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„Diet-dependent egg retention by the plant-inhabiting predatory mite *Amblyseius swirskii*"

verfasst von / submitted by

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1. Introduction

1.1 Reproductive modes

Across animals there are different kinds of reproductive modes, with the major types being **ovipary**, **ovovipary** and **vivipary.** Among the different kinds of reproductive modes, **ovipary** (egg laying) is the most fundamental, widespread, and already documented by fossils (Ji et al., 2004; Jackson and Varricchio, 2016; Legendre et al., 2020). After fertilization, which may take place inside or outside the body, the embryonic development happens inside completed eggs. All the nutrients, which are necessary for embryonic development, are already in the yolk sac (lecitotroph) and self-sufficient, not requiring any further nutrient provisioning by the mother (Dettner and Peters, 2003). In most insects, the offspring need no extended parental care after oviposition. After hatching they are independent. The benefit in this case is that the mother can lay more eggs and she can use her entire energy for egg production (Kappeler, 2020). If extended parental care is necessary after egg laying or giving birth, additional energy is needed. The focal animal of my study, the plant-inhabiting predatory mite *Amblyseius swirskii,* is an oviparous mite and shares ovipary with most other arthropods (Chew and Robbins, 1984; Ampleford and Davey, 1989; Hilker, 1994).

Ovovivipary is an intermediate form of reproductive mode, between ovipary and vivipary. After fertilization, the embryos develop while the egg is still inside the mother's body and the hatching process happens inside the body or shortly after oviposition. Some spiders and insects (van Barren et al., 2003; Moreno et al., 2008; Cook and Dadour, 2011), most cartilaginous fishes (Kousteni and Megalofonou, 2016), some echinoderms (Byrne, