone (non-associative learning) and prey cues paired with a feeding reward (associative learning) early in life increased the predation rate by experienced adults when compared to prey-naïve subjects. Though reinforcement through feeding strengthened the learning effect, both processes alone allowed for improved foraging by experienced adult predators compared to non-experienced individuals (Schausberger and Peneder, 2017), which has similarly been shown for *A. swirskii* as well (Seiter and Schausberger, 2016).

The process of learning prey early in life without any reinforcement and with lasting effects is known as food imprinting (Immelmann, 1975; Healy, 2006). Imprinting is a type of non-associative learning that involves more specific criteria than other forms of learning: the process takes place in a sensitive phase, occurs without any reinforcement, and its effects are persistent (Immelmann, 1975). Konrad Lorenz's

(1935) work on geese first drew attention to the phenomenon, making filial imprinting the most well-known and well-studied of imprinting contexts (others being kin, sexual, and food imprinting). Only a small body of work exists on food imprinting and of those studies, only a fraction have definitively demonstrated altered foraging behavior as the result of juvenile prey experience *without* the association of feeding (Darmaillacq et al., 2006; Schausberger et al., 2010). Although the protocol of this study assumes associative learning, due to the presence of prey cues in association with feeding and satiation, the additional involvement of non-associative learning cannot be ruled out, due to the obvious complexities and connections among learning processes.

1.2.3 Linking Diet and Cognition

As with other traits, the ability to learn has a complex genetic basis (Johnston, 1982; Dukas, 2004; Alcock, 2009) but environmental factors can either facilitate or impede learning performance in immediate contexts (Mery, 2013). Therefore, any variability in the expression of learning can be due to either genetic (constitutive) or environmental (operational) factors (Seiter and Schausberger, 2016). In an experiment on *Drosophila melanogaster*, Mery and Kawecki (2002) showed that artificial selection can improve learning and memory over generations, revealing strong constitutive variation in *D. melanogaster* for those traits. Operational variation in the expression of learning ability has previously been observed among populations of a single species, either due to natural variation (Raine et al., 2006) or based on varying selective pressures (e.g. predation; Brown and Braithwaite, 2005), but studies investigating within-species learning variation based solely on diet are less common in the literature.

Diet is an important aspect of an individual's environment, as it is an animal's only source of vital nutrients that cannot be produced by the body itself. Although a plethora of micronutrients play roles in neurological function and learning, some seem clearly more important than others across animal taxa (reviewed in Nyaradi et al., 2013; Prado and Dewey, 2014). A considerable amount of literature exists regarding the connection of nutritional intake with cognition and brain development in vertebrate models (for reviews: Bourre, 2004; Laus et al., 2011; Nyaradi et al., 2013; Prado and Dewey, 2014). In rats, one of the most well-studied taxa, protein

malnutrition or undernutrition can result in abnormal brain development and impaired learning ability (Alamy and Bengelloun, 2012). Studies show similar findings in humans as well; for example, early childhood malnutrition has been generally linked to lower IQ levels, lower school achievement, and greater behavioral problems in school-aged children (Grantham-McGregor, 1995), however, nutritional supplementation can improve cognitive performance in some cases (reviewed in Laus et al., 2011).

Much less work has been done on the relationship between nutrition and cognition in non-mammals. One of the first studies within the realm of arthropods to examine nutritional effects on the expression of learning ability was conducted by Xia et al. (1997) on the relationship between malnutrition and operant visual learning in *D. melanogaster*. Flies reared on a Peking medium low in proteins and high in carbohydrates were not capable of visual learning and memory formation in a flight simulator compared to conspecific flies reared on a standard medium. The authors found that after diet switches in both populations, the flies previously fed on Peking medium regained their learning ability within 5 generations and flies previously fed standard medium lost their learning ability within 3 generations. The observed population-wide gain and loss of learning ability after diet switches not only uncovered the importance of dietary intake with regard to learning in fruit flies, but also revealed a model for transgenerational dietary (operational) effects, including the bi-generational phenomena known as parental (i.e. maternal, paternal) effects.

1.3 Maternal Effects

1.3.1 An Overview

Maternal effects are defined as influences of a mother's phenotype, or the environment she experiences, on the phenotype of her offspring, independent of her genotype (Bernardo, 1996; Mousseau and Fox, 1998a; Wolf and Wade, 2009). Found in a diverse range of plant and animal species, maternal effects contribute in many ways to offspring phenotypic variation and may affect fitness, development, and survival of an organism (Mousseau and Fox, 1998a). Maternal effects are as diverse as the taxa in which they are found (Bernardo, 1996); they may affect the expression of offspring traits within any developmental stage (Wolf and Wade, 2009)

and have been linked to numerous traits including sex, morphological characters, growth rate, immune function, behavioral strategies, and food, habitat, or mate preferences (Schwabl and Groothuis, 2010). As such, these effects are context dependent and highly complex. Maternal effects can be adaptive, if maternally induced phenotypic change increases survival and reproduction (i.e. fitness) of progeny (Mousseau and Fox, 1998b), or non-adaptive, when maternal experience negatively affects offspring fitness (reviewed in Marshall and Uller, 2007). While in most cases these non-genetic transgenerational influences are maternally induced, it has recently become clear that paternal effects may be just as important, though they are by far not as well understood at this time (Crean and Bonduriansky, 2014).

Though defined decades ago, and now a field of burgeoning interest, some confusion remains as to which phenomena can be classified as maternal effects, due to the fact that some literature still interchanges the term with the much more broadly defined non-genetic maternal inheritance (Wolf and Wade, 2009). Authors Wolf and Wade (2009) explicitly exclude such phenomena as cytoplasmic inheritance, genomic imprinting, extra-chromosomal inheritance, and mitochondrial inheritance from the definition of maternal effects, because these have quite different genetic and evolutionary consequences. Historically, quantitative geneticists used the term maternal effects to explain phenotypic variation that could not otherwise be accounted for by genetics and/or developmental environment and, as a result, maternal effects were often viewed simply as bothersome noise in breeding and artificial selection (Schwabl and Groothuis, 2010). Advancements in the 1990s in disciplines like Eco-Evo-Devo and molecular biology began to shift the perspective on the subject and maternal effects are now appreciated as highly influential non-genetic effects with significant implications for evolutionary dynamics and potential adaptive advantages for offspring (Mousseau and Fox, 1998a,b; Schwabl and Groothuis, 2010).

The manifestation of maternal phenotypic effects can be demonstrated through one of two different pathways: indirect genetic effects, in which maternal genetics indirectly influence an offspring's developmental environment (reviewed in Wolf et al., 1998), or indirect environmental effects, in which a mother's environmental experience affects her progeny (reviewed in Schwabl and Groothuis, 2010), as is the case in the current study. Maternal behavior and/or experience of abiotic factors,

nutritional elements, or other ecological features of an environment can be transmitted to offspring in various ways (Rossiter, 1996; Mousseau and Fox, 1998b). In some cases, mothers are able to modify offspring phenotypes in anticipation of the surroundings their young will experience, which may be adaptive if environments are matching between generations (reviewed in Marshall and Uller, 2007; Schwabl and Groothuis, 2010; Uller et al., 2013). For example, in diapausing insects, mothers can control the induction and duration of offspring diapause based on environmental cues such as temperature and photoperiod (which often indicate seasonal changes in food availability), so that progeny may either further exploit resources when environments are favorable or to ensure offspring survival through diapause during increasingly unfavorable times (Saunders, 1965; Huestis and Marshall, 2006). In the presence of a conspecific alarm pheromone (emitted as the result of predation on a conspecific), aphid mothers produce an increased number of winged offspring, i.e. with dispersing ability (Podjasek et al., 2005) and fall field cricket females exposed to predators produce offspring that exhibit greater anti-predator behavior and survival in the presence of predators later in life (Storm and Lima, 2010). Offspring can also display enhanced immunity against a pathogen experienced by their mother (Moret, 2006), showing how maternal experience of the environment can be used to program offspring developmental traits in numerous ways, for potential adaptive advantages throughout life.

Maternal phenotypic influences may emerge through various mutually non-exclusive and often interrelated mechanisms (reviewed in Bernardo, 1996; Mousseau and Fox, 1998b; Schwabl and Groothuis, 2010). Firstly, the provisioning of biomolecules such as nutrients, hormones, growth factors, or chemical defenses by mothers into eggs may directly affect physiological, cognitive, or behavioral development of offspring and can impact growth and health over a lifetime (e.g. Dussourd et al., 1988; Rossiter, 1991; Diss et al., 1996; Kojima and Mori, 2014; Newcombe et al., 2015). For example, many avian species manipulate yolk androgen levels, both within and among clutches, providing a type of non-genetic influence on offspring behavior, growth, morphology, and immune function (reviewed in Groothuis et al., 2005; Schwabl and Groothuis, 2010). Also, gypsy moths differentially allocate egg provisions to create a range of egg, and thereby offspring, sizes (Rossiter, 1991). Secondly, in egg-laying species, a mother's decisions about oviposition site, timing, or clutch size can largely influence both pre- and postnatal developmental

environments. On the one hand, abiotic factors reflective of oviposition location can influence offspring traits, as, for example, in some oviparous reptiles with temperature-dependent sex determination (Doody et al., 2006). Oviposition behavior can also affect inter- or intraspecific interactions within the hatching environment, such as predation and intra-specific competition (Resetarits and Wilbur, 1989) or sibling competition (Parker and Begon, 1986). Thirdly, maternal effects can be transmitted through behavioral processes such as parental care. A classic example comes from rats, in which maternal grooming behaviors are transmitted to female offspring via non-genetic pathways (Champagne et al., 2003). Maternal care behavior can also influence offspring morphology; in a cross-fostering study on burying beetles with maternal care, Steiger (2013) found that offspring cared for by large females grew larger than offspring raised by small females, independent of hatching weight and genotype, showing how postnatal care can promote behavioral and/or phenotypic similarities in caregivers (i.e. parents) and offspring through purely non-genetic mechanisms. Fourthly, physical traits, such as maternal size and/or age, may also affect offspring phenotype, which has been found in swordtail fish (Kindsvater et al., 2012) and salamanders (Semlitsch and Gibbons, 1990). Finally, maternal experiences may be passed to offspring through epigenetic processes (e.g. Champagne, 2008; Bale, 2014). Certain maternal behaviors, diets, or experiences within a specific stage of offspring development can potentially cause epigenetic changes to progeny gene expression. Such effects are, however, very complex and not yet completely understood.

1.3.2 Maternal Nutritional Effects

The mechanisms by which mothers influence offspring developmental traits may encompass many different elements of the maternal phenotype or environment. Diet is a key aspect of the maternal environment, by which nutrients are transferred to the next generation (reviewed in Rossiter, 1996) and the availability of certain micronutrients within particular developmental stages may affect offspring phenotype through maternal effects. In humans, maternal nutritional deficiencies during pregnancy can influence offspring development in a number of ways (reviewed in Nyaradi et al., 2013; Morrison and Regnault, 2016) and has been linked to multigenerational health effects (Painter et al., 2008). Both maternal undernutrition,

as seen in developing countries, and higher rates of obesity (a specific form of malnutrition), prevalent in developed countries, can impact growth and behavior later in life (Morrison and Regnault, 2016).

Evidence also exists for arthropods, showing how maternal diet deficiencies can impact behavioral or morphological traits within the following generation. In gypsy moths, for example, maternal nutritional stress affects the tendency of offspring ballooning (dispersal via wind) as a consequence of low amounts of nutrients available for mothers to provision to eggs (Diss et al., 1996). In predatory mites, maternal food stress can result in sex-specific changes in egg size and sex ratio, due to the unequal energy costs of producing sons and daughters (Walzer and Schausberger, 2015). In rove beetles, maternal low-quality aphid diets cause prolonged offspring development and smaller eggs in both offspring and grand-offspring, showing that even grandmaternal diets may have important phenotypic effects (Kyneb and Toft, 2006). These examples demonstrate that not only quantity but also nutritional quality of the maternal diet affect how a mother passes along nutrients to her young and ultimately affect variation in offspring traits.

As mentioned previously, the work by Xia et al. (1997) on *D. melanogaster* demonstrates the multigenerational effects of diet quality on learning ability. Quite recently, maternal nutritional effects on learning ability have been demonstrated in predatory mites as well. In a study addressing early prey learning in the predatory mite *A. swirskii*, Reichert et al. (2017) found a recovery of offspring learning ability in a population previously not capable of learning, following a multigenerational maternal diet switch from pollen to live prey. In a population reared on only pollen for over 50 generations, mites fully regained their ability to learn in foraging contexts after both multigenerational and relatively short term (i.e. 3- and 10-day) maternal diet switches to live spider mite prey. For omnivorous predators such as *A. swirskii*, food sources can vary in their dietary value and composition; considering the nutritional differences between animal- and plant- based food sources (Murphy and Allen, 2003), the authors speculated a nutritional deficit in pollen-fed mites, responsible for the observed loss of learning ability over many generations. The studies by both Xia et al. (1997) and Reichert et al. (2017) show the implications of diet and nutrition on learning ability, not only within an individual, but also for future generations, providing the basis for this thesis.

1.4 Aim of Study

This study endeavored to determine whether nutritional supplementation in pollen-reared *A. swirskii* mothers is able to restore offspring learning ability and, if so, whether this restoration is comparable to that of offspring whose mothers were reared on live prey. Past studies show that early learning by *A. swirskii* is highly dependent on maternal diet type (Seiter and Schausberger, 2016; Reichert et al., 2017). Offspring of mothers reared on live spider mite prey, who experience wester flower thrips (WFT) in juvenile life stages, are able to more quickly recognize and attack this alternative prey as adults than thrips-naïve mites. In contrast, offspring of mothers reared on pollen alone do not show improved foraging on WFT as adults, following early-life thrips experience. It is possible that certain nutrients, or combinations thereof, necessary for the proper functioning of neural pathways involved in learning in predatory mites may be lacking in pollen but are present in animal prey and are responsible for the observed difference in learning ability between the two groups.

In this study, I tested five nutritional substances known to be involved in cognitive and neural function across animal taxa (Nyaradi et al., 2013; Prado and Dewey, 2014), and which differ between animal- and plant-based diets (Murphy and Allen, 2003): iron, vitamin B12, L-phenylalanine, L-tryptophan, and omega-3 fatty acids. Iron is essential for nervous system function and development and is used in many enzymes throughout the body. Though available in plant-based food sources, the mineral is more readily available in animal foods (Murphy and Allen, 2003) and heme-iron, used in hemoglobin and myoglobin, is found only in animal tissue (Insel et al., 2016). Iron deficiency is the most common nutrient deficiency among humans and is linked to impaired learning performance and memory development in both human and rodent models (Fretham et al., 2011). Vitamin B12 is essential for nervous system function, DNA synthesis, and cell metabolism and is found exclusively in animal food sources. In humans, low B12 levels are associated with neurodegenerative disease and cognitive impairments, some of which may be reversible, however, with B12 therapies (Moore et al., 2012). Deficiencies often arise in people consuming heavily plant-based diets, but relatively small amounts of animal source foods can help re-establish adequate B12 levels in the body (Murphy and Allen, 2003). Omega-3 fatty acids have numerous cognitive health benefits (Insel et

al., 2016) and adequate levels are needed for normal cognitive development in both humans (Richardson, 2003) and insect models (Arien et al., 2015). Omega-3s are found in both plant and animal foods; α-linolenic acid (ALA) can only be acquired through plant oils and is required to make the longer chain fatty acid docosahexaenoic acid (DHA), which may be obtained directly from animal sources (predominantly seafood) and is a structural component of the brain. The essential amino acids phenylalanine and tryptophan are used in the synthesis of proteins and other biomolecules, and must also be obtained through diet. Both molecules are precursors to various neurotransmitters, have been extensively studied in invertebrate model organisms such as the sea snail *Aplysia*, honey bees, and the fruit fly *D. melanogaster* (Yarali and Gerber, 2010; Lee et al., 2011; Menzel and Benjamin, 2013), and are, for example, associated with mood and memory in humans (Leyton et al., 2000; Riedel et al., 2003).

Each of the five described micronutrients was chosen for this thesis with the assumption that they are involved in neural and cognitive function in predatory mites. Due to ample evidence in the literature surrounding the association of these and other nutrients with cognitive processes in various animal models, and because these nutrients are either sparse or absent from plant- compared to animal-based food sources (Johri and Vasil, 1961; Murphy and Allen, 2003), it is plausible that they may contribute to pollen deficiencies in pollen-reared maternal mite lines, causing the

Figure 1: Predatory mite *Amblyseius swirskii* (© Alena Litin)

observed loss of learning ability in offspring. In this study, the five nutritional substances were presented to *A. swirskii* mothers in supplement to pollen diets with the hypothesis that supplementation results in offspring learning restoration comparable to that seen in offspring of mothers experiencing diet switches from pollen to live prey (Reichert et al., 2017). Adult offspring of mothers fed one of three maternal diets – (i) pollen only (PO), (ii) pollen plus nutritional supplements (SUP), and (iii) spider mites (SM) – were either allowed to experience WFT prey as juveniles (thrips-experienced) or not (thrips-naïve). Learning ability was quantified using a behavioral assay. Attack latency and other parameters were compared within maternal diet treatments between thrips-experienced and -naïve offspring, in order to determine if learning took place in *A. swirskii* progeny as the result of maternal nutritional supplementation. Maternal SM and PO diets were used as controls of learning ability, since previous work clearly shows the expression of learning ability in offspring of the former and lack of learning in offspring of the latter maternal food line (Seiter and Schausberger, 2016; Reichert et al., 2017).

2. MATERIALS AND METHODS

2.1 Study Organisms

2.1.1 Predatory Mite *Amblyseius swirskii*

The predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae; Table 1) is a type III-b generalist predator (McMurtry and Croft, 1997; McMurtry et al., 2013) originating in the Eastern Mediterranean area, the Nile Delta, Greece, and Israel (Heyler et al., 2014). The species is sometimes alternatively referred to as *Typhlodromips swirskii* (synonym; Figure 1). Within its original distribution, it is found on crops including citrus, apples, apricots, cotton, and vegetables, but it has recently been introduced in over 50 countries throughout the world as a biocontrol agent, both indoors and outdoors (Calvo et al., 2015; EPPO, 2018). Its wide prey spectrum includes herbivorous mite and insect species such as spider mites, thrips, whiteflies, and other small insects (Gerson and Weintraub, 2007; Calvo et al., 2015). Along with its arthropod prey, it can also feed on plant-derived substances such as pollen (Ragusa and Swirski, 1975), and can survive on insect eggs (El-Sawi and Momen, 2005), factitious prey (Riahi et al., 2017), and various artificial diets (Nguyen et al., 2013; Vangansbeke et al., 2016) under laboratory conditions. Additionally, *A. swirskii* engages in cannibalism (reviewed in Schausberger, 2003; Rasmy et al., 2004) and intra-guild predation (Buitenhuis et al., 2010) on juvenile life stages.

Table 1: Taxonomy of *Amblyseius swirskii* (after http://www.uniprot.org/taxonomy/)

Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Chelicerata
Class	Arachnida
Subclass	Acari
Superorder	Parasitiformes
Order	Mesostigmata
Superfamily	Phytoseioidea
Family	Phytoseiidae
Genus	Amblyseius
Species	*Amblyseius swirskii* Athias-Henriot, 1962

Amblyseius swirskii is well adapted to warm and humid climates. These predatory mites can develop and reproduce in temperature ranges between 15 to 36°C at 60% relative humidity (RH), with optimum temperatures around 25°C, reflective of the species' sub-tropical origins (Lee and Gillespie, 2011). They are typically found on the underside of leaves along the midrib, in domatia, or other protected areas such as those formed by the junctions of major leaf veins (McMurtry and Croft, 1997). *Amblyseius swirskii*, like all other phytoseiid mites, are eyeless but light sensitive; some studies suggest that prevailing light conditions may affect behavior, oviposition rate, and activity within certain phytoseiid species (Gerson et al., 2003). Local prey detection occurs primarily through chemical cues such as contact or volatile kairomones emitted by prey (Sabelis and Dicke, 1985). Additionally, at the regional spatial scale, predators may react to volatiles emitted by plants in response to herbivore infestation, although these herbivore-induced-plant-volatiles (HIPVs) differ depending on the plant and pest species involved (Gerson et al., 2003).
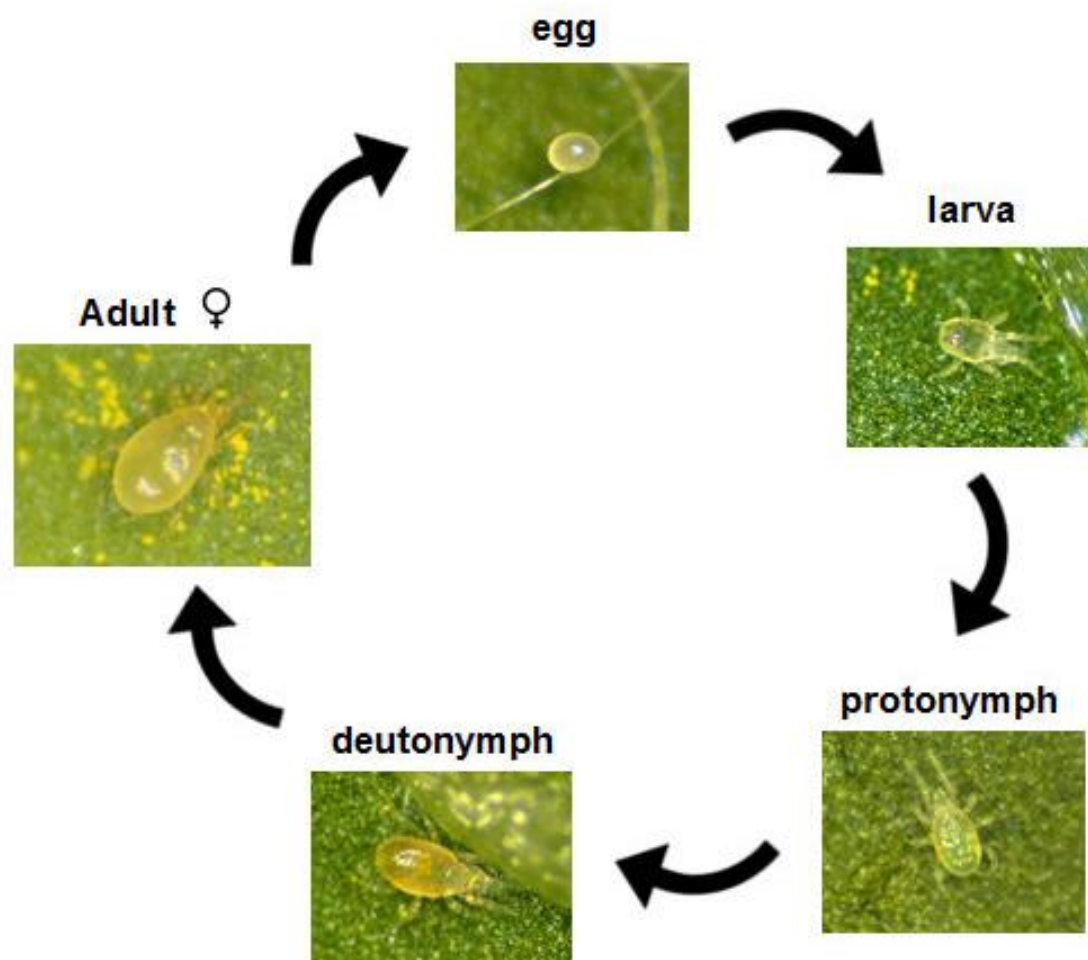
Figure 2: Life cycle of *Amblyseius swirskii* (© Alena Litin)

Figure 3: *Amblyseius swirskii* eggs (© Alena Litin)

Like most other Acari, *A. swirskii* has five life stages: egg, larva, protonymph, deutonymph, and adult (Figure 2). Development from egg to adult occurs in about a week in optimum temperature conditions of 25 to 28°C and 60-70% RH (Heyler et al., 2014). Eggs are deposited singly, typically on trichomes (leaf hairs) on the underside of leaves; they are translucent, oviform, and about 0.15 mm long (Figure 3). Six-legged whitish larvae hatch after 1 to 2 days in optimal conditions, depending on temperature. Larvae of this species are facultative feeders, i.e. they do not need to feed to molt to the protonymphal stage, but will if food is available (Zhang and Croft, 1994; Wimmer et al., 2008). The 8-legged protonymph stage is the first in which feeding becomes obligatory for continued development (Wimmer et al., 2008). The protonymph molts to the second of two nymphal stages, the deutonymph, growing in the process. Each developmental stage is preceded by molting, which leaves behind ghost-like exuviae in the form of the previous stage.

Adult females measure up to 0.5 mm in length and males, at 0.3 mm, are the smaller of the two sexes. Adults are characterized by their teardrop body shape, smooth and shiny dorsal shield, and relatively few setae (compared to non-phytoseiid mites). With the naked eye, and even under the stereomicroscope, *A.swirskii* is virtually indistinguishable from closely related species; proper species identification requires examination of the number and position of setae on the dorsal and ventral shields and the morphology of spermathecal structures and can therefore only be accomplished with mounted specimens using a transmitted light microscope. Body color is typically whitish, yellowish, or tan, depending on diet, but the body often

becomes darker after feeding on live prey. *Amblyseius swirskii* are obligatory sexually reproducing but parahaploid, i.e. both sexes develop from fertilized eggs but the paternal chromosome set is eliminated from males sometime during embryogenesis, resulting in haploid individuals (also dubbed pseudo-arrhenotoky; Sabelis and Nagelkerke, 1988). Males are attracted to premature females by a sex pheromone (Gerson et al., 2003) and will often display a behavior known as mate guarding, in which they mount the backs or undersides of deutonymph females in anticipation of sexual maturity. A female reared on pollen typically lives about 40 days and lays 25 to 40 eggs over a lifetime, but longevity and fecundity can vary greatly depending on temperature (Lee and Gillespie, 2011) and diet (Nguyen et al., 2013). Adult females produce up to 2 eggs per day in optimum conditions (Helyer et al., 2014), but egg production often declines in times of nutritional deficits. Like in other phytoseiid species, the sex-ratio is typically female biased, at about 2.5:1 (Hoy, 2011), but it may vary based on maternal environmental stress (Walzer and Schausberger, 2015).

In recent decades this polyphagous predator, along with other phytoseiid species, has attracted significant interest as a biological control agent of various herbivorous pests in greenhouses, due to its wide range of host species and its ability to survive on non-prey food sources. Increasing restrictions on pesticides and extensive pesticide resistance in some herbivores have made biological control a favorable alternative to chemical control in ornamental and vegetable crops (Gerson and Weintraub, 2007). Phytoseiid mites are the most well-known group of acarine biocontrol agents (ABAs) and are currently being sold by about 50 commercial biocontrol companies worldwide (Gerson et al., 2003). Most notable is the Dutch company Koppert, which has been selling *A. swirskii* since 2005 (see http://www.koppert.com/). With enough food present, a population of *A. swirskii* can grow quickly and is able to control multiple pests simultaneously due to its wide range of prey. The species is extremely beneficial for combat of thrips (Messelink et al., 2005; van Houten et al., 2005) and whiteflies (Bolckmans et al., 2005) on crops such as cucumber, sweet pepper, and eggplant. It can be used to control spider mites, albeit most effectively in combination with other predators due to *A. swirskii*'s inability to enter and move through dense spider mite webbing (van Houten et al., 2007).

With so many possible food sources, and with its ever-growing popularity as an ABA, it is no surprise that *A. swirskii* has been the subject of many laboratory studies analyzing life table parameters when reared on various food sources (Momen and El-Sawi, 1993; Park et al., 2001; El-Sawi and Momen, 2005; Xu and Enkegaard 2010; Onzo et al., 2012; Riahi et al., 2017). However, these data are often difficult to translate to biological control contexts, since results are highly dependent on laboratory conditions under which they were obtained (Hoy, 2011). Pollen supplementation is a widely used method in greenhouses, in order to limit cannibalism and to improve efficacy of predators (van Rijn et al., 2002), and comes with the additional advantages that predator populations can be established on plants before prey is present and in times of low prey densities (reviewed in Calvo et al., 2015). Due to its wide range of prey items and its importance as an ABA worldwide, *A. swirskii* is an ideal test subject for studies investigating predator performance based on differences in rearing (or in this case, maternal) diets.

2.1.2 Two-Spotted Spider Mite *Tetranychus urticae*

The two-spotted spider mite (TSSM) *Tetranychus urticae* Koch (Acari: Tetranychidae; Table 2) is a destructive pest of hundreds of host plant species and is one of the most abundant, widespread, and economically important herbivorous species worldwide (Hoy, 2011). TSSM are characterized by two internal dark spots on either side of the body (resulting in their common name) and, unlike phytoseiid mites, have

Table 2: Taxonomy of *Tetranychus urticae* (after http://www.uniprot.org/taxonomy/)

Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Chelicerata
Class	Arachnida
Subclass	Acari
Superorder	Acariformes
Order	Trombidiformes
Suborder	Prostigmata
Superfamily	Tetranychoidea
Family	Tetranychidae
Genus	Tetranychus
Species	*Tetranychus urticae* Koch, 1836

Figure 4: *Tetranychus urticae* adult female and egg (© Peter Schausberger)

eyes (Figure 4). Spider mites display the same sequence of life stages as described above for *A. swirskii,* with an inactive chrysalis stage preceding each molt. The life cycle is usually completed in one week at a favorable temperature of 30°C and 50% RH (Zhang, 2003). Members of this species produce dense silk webbing, which can enshroud large sections of plants. The webbing provides mites protection from some predators, abiotic hazards (e.g. desiccation or pesticides; Hoy, 2011) and is advantageous to dispersal and colony establishment (Gerson, 1979). Leaf damage is characterized by pale flecks caused by the penetration of leaf tissue and emptying of parenchyma cell contents (Figure 5). Adult *T. urticae* are equal in size to *A. swirskii* adults, making smaller juvenile spider mites easy target prey for the predators to attack and overpower. In this study, mixed life stages of *T. urticae* were used as maternal prey.

Figure 5: Bean leaves showing damage of spider mite infestation (© Alena Litin)

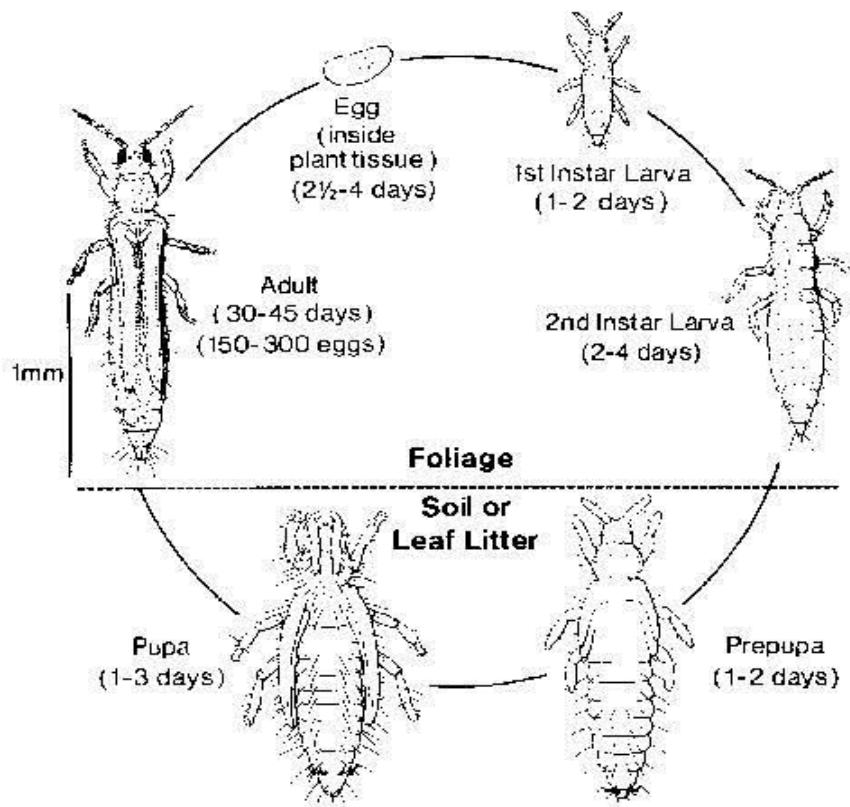## 2.1.3 Western Flower Thrips *Frankliniella occidentalis*

The western flower thrips (WFT) *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae; Table 3) is one of the most destructive pests of greenhouse crops worldwide, owing not only to its wide range of host plants but also its ability to vector destructive plant viruses (Reitz, 2009). Thrips are fringe-winged insects of the order Thysanoptera with unique asymmetrical mouthparts and a hemimetabolous life cycle (Figure 6). Thrips have four nymphal instars but only the first two larval stages actively feed, causing most of the physical damage done to plants (Cranshaw and Shetlar, 2018). The latter two instars are inactive non-feeding stages and are misleadingly known as prepupa and pupa, respectively, because of their analogy to the pupal stages of holometabolous insects. The optimum temperature range for WFT is 20 to 25°C (Zhang et al., 2012). Eggs are deposited into plant tissue and whitish to yellowish larvae hatch after 3 to 5 days (Figure 7a). Adulthood is reached in approximately 15 days at 25°C, at which point males are about 1 mm long and females about 1.5 mm long (Figure 7b).

This particular thrips species is native to the western United States but has spread throughout almost the entire world in recent decades due to the transport of infested horticultural material (Kirk and Terry, 2003). However, they cannot permanently establish outdoors in temperate regions with cold winters because of lacking diapause ability. The insects use their rasping-piercing mouthparts to make holes in superficial epidermal plant cells, from which they subsequently extract the liquid contents. The damaged and sucked-out cells fill with air causing characteristic silvery

**Table 3:** Taxonomy of *Frankliniella occidentalis* (after http://www.uniprot.org/taxonomy/)

Kingdom	Animalia
Phylum	Arthropoda
Superclass	Hexapoda
Class	Insecta
Subclass	Pterygota
(unranked)	Hemimetabola
Order	Thysanoptera
Superfamily	Thripoidea
Family	Thripidae
Genus	Frankliniella
Species	*Frankliniella occidentalis* Pergande, 1895

patches on leaves, interspersed with dark fecal droplets (Figure 8). Adults and larvae feed primarily on plant tissue, but can survive on pollen and are known to be opportunistically predaceous on the eggs of other herbivores and predatory mites (Trichilo and Leigh, 1986; Walzer and Schausberger, 2009) or be cannibalistic (A. Litin personal observation).

Figure 6: Life cycle of western flower thrips *Frankliniella occidentalis* (© John P. Sanderson; from <http://www.pestnet.org/fact_sheets/ western_flower_thrips_183.htm>)

Frankliniella occidentalis can grow to be up to four times larger than their phytoseiid predators, so predatory mites typically feed only on first instar (L1) larvae (0.5 to 0.7 mm in length; Figure 9) due to size constraints. Despite their early developmental stage, L1 thrips are not the easiest of prey to overpower. Thrips display defensive behaviors in response to mite attacks, such as jerking their abdomen or secreting allomones in the form of fecal droplets, which will often cause mites to break off attacks (Bakker & Sabelis, 1989). Thrips also attack and kill predatory mite eggs; not only has this behavior been shown against some of their phytoseiid predators (for *Iphiseius degenerans*: Faraji et al., 2001; Janssen et al., 2003; for *Neoseiulus*
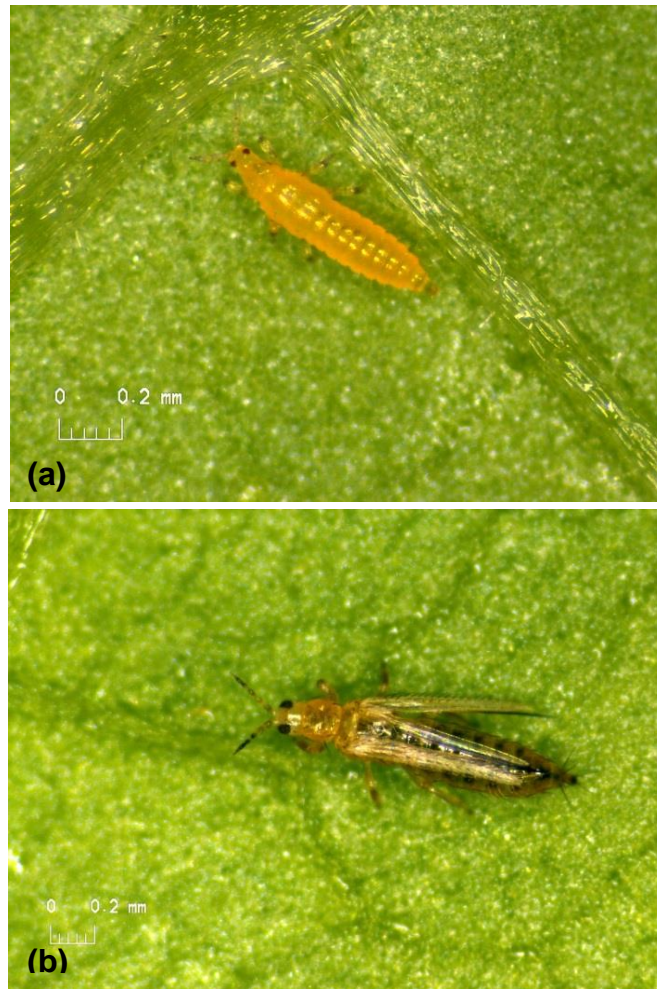
Figure 7: *Frankliniella occidentalis* **(a)** larva; and **(b)** adult female (© Alena Litin)

californicus: Walzer and Schausberger, 2009) including *A. swirskii* (I. Christiansen personal observation), but thrips have been shown to attack the eggs of organisms that pose them no apparent predation threat, such as *Phytoseiulus persimilis*, a specialist predator of spider mites (Janssen et al., 2003; Walzer and Schausberger, 2009). Because thrips are facultatively omnivorous, it is possible that they attack predator eggs for the sole purpose of food; nevertheless, this behavior has been shown to further benefit the insects by deterring adult predatory mites and reducing their risk of predation (Janssen et al., 2002; Walzer and Schausberger, 2009), showing multiple benefits of the behavior. Due to these defensive and anti-predator behaviors, thrips are considered difficult-to-grasp and high-risk prey for *A. swirskii* (Christiansen et al., 2016) and are therefore ideal for use in studies such as this thesis, where learning ability can improve foraging performance on this prey later in life. In this study, WFT were used as alternative prey to be learned by offspring.

Figure 8: Leaves showing damage by *Frankliniella occidentalis* (© Raymond A. Cloyd; from <https://gpnmag.com/article/developing-effective-western-flower-thrips-management-program/>)

2.2 Rearing

2.2.1 Predatory Mite Rearing

Two stock populations of *A. swirskii* were used in this study, from different origins. The first was obtained from Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands) in October, 2013. The second population was collected from citrus fruit trees in Ramat Yishay, Israel in 2013 and was used to found a laboratory population in December of that year. Both populations, upon arrival in the laboratory, were reared consistently on Nutrimite™ (Biobest N.V., Belgium), a diet consisting solely of cattail pollen *Typha angustifolia*, dusted onto the arena once per week (Figure 10a). Mothers used in this experiment were collected from both the Israel and Koppert pollen-reared stock populations.

Figure 9: *Amblyseius swirskii* adult females feeding on *Frankliniella occidentalis* larva (© Koppert Biological Systems)

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2.2.2 Spider Mite Rearing

The stock population of *T. urticae* was obtained from Biohelp (Vienna, Austria) >10 years ago. Spider mites were reared on whole bean plants *Phaseolus vulgaris* at room temperature (24 ± 4°C; Figure 10b).

2.2.3 Thrips Rearing

Prey to be learned by the predators in the experiment was WFT, *F. occidentalis*. The thrips stock population was founded by a mixture of individuals obtained from Dr. William Kirk (Keele University, United Kingdom) in March 2017, reared on chrysanthemums, and individuals collected from greenhouse-grown aubergine plants in Vienna, Austria. Upon receipt in the laboratory, thrips were reared on whole green bean pods within glass jars covered with fine netting at room temperature (23 ± 1°C; Figure 10c). Fresh pods were added to the jars, and dried-out pods removed from the jars, in 3 to 4 day intervals.

To obtain even-aged larvae, adult thrips were taken randomly from the stock population and transferred to oviposition arenas in Petri dishes (14.5 cm ø x 2 cm). Oviposition arenas consisted of detached leaves of *P. vulgaris* placed abaxial side up into 1.0-1.5% water agar solution (Sigma-Aldrich, Germany), with the stem detachment site immersed in agar to maintain leaf turgidity (Figure 11). Circular openings (2 cm ø) were cut into Petri dish lids and covered with fine netting to allow ventilation to the agar plates. To prevent adult thrips escaping, Parafilm M® was wrapped around the contact zone of the Petri dishes and their lids. After 24 hours, adult thrips were removed and Petri dishes were stored at room temperature until larvae began to emerge 3 to 4 days later. Once first instar larvae (L1) were observed on the leaves, agar plates were transferred to the refrigerator (8°C), where development was slowed until L1 thrips were needed either as dead (frozen) or live prey in the experiment. To ease feeding on thrips by early predator life stages in the experience phase, juvenile predators were offered dead thrips, killed by deep-freezing at -18°C for 75 to 90 minutes (Reichert et al., 2017).

Figure 10: Stock populations of **(a)** pollen-reared *Amblyseius swirskii*; **(b)** *Tetranychus urticae*, reared on whole bean plants; **(c)** *Frankliniella occidentalis,* reared on whole green bean pods (© Alena Litin)

2.3 Experimental Procedure

The experiment consisted of four chronological phases: maternal nutritional conditioning, prey experience by offspring, consolidation of prey experience, and a behavioral assay. Maternal nutritional conditioning and consolidation of prey experience by offspring took place on detached leaf arenas; prey experience and the behavioral assay took place in acrylic cages.

Figure 11: *Frankliniella occidentalis* oviposition arena. Arena consisted of detached bean leaf *P. vulgaris*, abaxial side up in solidified agar solution (© Alena Litin)

2.3.1 Leaf Arena Set-up

Mothers of all experimental subjects were reared on detached bean leaf arenas of *P. vulgaris*. Detached primary leaves were placed abaxial side up onto filter paper squares on top of water-saturated foam cubes. The foam cubes were set into water-filled plastic containers (10 cm x 10 cm x 6 cm) to ensure continued saturation of the foam and to isolate the arenas. Moistened tissue paper strips were placed over the leaf stem to maintain leaf turgidity and around the edges of the leaf as a source of free water and to prevent the mites from escaping (Schausberger, 1997). Before transferring predatory mites to the arenas, the tissue paper borders were checked under a stereo microscope and any open pockets were smoothed out to keep animals from seeking shelter or laying eggs in one of these cavities. Each arena contained small bunches of cotton strands underneath a microscope cover slip for shelter and oviposition sites for the predatory mite females, as a simulation of the domatia on the undersides of leaves on which they usually reside in natural settings. Acrylic hoods (28 cm x 22 cm x 16 cm), with circular openings (14 cm ø) covered with fine netting for ventilation, were placed over the arenas to avoid contamination. Arenas were kept in a climate chamber at 23 ± 1°C, 60 ± 5% RH, and 16:8 hour light:dark photoperiod.

2.3.2 Maternal Diets

Predatory mite mothers were reared on leaf arenas with one of three diets made available: pollen only (subsequently referred to as PO line), TSSM *T. urticae* (SM line), or pollen plus nutritional supplements (SUP line; Figure 12a-c). Spider mites of mixed life stages were brushed from infested leaves onto the SM arena at the time of arena setup. Spider mites were not replenished throughout the maternal oviposition phase due to the self-sustaining nature of the SM arena. Pollen was renewed on PO and SUP arenas every 2 to 5 days. Supplement mixture made available to the SUP line consisted of 300 mg L-tryptophan (Stadt-Apotheke Judenburg; Judenburg, Austria), 300 mg L-phenylalanine (Stadt-Apotheke Judenburg; Judenburg, Austria), 15 mg iron (Pure Encapsulations; Sudbury, MA, USA), and 500 µg B12 vitamin (Pure Encapsulations; Sudbury, MA, USA) dissolved in 500 mL of tap water. Dosages of all four supplements were determined based on recommended supplemental intake for adult humans; iron and B12 were pre-packaged in the form of digestible capsules (already containing the recommended dosage), which were broken open and capsule cases omitted from the water-supplement mixture. To confirm the uptake of the four water-soluble dietary supplements by the predatory mites, blue food coloring (Wilton Industries, Inc.; Woodridge, IL, USA) was added to the water-supplement mixture. The semi-translucent body of *A. swirskii* allowed for easy visibility of the blue color throughout the mites' digestive tracts, serving as a visual indicator of supplement ingestion (Figure 13). To avoid potential confounding factors, the water sources of the PO and SM lines were likewise colored blue with food coloring. Additionally,

(a) (b) (c)
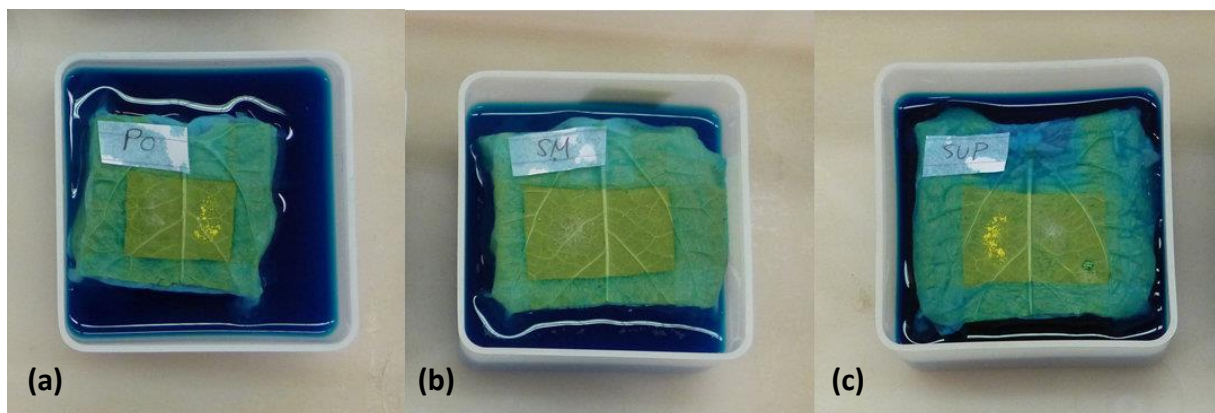
Figure 12: Maternal diet arenas. *Amblyseius swirskii* mothers were reared on one of three diets, **(a)** pollen only; **(b)** mixed life stages *Tetranychus urticae*; or **(c)** pollen plus nutritional supplements (© Alena Litin)
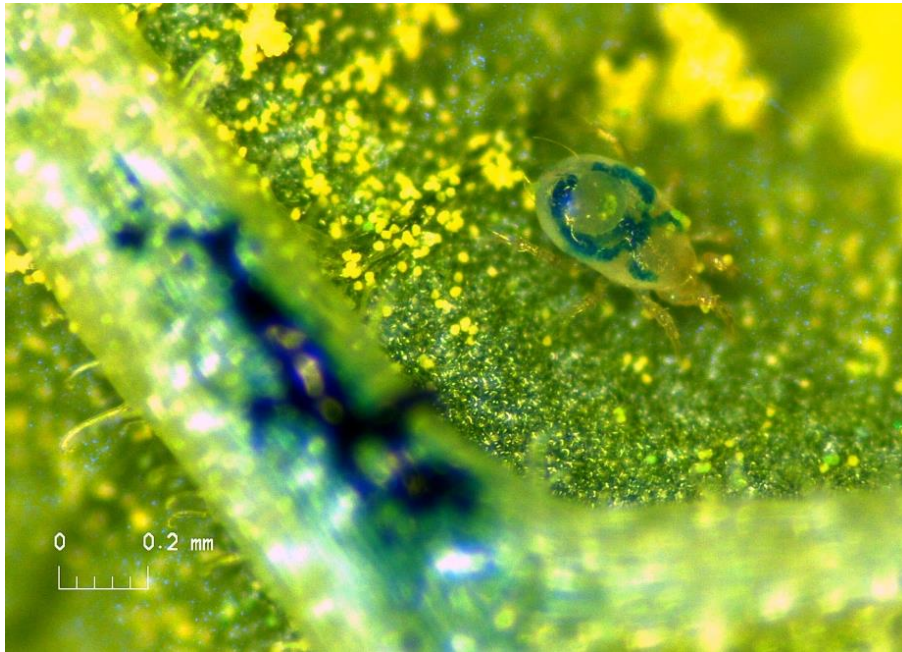
Figure 13: *Amblyseius swirskii* female with digestive tract dyed blue (© Alena Litin)

omega-3 fatty acid (Dr. Böhm, Apomedica; Graz, Austria) was made available on the SUP arenas as a droplet on a fragment of cover slip placed in a corner of the arena. Preliminary trials with food coloring mixed into droplets of omega-3 clearly showed that animals were ingesting the oil; however, due to the inability to emulsify the mixture, the blue color was subsequently omitted from the omega-3 droplets for practical reasons.

Five to seven gravid females were randomly taken from either the pollen-reared Koppert or Israel stock population and transferred to each of the three maternal arenas (PO, SM, and SUP) using a moistened fine-tipped brush. Maternal diet switches in *A. swirskii* mothers affect offspring learning ability within as little as 3 days on a new diet (Reichert et al., 2017); consequently, mothers were given 5 days on the maternal arenas for the diet switch to take effect in offspring production. Maternal arenas were checked in 24 h intervals for eggs laid by the predatory mites and eggs removed from the arena; eggs collected during the first 5 days after initial arena set-up were discarded. Starting on the sixth day, collected eggs (<24 h old) were transferred to acrylic cages and stored above water-saturated paper towels to warrant elevated humidity inside the cages. Cages consisted of cylindrical cavities (15 mm ø) laser-cut into 3 mm thick acrylic slides, covered with fine netting on the
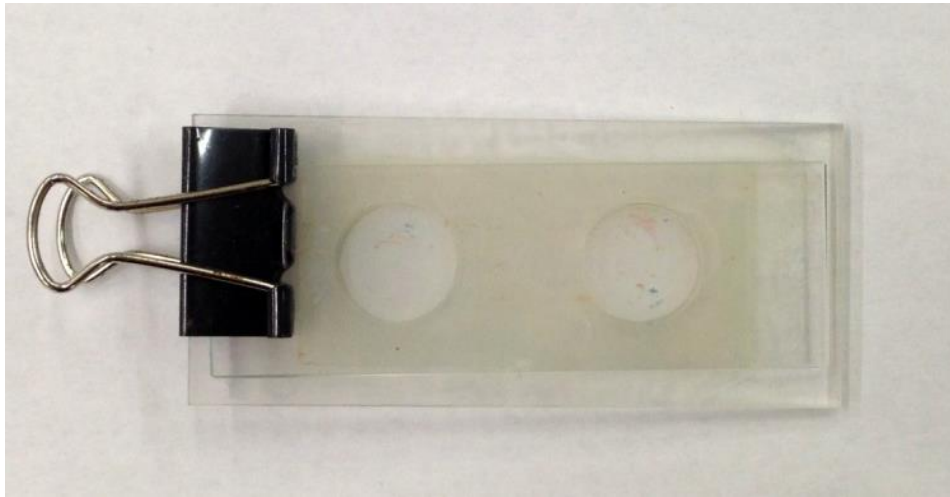
Figure 14: Acrylic cage set-up. Cages were used in the prey experience phase and behavioral assay and consisted of acrylic slides with laser-cut cavities, covered with fine netting and with a microscope slide on top, secured in place with a binder clip. (© Alena Litin)

bottom for ventilation and with a microscope slide on top, secured in place with a binder clip (Figure 14) (Schausberger, 1997). Eggs collected over multiple days, from day 6 onwards, were stored in the refrigerator (8°C) between collections to halt development. In experimental conditions, *A. swirskii* eggs take 1.5 to 2 days to hatch, therefore, 24 h prior to the start of the prey experience phase, cages containing predator eggs were removed from the refrigerator and kept in a walk-in climate chamber at 23 ± 1°C, 60 ± 5% RH, and 16:8 hour light:dark photoperiod.

Eggs were collected from maternal arenas for one to two experimental series, at which point fresh leaf arenas were set up with new mothers taken from the stock populations.

2.3.3 Prey Experience and Consolidation Phases

In the prey experience phase, previously collected eggs from each of the three maternal diet lines were randomly divided into prey-naïve (labeled "-") and prey-experienced (labeled "+") for a total of six treatments (Figure 15). Eggs were placed into six separate acrylic cages, which were subsequently loaded with either pollen, to generate thrips-naïve predators, or 5 first and second instar thrips (killed by deep-freezing), to generate thrips-experienced predators. Thrips experience occurred in the learning sensitive larval and early protonymphal stages of the predators in the

presence of conspecifics (Christiansen and Schausberger, 2017). Because *A.swirskii* larvae feed facultatively (Wimmer et al., 2008), mites were kept in the presence of thrips as prey until the appearance of the protonymphal stage, to ensure that thrips feeding had taken place (Seiter and Schausberger, 2016). Each day, freshly frozen thrips were renewed in the three prey-experienced (+) cages, to ensure a continued supply of turgid prey items on which newly hatched or molted predator individuals could feed. Cages were kept in a climate chamber over water-saturated paper towels to warrant elevated humidity inside cages, and were checked daily for the developmental progress of the predatory mites.
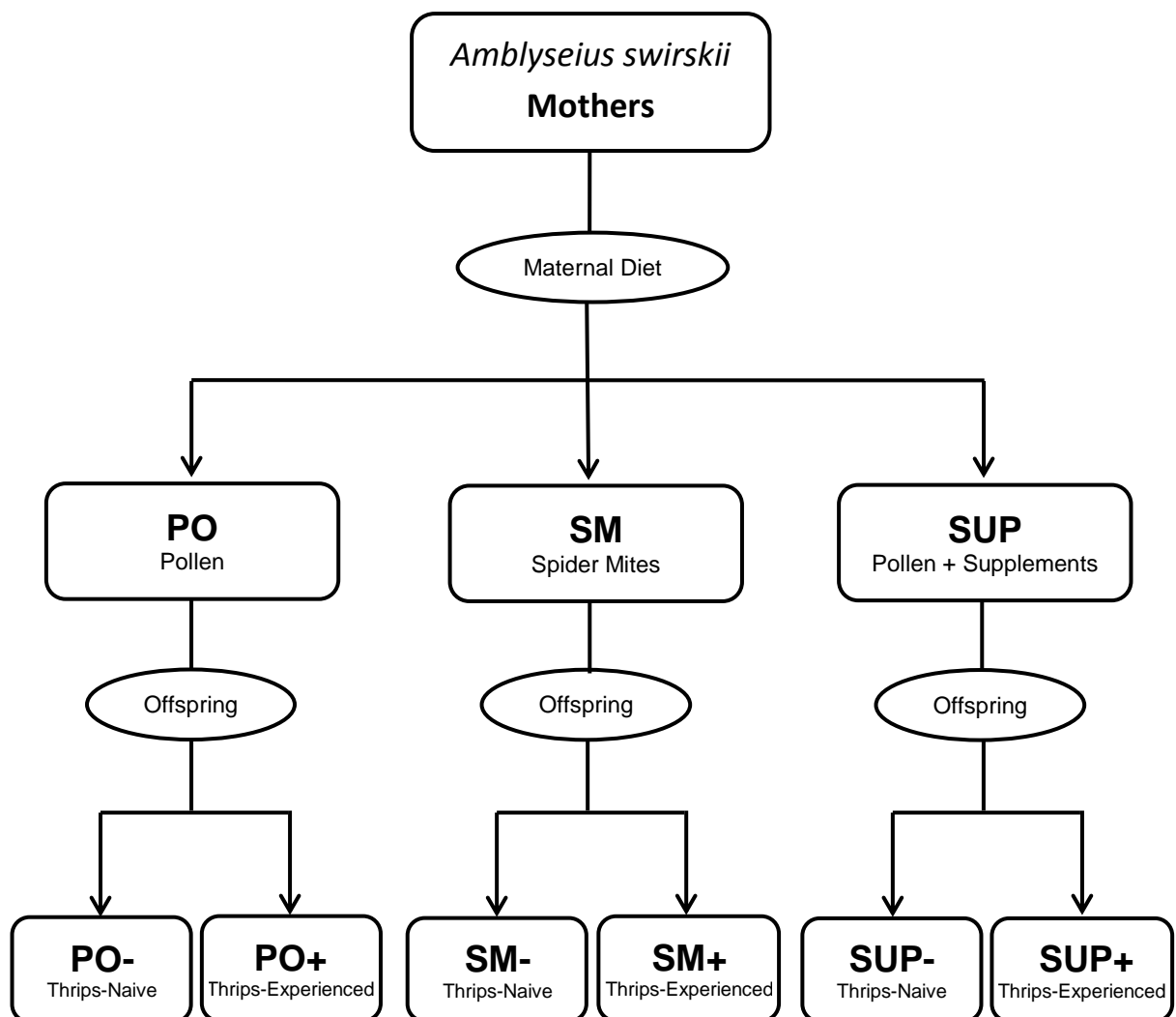
Figure 15: Overview of experimental treatments. Eggs were collected from each of three maternal diet lines (PO, SM, and SUP) and divided into thrips-experienced (+) and thrips-naïve (-) test subjects, for a total of six treatments.

Following the prey experience phase, predator protonymphs were transferred from their prey experience cages to six separate detached bean leaf arenas (one for each treatment) for the consolidation phase, in which they were reared on pollen only and in the presence of conspecifics, until reaching adulthood (Figure 16).

The developmental progress of the experimental animals on consolidation arenas was checked once per day until all experimental individuals had reached adulthood (lasting 4 to 6 days), at which point their sex could be determined. If at least one male was present within the treatments, these were allowed to fertilize the females on the respective arenas; if no male was present, one to two males were taken from the stock population for mating at least 24 h before the start of the behavioral assay.

Figure 16: Consolidation arenas. After prey experience phase, predator protonymphs were transferred to six separate detached bean leaf arenas, depending on treatment, and were reared on pollen only and in the presence of conspecifics until adulthood (© Alena Litin)

2.3.4 Behavioral Assay and Parameters

After fertilization (males present on consolidation arenas for at least 24 h), gravid adult females from each treatment were individually tested for learning (i.e. memory of prey experience obtained in early life) by measuring their attack latency on live L1 thrips larvae. Attack latency was chosen as a behavioral indicator of learning ability, since learning prey cues leads to reduced attack times on that prey later in life (e.g. Rahmani et al., 2009; Schausberger et al., 2010; Christiansen et al., 2016; Seiter and Schausberger, 2016; Reichert et al., 2017). Latency to attack was defined as the time elapsed until the first successful attack on thrips by *A. swirskii* females after offering thrips as prey. Test subjects were singly transferred to acrylic cages as described above, which had previously been loaded with 3 live first instar thrips. To determine attack latency of thrips-naïve (-) vs. thrips-experienced (+) predators, cages were inspected in 15-min intervals for 3 h, starting 15 min after the addition of the predator, again at 6 h, and finally at 24 h. At each observation point, mite activity (stationary or moving), number of killed thrips, and number of eggs laid was recorded. Moving was defined as locomotor activity, regardless of velocity, and stationary was considered a non-mobile and non-feeding state. Thrips were recorded killed either at the first instance of observed feeding by the predator or at the first discovery of thrips remains (i.e. feeding was not directly observed but attack must have occurred within the preceding 15-min interval).

During the initial 6 h observation period, cages were left at room temperature (23 ± 1°C) but were returned to the climate chamber (23 ± 1°C) between 6 h and 24 h observation points. Cages were kept above water-saturated paper towels and under acrylic hoods to warrant elevated humidity inside cages. Each of the six treatments was replicated between 10 and 18 times (SUP-, n=13; SUP+, n=12; SM-, n=12; SM+, n=10; PO-, n=18; PO+, n=16).

2.4 Statistical Analyses

Statistical analyses were carried out using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp.; Armonk, NY, USA). Based on the directional expectation that early thrips experience by the predators enhances foraging on thrips (Schausberger et al., 2010; Christiansen et al., 2016; Seiter and Schausberger, 2016; Christiansen and

Schausberger, 2017; Reichert et al., 2017), all tests were one-tailed. Generalized linear models (GLMs) were used to analyze the effects of early thrips experience on attack latency (normal distribution, identity link), attack likelihood (binomial distribution, logit link), total egg count (Poisson distribution, log link), and activity (binomial distribution, logit link) within each of the three maternal diet lines. For analysis of attack likelihood, a stopping time of 360 minutes was used (individuals that did not kill a single thrips within this time were omitted from the analysis). For analysis of activity, the fourteen observation points were aggregated into one value for each individual, i.e. activity was defined as the number of times a predator was observed moving out of 14 total observation points. Cumulative thrips kill over time (Poisson distribution, log link) as affected by early thrips experience was analyzed by generalized estimating equations (GEE; autocorrelation structure between observation points).

3. RESULTS

3.1 Attack Latency and Likelihood

Attack latency (time until first attack) of *Amblyseius swirskii* females on L1 thrips prey ranged between 284.06 ± 115.87 min and 881.25 ± 199.55 min across all experimental treatments (Figure 17). Within the maternally nutritionally supplemented line (SUP), thrips experience in larval and early protonymphal stages did not influence attack latency (GLM; Wald χ^2_2 = 0.282, p = 0.298) nor attack likelihood (Wald χ^2_2 = 0.008, p = 0.465) of predators on thrips prey (Figure 17a). Early thrips experience also had no significant effect on attack latency (Wald χ^2_2 = 1.142, p = 0.143) or likelihood (Wald χ^2_2 = 1.156, p = 0.141) within the pollen-fed (PO) line (Figure 17c). In contrast, thrips-experienced predators from the spider mite-fed (SM) line attacked thrips prey significantly faster (Wald χ^2_2 = 3.166, p = 0.038) and were more likely to attack thrips (Wald χ^2_2 = 3.066, p = 0.040) than thrips-naïve predators of the same line (Figure 17b). Mean attack latency of experienced SM line predators was about half that of naïve SM line predators. Experienced SM line predators were almost twice as likely to attack the first prey item within the first 360 min of the behavioral assay as their thrips-naïve counterparts.

3.2 Cumulative Thrips Killed

Between 0.58 and 1.50 thrips were killed and consumed by *A. swirskii* predators across all experimental lines within the first 360 min of the 24 h behavioral assay (Figure 18). In the SM line, experienced predators consumed an average of 1.2 thrips compared to only 0.58 thrips consumed by naïve predators during the first 360 min of the behavioral assay (GLM; Wald χ^2_2 = 2.824, p = 0.047; Figure 18b). In the SUP (Wald χ^2_2 = 0.956, p = 0.164) and PO (Wald χ^2_2 = 0.246, p = 0.310) lines, early thrips experience made no difference to cumulative thrips killed by predators throughout the behavioral assay (Figure 18a,c).
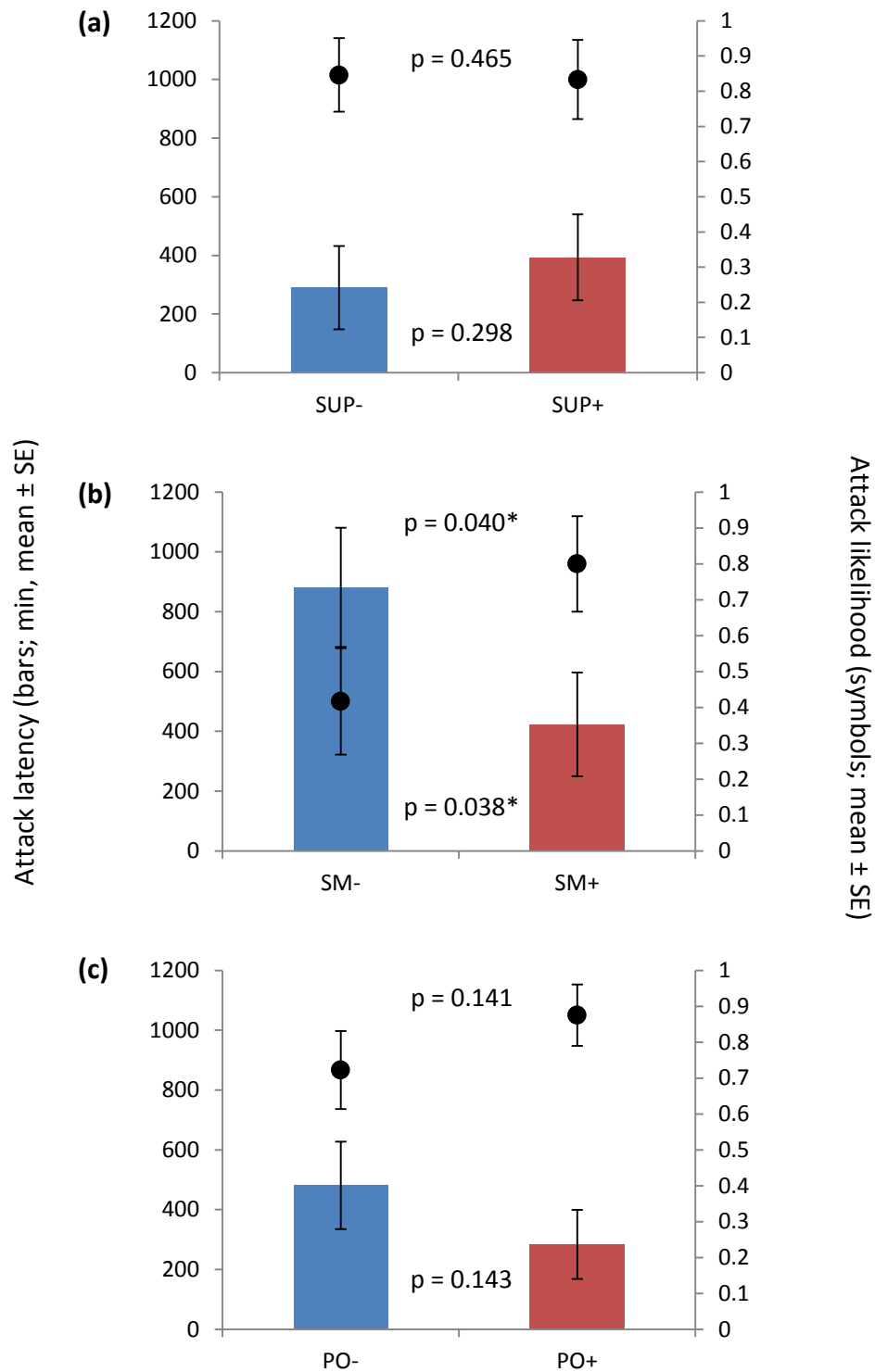
Figure 17: Attack latency and likelihood. Time elapsed until first attack (bars) and likelihood of attack within 360 min (symbols) of *Amblyseius swirskii* females offered first instar (L1) thrips *Frankliniella occidentalis* as prey during a 24 h behavioral assay. Experimental individuals were singly caged in an acrylic cage and offered 3 live L1 thrips. Individuals were offspring of mothers reared on one of three experimental diets: **(a)** pollen plus nutritional supplements (SUP), **(b)** spider mites (SM), or **(c)** pollen only (PO). Thrips-naïve (-) predators were offered only pollen throughout juvenile development, whereas thrips-experienced (+) predators were allowed to contact and feed on dead (killed by deep-freezing) L1 thrips in larval and early protonymphal stages. P values nearer to the x-axis correspond to attack latency (bars) and p values nearer to the top of each graph correspond to attack likelihood (symbols). * indicates statistically significant differences at the alpha=0.05 level (GLM).
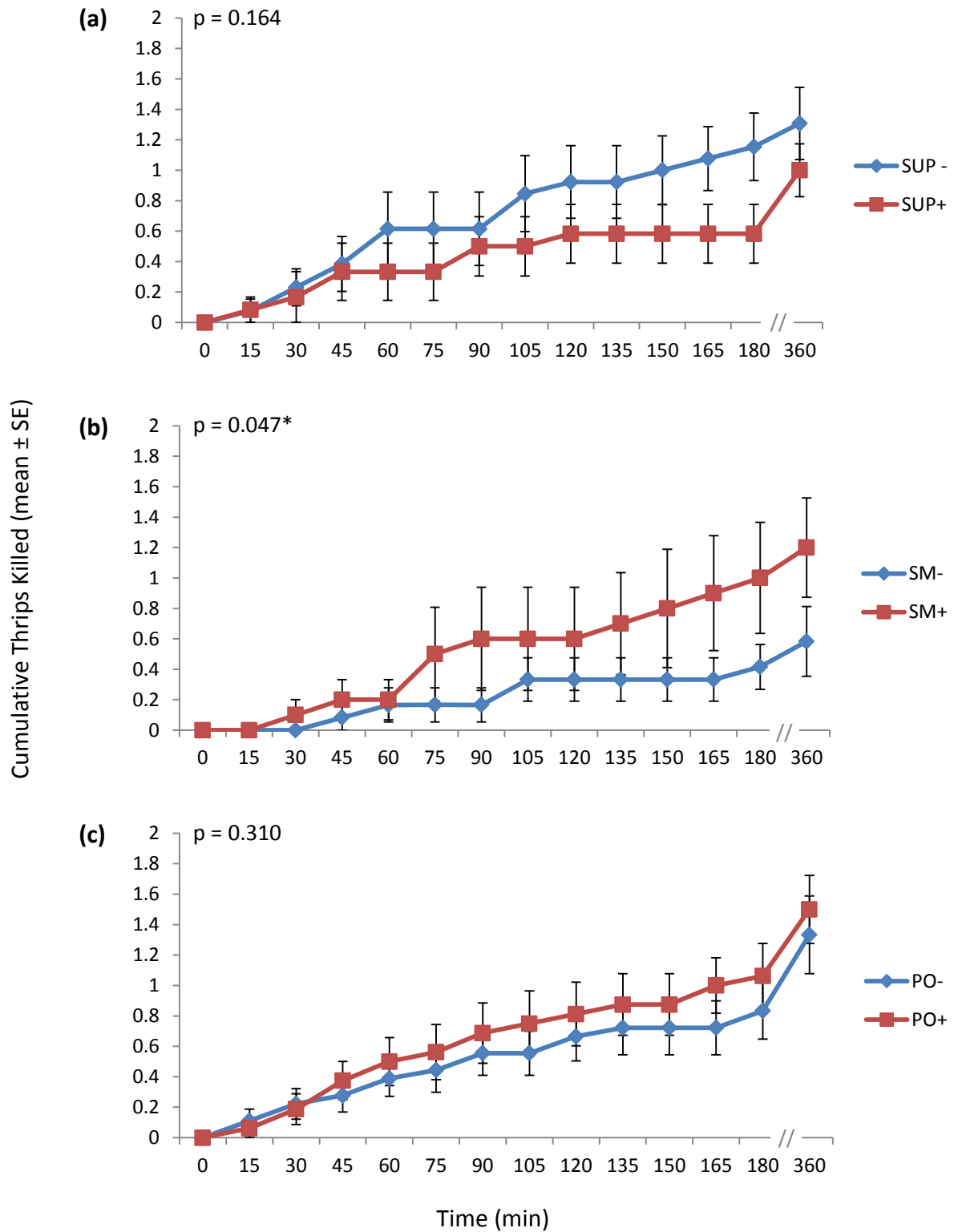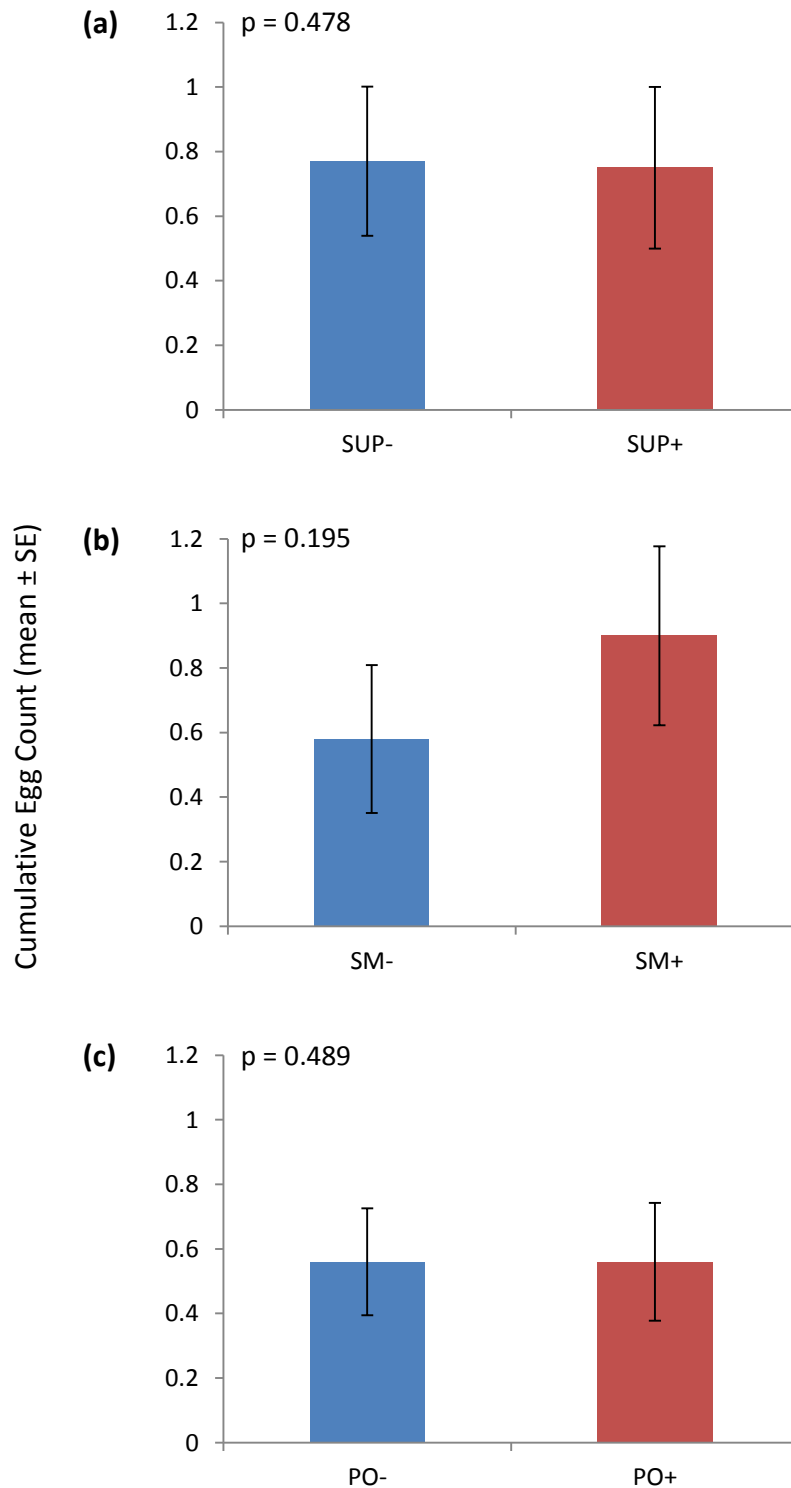
Figure 18: Cumulative thrips killed. Total number of first instar (L1) thrips *Frankliniella occidentalis* killed by *Amblyseius swirskii* females in the first 6 h of 24 h behavioral assay. Each experimental individual was singly caged in an acrylic cage and offered 3 live L1 thrips. Experimental individuals were offspring of mothers reared on one of three experimental diets: **(a)** pollen plus nutritional supplements (SUP), **(b)** spider mites (SM), or **(c)** pollen only (PO). Thrips-naïve (-) predators were offered only pollen throughout juvenile development, whereas thrips-experienced (+) predators were allowed to contact and feed on dead (killed by deep-freezing) L1 thrips in their larval and early protonymphal stage. * indicates statistically significant differences at the alpha=0.05 level (GEE).

3.3 Total Eggs Produced

Predators of all three maternal nutritional lines laid on average between 0.56 and 0.90 eggs during the 24 h behavioral assay (Figure 19). Thrips experience in early life did not affect cumulative egg count within any of the three lines. In the SUP (Wald $\chi^2_2 = 0.003$, p = 0.478) and PO (Wald $\chi^2_2 = 0.001$, p = 0.489) lines, there was almost no difference in the number of eggs laid between thrips-experienced and thrips-naïve animals (Figure 19a,c). Thrips-experienced predators of the SM line did lay more eggs than thrips-naïve predators (Wald $\chi^2_2 = 0.740$, p = 0.195) but the difference was not statistically significant (Figure 19b).

3.4 Activity

Within all three maternal nutritional lines, SUP (Wald $\chi^2_2 = 0.554$, p = 0.229), SM (Wald $\chi^2_2 = 1.023$, p = 0.156), and PO (Wald $\chi^2_2 = 0.238$, p = 0.313), early thrips experience had no effect on the overall activity of *A. swirskii* females during the behavioral assay (Figure 20a-c). Predators across all three maternal nutritional lines generally spent half their time active and half their time stationary; locomotor activity was observed at a minimum average of 6.19 and a maximum average of 8.50 observation points out of a total of 14 recorded observation points (Figure 20).

Figure 19: Total eggs produced. Number of eggs laid by *Amblyseius swirskii* females offered first instar (L1) thrips *Frankliniella occidentalis* during a 24 h behavioral assay. Each experimental individual was singly caged in an acrylic cage and offered 3 live L1 thrips. Experimental individuals were offspring of mothers reared on one of three experimental diets: **(a)** pollen plus nutritional supplements (SUP), **(b)** spider mites (SM), or **(c)** pollen only (PO). Thrips-naïve (-) predators were offered only pollen throughout juvenile development, whereas thrips-experienced (+) predators were allowed to contact and feed on dead (killed by deep-freezing) L1 thrips in their larval and early protonymphal stage. P levels refer to GLM within each diet line.
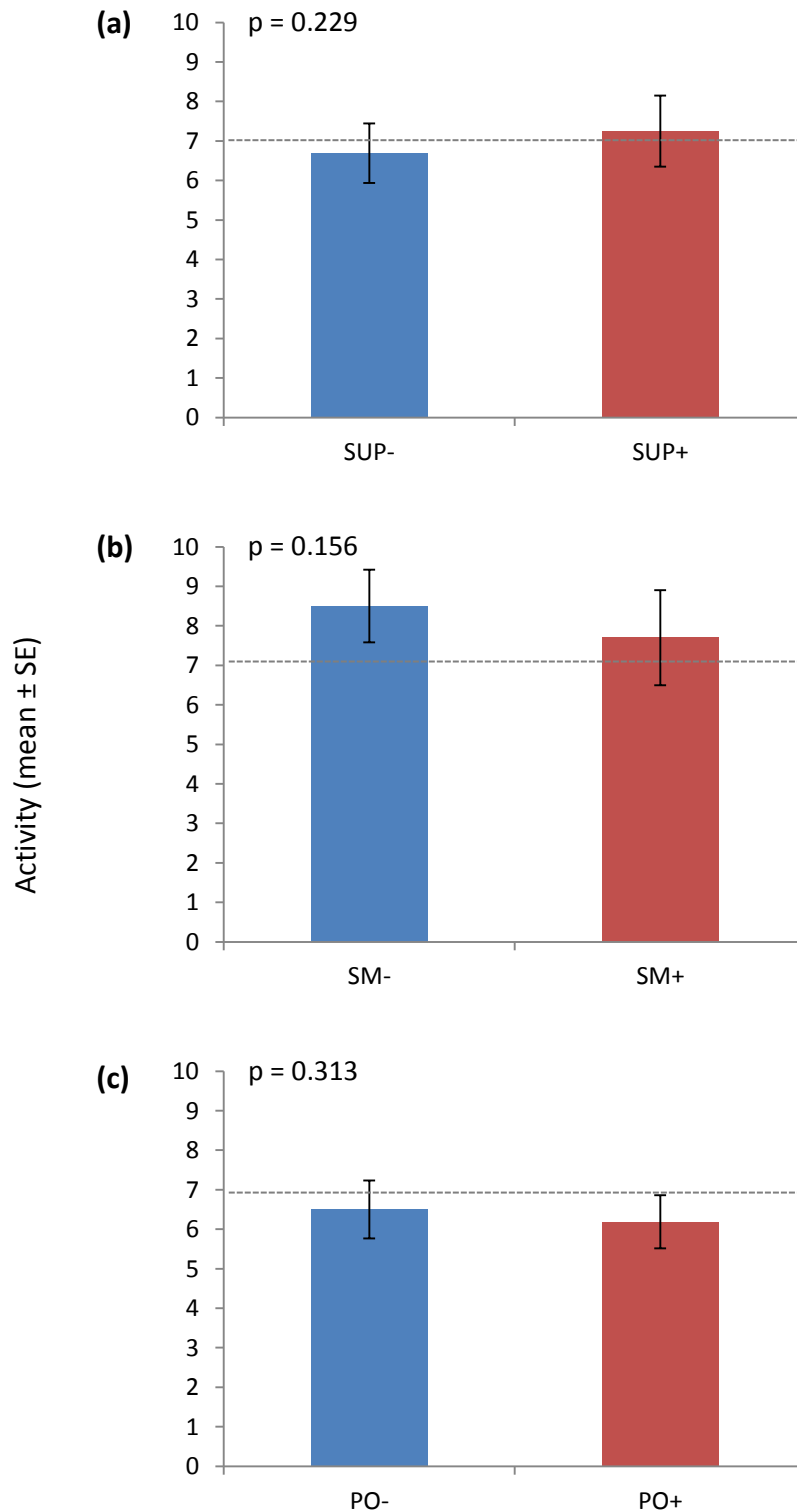
Figure 20: Locomotor activity. Number of observation points during a 24 h behavioral assay in which *Amblyseius swirskii* females, offered first instar (L1) thrips *Frankliniella occidentalis* as prey, showed locomotor activity. Each experimental individual was singly caged in an acrylic cage and offered 3 live L1 thrips. Experimental individuals were offspring of mothers reared on one of three experimental diets: **(a)** pollen plus nutritional supplements (SUP), **(b)** spider mites (SM), or **(c)** pollen only (PO). Thrips-naïve (-) predators were offered only pollen throughout juvenile development, whereas thrips-experienced (+) predators were allowed to contact and feed on dead (killed by deep-freezing) L1 thrips in their larval and early protonymphal stage. Dotted lines indicate the halfway mark out of the total number of observation points (n=14). P levels refer to GLM within each diet line.

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4. DISCUSSION

An animal's ability to use learned cues to recognize kin, find suitable food, court a mate, confront rivals, or avoid predators can positively affect evolutionary fitness in many ways (Papaj and Lewis, 1993; Dukas, 1998). Although learning ability has a genetic basis, its expression is state-dependent and often influenced by immediate environmental circumstances (Mery, 2013), including diet and nutritional intake (reviewed in Scrimshaw, 1998; Nyaradi et al., 2013; Prado and Dewey, 2014), and/or may be influenced by transgenerational environmental effects, such as maternal effects. Maternal effects can be potent forces shaping offspring phenotypes, based on maternal environmental experience (Rossiter, 1996; Mousseau and Fox, 1998b). In the predatory mite *Amblyseius swirskii*, the expression of learning ability is highly dependent upon maternal food type: mothers derived of populations reared on live spider mite prey for multiple generations produce offspring capable of learning prey cues early in life, whereas mothers derived of populations reared on only pollen for multiple generations produce non-learning offspring (Seiter and Schausberger, 2016; Reichert et al., 2017). Maternal diet switches to live prey of as little as 3 days is enough for a restoration of learning ability in offspring of the pollen-reared population (Reichert et al., 2017). This diet-based variance in the phenotypic expression of learning ability among offspring of the two maternal diet populations provides an excellent example of operational within-species variation of a cognitive trait and of the far-reaching influences of diet and nutrition across generations.

The aim of this thesis was to investigate whether maternal nutritional supplementation, when administered alongside a pollen diet, is able to restore offspring learning ability in *A. swirskii* and, if so, whether learning ability is comparable to that seen in offspring of spider mite-fed mothers. Individuals from three maternal diet lines – i.e. (i) coming from the pollen-reared population and exclusively fed on pollen (PO), (ii) coming from the pollen-reared population and fed on pollen plus nutritional supplements (SUP), and (iii) coming from the pollen-reared population and fed on spider mites (SM) – were either allowed to experience the difficult-to-grasp prey western flower thrips (WFT) *Frankliniella occidentalis* in early juvenile life or not, with the expectation that prey experience improves prey

recognition and reduces attack times on prey later in life within learning-capable individuals. The combination of maternal nutritional supplements tested (iron, B12 vitamin, L-phenylalanine, L-tryptophan, and omega-3 fatty acids) did not result in restoration of learning ability in offspring of pollen-fed *A. swirskii* lines (PO and SUP), as was reflected in similar attack latencies on thrips between thrips-naïve and -experienced treatments of the PO and SUP lines. In contrast, thrips experience shortened the attack latencies on thrips by offspring of the SM line, relative to thrips-naïve individuals. Altogether, my study corroborates previous findings that learning in predatory mites takes place in a sensitive phase early in life (Schausberger et al., 2010), and that offspring from pollen-fed mothers coming from a population reared on pollen for multiple generations are not capable of learning, whereas offspring of mothers reared on live prey are capable of learning in foraging contexts (Christiansen et al., 2016; Seiter and Schausberger, 2016; Reichert et al., 2017).

4.1 Learning and Behavioral Parameters

4.1.1 Early Sensitive Phases

For many animals, early learning phases are critical to establishing long-lasting neural connections, whose effects on behavior may be important for extended periods of time, in some cases throughout a lifetime (Knudsen, 2004). As eyeless predators, phytoseiid mites use primarily chemosensory signals to recognize conspecifics (Schausberger, 2007; Christiansen and Schausberger, 2017) or to localize prey (Sabelis and Dicke, 1985). Recognizing these cues can be innate and/or learned, with learning often occurring in early sensitive periods and used for recognition later in life (Schausberger, 2007; Schausberger et al., 2010; Seiter and Schausberger, 2016; Christiansen and Schausberger, 2017). Early learning with regard to foraging has been previously shown in phytoseiid predators including *Phytoseiulus persimilis* (Rahmani et al., 2009), *Neoseiulus californicus* (Schausberger et al., 2010; Christiansen and Schausberger, 2017; Schausberger and Peneder, 2017), and the focal animal of this thesis, *Amblyseius swirskii* (Christiansen et al., 2016; Seiter and Schausberger, 2016; Reichert et al., 2017). The current study further supports the existence of early learning sensitive phases in *A. swirskii*, since adult performance in learning-capable offspring of SM lines improved

with prey experience in juvenile life stages, with memory persisting through multiple molting events (Schausberger et al., 2010). In their study on *N. californicus*, Schausberger and Peneder (2017) showed that both associative learning (early thrips feeding) and non-associative learning (thrips cues without feeding) in juvenile life stages improve adult foraging on thrips, with non-associative learning producing weaker effects, but also fewer costs, than associative learning. In this study, *A. swirskii* larvae were allowed to feed on dead thrips prey in juvenile learning phases, giving the animals an association of chemosensory prey cues to feeding and satiation, which likely strengthened the effects of the learning process (Seiter and Schausberger, 2016; Schausberger and Peneder, 2017), but non-associative learning should not be ruled out.

4.1.2 Attack Latency and other Parameters

Since learning involves neurological processes, the only way to observe whether or not it has taken place is through its impact on behavior. Thus, learning ability is measured indirectly through the quantification of behavioral responses in tasks that are expected to be influenced by learning processes (Dukas, 2008; Mery, 2013). In studies examining the expression of learning in foraging carnivorous predators, attack latency is a powerful parameter used to quantify learning ability, because reduced prey handling times are assumed to benefit animals by increasing net energy gain, i.e. prey profitability (MacArthur and Pianka, 1966). Learning-capable individuals, who have experienced prey cues early in life and can recall these cues as adults, are expected to attack prey faster than naïve predators without any experience of prey cues. In non-learning individuals, on the other hand, no difference in attack latency between naïve and experienced individuals is expected and adult attack times remain unaffected by prey experience. This study's findings, that thrips-experienced offspring from mothers of the SM line attacked thrips twice as fast and twice as likely as thrips-naïve offspring from mothers of this line, supports previous findings that learning by offspring of prey-fed mothers has positive effects on prey recognition and acceptance times (Seiter and Schausberger, 2016; Reichert et al., 2017). The fact that offspring of the PO and SUP lines showed no difference in attack latency or likelihood between the thrips-naïve and -experienced treatments corroborates findings that maternal pollen diets are not conducive to offspring

learning (Reichert et al., 2017) and shows that the particular combination of supplements tested in my thesis is unable to restore learning ability in predatory mites.

Assuming learning is adaptive, the ability to learn should have positive effects on individual fitness, which can be measured experimentally through the quantification of fitness-relevant life history traits. Attack latencies, cumulative prey count, egg production, and predator activity were analyzed, in order to investigate how learning translates into overall performance and fitness of predators. In addition to the shorter attack latencies observed in experienced offspring of the SM line, individuals of this treatment also consumed over twice as many thrips in the first 360 minutes of the behavioral assay compared to naïve offspring of the SM line. In the other two maternal diet lines, PO and SUP, early thrips experience made no difference to the amount of thrips attacked by offspring of adult females, meaning that in non-learning offspring lines, both attack latency and overall predatory performance were unaffected by early thrips experience. These results show that learning-capable individuals are able to increase prey acquisition as a result of faster recognition, but that early prey experience within non-learning lines makes no difference with regard to shortened attack latency or amount of prey consumed. Shortened attack times (resulting in greater net energy gain per unit time) should also translate into higher egg production (Schoener, 1971), which is commonly used as one important indicator of evolutionary fitness. Shorter attack latencies are commonly correlated with higher egg counts in offspring of spider mite- but not pollen-reared phytoseiid mothers, indicating a positive effect on fecundity in learning-capable animals (Seiter and Schausberger, 2016; Christiansen and Schausberger, 2017). Though not statistically significant, the results of my thesis point in the same direction, namely slightly higher egg production in thrips-experienced versus -naïve offspring of the SM line, which would have possibly shown a stronger trend with a higher number of replicates. The final parameter tested, locomotor activity, also showed no significant variation among treatments. Although the data initially displayed a trend towards increased activity in individuals of the SUP line, this later leveled out, possibly due to confounding factors.

4.2 Possible Factors Influencing Learning Ability in *Amblyseius swirskii*

In recent decades, parental (i.e. maternal and/or paternal) effects have gained appreciation due to their far-reaching influences on numerous aspects of offspring phenotypes. The processes by which mothers induce these phenotypic changes are diverse, complex, and context-dependent (reviewed in Marshall and Uller, 2007), and the effects can persist through multiple generations, even in the absence of the original environmental triggers (reviewed in Harper, 2005). Recent work on maternal effects mechanisms has shown a variety of ways in which environmental information is passed from mothers to offspring, affecting progeny fitness (Mousseau and Dingle, 1991; Rossiter, 1996; Mousseau and Fox, 1998b; Marshall and Uller, 2007), and has shown how some mothers can "forewarn" offspring of coming conditions (Uller et al., 2013). Specifically, the phenomenon of diet-based maternal effects is found in a wide range of taxa and can lead to offspring phenotypic changes through a number of mechanisms (reviewed in Rossiter, 1996). Two mutually non-exclusive types of maternal effects that potentially cause the observed maternal diet-based variation in offspring learning ability in *A. swirskii*, nutrient deficiencies and/or epigenetic effects, are discussed below.

4.2.1 Nutrient Deficiencies

Mothers influence offspring development and phenotype through the process of egg provisioning, which determines the initial environment and resources provided to offspring. Maternal diet has been shown to influence egg composition through nutrient allocation (e.g. Rossiter, 1991; Newcombe et al., 2015) and is thus one source of dietary maternal effects. Low quality maternal diets sometimes negatively affect life history traits as a result of maternal provisioning. For example, maternal low quality diets reduce the likelihood and window of offspring dispersal in gypsy moths (Diss et al., 1996), and cause prolonged offspring development and smaller eggs in rove beetles (Kyneb and Toft, 2006). This thesis operated under the assumption that learning ability is reduced in *A. swirskii* as the result of nutritional deficiencies in maternal pollen-only diets, due to the fact that plant-based diets often vary in nutritional composition compared to prey. One explanation, therefore, is

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differential nutrient allocation to eggs, due to lower quality pollen diets, adversely affecting cognitive development and performance of offspring.

I tested the micronutrients iron, vitamin B12, omega-3 fatty acid, L-tryptophan, and L-phenylalanine, all of which are substances known to vary in amount and availability between animal- and plant-based diets (Johri and Vasil, 1961; Murphy and Allen, 2003), and which are known to be important for and positively affect cognition and neural function (Bourre, 2004). Although the majority of literature regarding the cognitive effects of diet involves mammalian models (for reviews: Nyaradi et al., 2013; Prado and Dewey, 2014), we can infer similar effects with regard to micronutrients in other animals, including *A. swirskii*, since the nutrients tested are implicated in cognitive function across animal taxa. The fruit fly *Drosophila melanogaster* is a widely used arthropod model system to gain understanding of neurological functioning across animals, including humans (Bellen et al., 2010). As previously mentioned, a pivotal study to deal with dietary influence on learning in an arthropod (Xia et al., 1997) showed that low protein/high carbohydrate diets reduce learning ability across generations of *Drosophila melanogaster*. In this case, however, Xia and colleagues (1997) were investigating macro- rather than micro-nutrients. There exist numerous studies on the function of micronutrients for cognitive performance of arthropods such as honey bees and *Drosophila* sp. (reviewed in Menzel and Benjamin, 2013). However, to the best of my knowledge, only one experimental study has been conducted on the effects of a micronutrient deficiency on cognitive function and brain physiology in an arthropod, in which omega-3 deficiency was shown to reduce learning ability and decrease hypopharyngeal gland size in honey bees (Arien et al., 2015).

My thesis provides no evidence that the tested combination of micronutrients restores learning ability or affects life history traits of offspring from pollen-reared *A. swirskii* mothers receiving nutritional supplementation. The five nutrient supplements tested in my thesis were chosen for multiple relevant reasons (as discussed above) but are nonetheless only five of many nutrients that may be involved in learning processes in predatory mites. The fact that this particular combination of diet supplements was not able to restore offspring learning ability in *A. swirskii* does not reject the possibility that other types or combinations of substances may not in fact do so. Nutrients such as zinc, vitamin A, choline, calcium, and iodine have been

studied extensively with regard to cognitive development and brain function (for reviews: Scrimshaw, 1998; Nyaradi et al., 2013; Prado and Dewey, 2014) and also tend to vary in availability between plant and animal diets (Murphy and Allen, 2003). Assuming that nutritional deficits of pollen are responsible for the loss of learning ability in predatory mites, it is possible that one or more of these micronutrients, whether in addition to or *in lieu* of the five nutrient supplements tested, may be involved in neural functioning in *A. swirskii.*

Pollen suitability varies for phytoseiid predators (Ragusa and Swirski, 1975; Goleva and Zebitz, 2013; Samaras et al., 2015), so one might claim that the alleged nutritional deficiencies following multi-generational rearing on pollen-only diets may have weakened predator vigor and negatively affected attack times in PO treatments. This was not the case, however, since offspring of pollen-reared lines attacked prey with overall similar attack latencies as offspring of spider mite-reared lines (Seiter and Schausberger, 2016; Reichert et al., 2017). If long-term pollen feeding resulted in decreased vigor, we would expect to see the first attacks of offspring of spider mite-reared lines to occur far sooner than those of offspring of pollen-reared lines. Instead, however, we find a normal distribution of prey attack times across treatments, regardless of prey experience or learning ability, including discrete instances of offspring of the pollen-reared lines (i.e. non-learning) attacking prey sooner than offspring of the spider-mite reared lines (i.e. experienced and learning-capable). This contradicts the suggestion that pollen rearing decreases predator vigor, since no physiological weaknesses can be observed based on overall predator attack latencies in this or previous studies.

Theoretically, the observed absence of learning restoration in offspring of SUP lines may have been due to unsuitable dosage of micronutrients used in the supplement mixture, but this explanation is unlikely, since three out of five nutrients were prepared and pre-encapsulated for human consumption and the other two nutrient dosages (L-tryptophan and L-phenylalanine) were calculated (rather generously) based on recommended human intake. It is, therefore, improbable that an underdosing occurred. We can also dismiss the possibility of laboratory selection as the cause for reduced learning ability in pollen-reared lines, since previous work (Reichert et al., 2017) shows a full restoration of learning ability after diet switches to live prey in predatory mites taken from multi-generational pollen-fed stock

populations. Thus, further investigation is required to determine whether other combination(s) and/or quantities of nutrients might be able to restore learning ability in offspring of pollen-fed *A. swirskii* lines, assuming that the loss of ability is caused by maternal nutritional deficiencies.

4.2.2 Epigenetic Effects?

An additional or alternative explanation for the observed differences in learning ability between offspring from pollen- vs. animal-fed mothers might be due to epigenetic factors. Epigenetics encompasses changes in gene expression that arise without changes to the primary DNA sequence and usually involves the addition or removal of molecules to or from the DNA strand to either boost or reduce gene expression (Harper, 2005). Epigenomic marks are commonly susceptible to dietary changes (McKay and Mathers, 2011). Certain nutrients (e.g. B12) are known to be tied to epigenetic processes, such as DNA methylation (Geraghty et al., 2015), in which the addition of a methyl group along a DNA strand represses gene expression, however other environmental factors may influence epigenetic processes as well. A classic case of diet-based modification of the epigenome comes from honey bee queens: individuals receiving special diets of royal jelly during development experience changes to the epigenome via DNA methylation, resulting in vastly different physiological, behavioral, and morphological characteristics between fertile queens and genetically identical sterile workers (Kucharski et al., 2008). This represents a noteworthy example of an environmentally, specifically diet, induced epigenetic switch, albeit these effects are displayed only within individuals rather than spanning generations.

Epigenetic effects may be translated across generations as well, via maternal (and/or paternal) environmental experiences (Harper, 2005). Stress, for example, is well-studied in both humans and non-human animals, and is one way in which a parental environment can bring about epigenetic modifications in offspring (e.g. neurodevelopmental changes, reviewed in Bale, 2014). Other transgenerational behavioral effects are also relatively well investigated in mammals (Maestripieri and Mateo, 2009), including the epigenetic transmission of maternal care behavior in humans, monkeys, rats, and other taxa (reviewed in Champagne, 2008). Research

regarding epigenetic involvement in maternal effects is less prevalent within arthropod taxa, however, examples of persistent environmentally-induced offspring phenotypic changes are abundant in the literature (Mousseau and Dingle, 1991; Rossiter, 1996), some of which may possibly be associated with epigenetic modifications but be not yet methodologically accessible. In many insects, for example, mothers use environmental cues to program the induction and duration of offspring diapause (Saunders, 1965; Huestis and Marshall, 2006). Maternal exposure to predators can improve offspring anti-predator behavior (Storm and Lima, 2010) or induce offspring phenotypic changes (Agrawal, 1999; Podjasek et al., 2005), and maternal experience of pathogens can enhance offspring pathogen immunity (Moret, 2006). Some parents are even able to provide defenses to their offspring by inducing chemical or morphological defenses in response to predator or herbivore attacks (Agrawal et al., 1999), or by sequestering plant-derived alkaloids (Dussourd et al., 1988) or toxins from prey (Kojima and Mori, 2014) into their eggs.

4.2.3 Synthesis of Maternal Diet-Related Mechanisms

Offspring phenotypes may also be triggered by specific diets or foraging conditions experienced by mothers, and a number of studies have focused on the correlation between maternal diet quality and/or quantity and offspring performance. Most often, high-quality and/or quantity maternal diets are associated with positive impacts on offspring performance and vice versa. In black widow spiders, for example, offspring of mothers experiencing a high-quantity diet display faster developmental times as opposed to those from mothers with a low-quantity diet (Johnson et al., 2014). In grasshoppers, high-quality maternal diets have positive effects on development, body mass, and song amplitude of offspring over and above the direct effects of the same diets on these traits, when only experienced by offspring themselves (Franzke and Reinhold, 2013), showing an example of how maternal diets can be even more influential to offspring development than the diet they themselves experience. Interestingly, nutritional effects on offspring performance can vary dramatically, depending which parent experiences the diet; for example, maternal protein diets in a neriid fly enhance larval hatching success while paternal protein diets reduce offspring success for the same parameter (Bonduriansky et al., 2016).

In some cases, maternal programming through diet experience can benefit offspring experiencing the same environmental conditions as their parent(s), referred to as "anticipatory maternal effects" (reviewed in Marshall and Uller, 2007; Uller et al., 2013). For example, cabbage white butterfly mothers reared on extremely protein-rich or -poor diets produce offspring with faster development times under the corresponding diet conditions experienced by their mothers (Rotem et al., 2003). European earwigs experiencing high- or low-quality diets show increased survival to adulthood when diets match those of their mothers (Raveh et al., 2016). Even plants can display adaptive advantages under similar environmental conditions as their mothers, in the form of higher germination and survival rates (Galloway and Etterson, 2007), showing that matching environments across generations can have adaptive advantages to offspring in an extremely wide range of taxa.

Based on these examples, it is possible that *A. swirskii* mothers may program their offspring (possibly via epigenetic mechanisms) based on their own dietary experiences during egg formation, in order to improve the foraging ability of their offspring in environments that are likely to match their own. In their investigation into the effects of maternal diet switches in *A. swirskii*, Reichert et al. (2017) found that mothers switched from pollen to live prey for as little as 3 days produced offspring capable of learning, but that predator vigor was further enhanced with time elapsed since maternal diet switches, as was evident in shorter attack latencies and higher prey consumption rates in offspring of mothers experiencing 10-day diet switches. The authors argue that these findings may indicate a maternal ability to program offspring based on diet experience prior to or during egg production. If this were the case, mothers experiencing briefer (i.e. 3-day) diet switches to live prey could be expected to program their offspring less severely compared to those experiencing longer (i.e. 10-day) diet switches, since maternal programming for matching environments comes at the risk of reducing offspring performance in non-matching environments (Uller et al., 2013).

One aspect of maternal diet experience, aside from diet quality and amount, which may be relevant to the current study, is maternal foraging activity. Mothers feeding on live and actively moving spider mites must seek out and attack moving targets, whereas pollen-feeders do not have the task of tracking and overpowering a live food source; this difference in experience may be enough to flip some epigenetic switch

controlling learning ability within the following generation. To my knowledge, no previous studies have investigated the effects of maternal experience of moving prey on offspring learning ability, however, maternal olfactory experiences have been found to epigenetically impact offspring behavior (including learning), and may therefore also be connected to the experience of prey in this scenario. Mice conditioned to associate a specific odor with fear produce offspring with increased behavioral sensitivity when exposed to the same odor (Dias and Ressler, 2014) and many mammals display increased postnatal preferences for flavors they have experienced in utero (Galef, 2009). This so-called prenatal learning is also present in the predatory mite *N. californicus*, in which maternal olfactory and gustatory experience of either neutral or flavor-enriched prey produces offspring that prefer flavored diets matching those of their mothers (Peralta Quesada and Schausberger, 2012). Therefore, maternal olfactory/gustatory experience of prey or experience of moving prey, both of which are lacking in pollen-fed mothers, may additionally be involved in maternal effects affecting learning ability in foraging contexts within *A. swirskii* offspring. The question then arises, whether maternal experience of spider mites versus thrips might produce differences in offspring learning ability when exposed to one or the other prey type as adults, which provides a possibility for future work on this subject.

If there really were some epigenetic switch involved in the expression of learning ability, then why should it only be flipped in certain situations? The benefits gained by learning can differ across environments (Dunlap and Stephens, 2009), so a particular learned ability that is just right in some settings might be either insufficient in more complex or a waste of energy in simpler environments. Learning clearly has adaptive advantages in *A. swirskii* for handling difficult prey such as thrips, but, due to its inherent costs (Christiansen et al., 2016) it might be more advantageous for mothers to forgo the costs of learning in their offspring within relatively stable, food-rich, or low-risk environments. Pollen as a food source is not hunted, cannot escape, nor display defensive behaviors, and may therefore be an easier food source for predators to acquire without the help of learning. Thus, it might benefit mothers experiencing lifelong pollen-only diets to pre-program their offspring for pollen environments, leading to an alternative explanation for the lack of learning ability in offspring of mothers who have only experienced pollen as a food source for their entire lives.

Changes to the expression of learning ability as a result of maternal experience have previously been shown in a number of species. Maternal stress exposure in rats and mice tends to produce learning and memory deficits in offspring (reviewed in Weinstock, 2008) and in three-spined stickleback fish, maternal predator exposure decreases offspring anti-predator behavior, survival (McGhee et al., 2012), and performance in a discrimination task (Roche et al., 2012). On the other hand, *P. persimilis* females experiencing intraguild predation (IGP) risk during egg formation may produce offspring with enhanced learning abilities and anti-predator behavior in IGP environments (Seiter and Schausberger, 2015), showing that maternal effects on learning ability can be either non-adaptive or adaptive, depending on species and context. Overall, it seems plausible that an epigenetic mechanism of maternal programming may be at work within *A. swirskii*, such that mothers who do not experience moving prey are able to switch off learning ability in offspring, in order to avoid the associated fitness costs and, conversely, that mothers who experience diet switches from pollen to live prey are able to switch on learning ability. This provides either an alternative mechanism for the observed loss of learning ability, or one that goes hand in hand with alleged nutritional deficiencies. Further investigation into the effects of maternal diet and epigenetic processes in predatory mites is needed, but this provides a wonderful opportunity for future work, e.g. the comparison of offspring learning ability from mothers consuming live and moving as opposed to recently killed prey (i.e. non-moving but prey olfactory and gustatory cues present).

4.3 Conclusion

In their study on the transgenerational effects of diet on learning ability in predatory mites, Reichert et al. (2017) speculated nutritional deficiency as one of the possible reasons behind the operational loss of learning ability in pollen-fed *A. swirskii* populations. In the current study, I investigated the effects of five micronutrients, administered alongside a maternal pollen diet, on learning ability of offspring. No evidence was found that this particular combination of nutritional supplements is able to restore learning ability in offspring of pollen-fed *A. swirskii* mothers, but this should not dismiss the possibility of learning restoration in pollen-fed mites with the help of nutritional supplementation, since many other relevant nutrient combinations could

still be tested. It is also possible that learning ability in predatory mites arises through epigenetic mechanisms brought about by maternal dietary factors, such as the maternal experience of moving prey or prey olfactory and/or gustatory cues, which would allow mothers to prime offspring for the environments they are likely to encounter in order to reduce some of the costs associated with learning, but such determination is outside the realm of this study. Regardless of the mechanism(s) involved, it is impossible to deny the extensive transgenerational influences of diet and nutrition on learning ability within this species.

The topic of learning is highly relevant regarding the use of natural enemies in biological control of herbivorous pests (Prokopy and Lewis, 1993) and, over the years, many phytoseiid species have become important biological control agents against herbivorous mites and insects (McMurtry and Croft, 1997). As previously mentioned, learning can increase the speed with which phytoseiid enemies attack their prey (e.g. Seiter and Schausberger, 2016; Reichert et al., 2017; Schausberger and Peneder, 2017) and is largely prey-specific (Schausberger et al., 2010; Christiansen et al., 2016). Thus, learning may be effectively used to prime biological control agents on their targets, through rearing methods that are conducive to learning a specific prey or host (Hare et al., 1997). Hare and Morgan (1997) investigated a priming technique that effectively increased host selection of a parasitoid wasp after exposure to a synthetic kairomone that mediates host selection, which could be used as an inexpensive way to mass-prime parasitoids for improved performance in biological control. Perhaps similar techniques may be useful in priming omnivorous predators like *A. swirskii*, for example, rearing mites on factitious prey with simultaneous exposure to target prey olfactory cues, in order to promote associative learning within mass-reared predator lines (Christiansen et al., 2016; Seiter and Schausberger, 2016; Schausberger and Peneder, 2017). It is my hope that this thesis contributes to the knowledge base for future investigations into the mechanisms at work behind (and the relationships among) nutrition, learning ability, and maternal effects in this important acarine predator.

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LIST OF FIGURES

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ABSTRACT

Learning is found in a wide range of animal taxa and commonly allows animals to improve their performance in any number of tasks, including foraging. The expression of learning can vary among individuals within species and populations, and may be influenced by a number of environmental factors, including diet, which may have consequences on phenotypic expression of learning both within individuals and between generations. Non-genetic transgenerational phenotypic effects include maternal effects, and have recently been found to operate within the plant-inhabiting predatory mite *Amblyseius swirskii*, in which maternal diet influences offspring learning ability. *Amblyseius swirskii* can survive and reproduce on both pollen and animal prey and, because plant- and animal-based foods vary in nutritional content and composition, this thesis hypothesized that nutritional deficits in pollen-reared mites are responsible for the previously reported loss of learning ability in offspring of pollen-fed mothers. This thesis investigated whether nutritional supplementation of pollen-reared mother mites can restore learning ability to offspring, the same way that diet switches from pollen to live prey are able to, which has been shown previously. A combination of five nutrient components were tested (iron, vitamin B12, L-phenylalanine, L-tryptophan, and omega-3 fatty acids), all known for their involvement with cognitive ability across animal taxa. Individuals from three maternal diet lines – i.e. (i) exclusively fed on pollen (PO), (ii) fed on pollen plus nutritional supplements (SUP), and (iii) fed on spider mites (SM) – were either allowed to experience the difficult-to-grasp prey western flower thrips *Frankliniella occidentalis* in early life or not. Learning in predatory mites has been shown to occur in an early sensitive phase and individuals exposed to prey in the juvenile life stage show improved foraging ability on that prey as adults. Learning and other behavioral and life history changes were quantified using four parameters, i.e. attack latency, cumulative predation, oviposition, and locomotor activity. The results show that this combination of nutrient supplements does not restore learning ability to offspring of pollen-reared mothers, as was evident from similar attack latencies by both thrips-experienced and -naïve predators of both the SUP and PO lines. In contrast, offspring of mothers whose diet was switched from pollen to spider mites for five days before offspring production (SM line) were well able to learn thrips early in life,

resulting in shorter attack latencies on thrips by experienced adult predator females. Nevertheless, this thesis corroborates previous findings that spider mite-fed *A. swirskii* mothers produce learning-capable offspring and that learning in these mites occurs in juvenile sensitive phases. Future studies should investigate the possibility of epigenetic maternal effects mechanisms, e.g. maternal exposure to prey olfactory/gustatory cues or maternal experience of moving vs. non-moving prey, and if/how these mechanisms are tied to maternal nutritional effects on learning ability in *A. swirskii.*

ZUSAMMENFASSUNG

Lernen wurde als Phänomen schon in vielen Taxa nachgewiesen und ermöglicht Tieren, ihre Leistungsfähigkeit in den verschiedensten Situationen und Aktivitäten, unter anderem bei der Nahrungssuche, zu verbessern. Die Expression der Lernfähigkeit variiert gemeinhin zwischen den Individuen innerhalb einer Art oder einer Population und kann stark von Umweltfaktoren beeinflusst werden. Zum Beispiel kann die Nahrung gravierende Auswirkungen auf den Phänotypus innerhalb eines Individuums oder zwischen den Generationen haben. Nicht-genetische generationsübergreifende phänotypische Effekte, u.a. mütterliche Effekte, wurden vor kurzem bei der auf Pflanzen lebenden Raubmilbe *Amblyseius swirskii* festgestellt, bei welcher die mütterliche Nahrung die Lernfähigkeiten der Nachkommen beeinflusst. *Amblyseius swirskii* kann sich sowohl von Pollen als auch tierischer Nahrung ernähren und fortpflanzen. In Verhaltensexperimenten wurde beobachtet, dass Nachkommen pollengefütterter Linien keine Lernfähigkeit zeigen, während die Nachkommen spinnmilbengefütterter Linien gut lernfähig sind. Da sich pflanzliche Nahrung in Nährstoffgehalt und -zusammensetzung von tierischer Beute unterscheidet, wurde vermutet, dass die Lernfähigkeit der Nachkommen der mit Pollen gefütterten Mütter aufgrund eines Nährstoffmangels verloren gegangen ist. Die vorliegende Studie untersuchte, ob eine Nahrungsergänzung bei mit Pollen gefütterten Tieren die Lernfähigkeit der Nachkommen in gleicher Weise wiederherstellen kann, wie die Umstellung von Pollen auf lebende Beutetiere, wie in einer früheren Studie bereits gezeigt wurde. Es wurden fünf Nahrungsstoffe untersucht (Eisen, Vitamin B12, Omega-3 Fettsäuren, L-Tryptophan und L-Phenylalanin), die für ihre Taxon-übergreifende Bedeutung für das Aufrechterhalten kognitiver Fähigkeiten bekannt sind. Nachkommen von drei mütterlichen Nahrungs-Linien – (i) ausschließlich von Pollen ernährt (PO), (ii) von Pollen und Nahrungsergänzungsmitteln ernährt (SUP) oder (iii) von Spinnmilben ernährt – durften in der frühen Jugendphase Erfahrung mit der schwer-zu-greifenden Beute Kalifornischer Blütenthrips *Frankliniella occidentalis* machen oder nicht. Wie bereits früher gezeigt, findet das Lernen nahrungssuchender Raubmilben in einer lernsensitiven Phase früh im Leben statt. Individuen, die früh im Leben Beuteerfahrung machen, sind schneller beim Attackieren dieser Beute im

erwachsenen Lebensstadium. Lernen und andere Verhaltens- und Lebenszyklus-änderungen wurden mittels vier Parameter quantifiziert: Angriffszeit, kumulativer Beuteerwerb, Eiablage und generelle Aktivität. Die Ergebnisse zeigen, dass die getestete Kombination von Nährstoffen die Lernfähigkeit der Nachkommen der mit Pollen gefütterten Mütter nicht wiederherstellen kann. Thrips-erfahrene und -naive Prädatoren der SUP und PO Linien zeigten ähnliche Angriffszeiten auf *F. occidentalis.* Im Gegensatz dazu konnten Thrips-erfahrene Nachkommen, deren Mütter fünf Tage vor der Eiablage eine Nahrungsumstellung von Pollen auf lebende Beutetiere erlebten (SM-Linie), als Erwachsene die Thripse schneller attackieren als Thrips-naive Nachkommen derselben Linie. Somit bestätigt diese Studie frühere Ergebnisse, die zeigten, dass *A. swirskii* Mütter, die mit tierischer Beute ernährt wurden, lernfähige Nachkommen erzeugen und dass diese Milben lernsensitive Phasen im frühen Leben besitzen. Zukünftige Studien sollten die Möglichkeit epigenetischer Mechanismen untersuchen, die eventuell mit der mütterlichen Erfahrung lebender Beute, oder mit oder anstatt der vermuteten mütterlichen Nährstoffmängel, das Lernen der Nachkommen beeinflussen können.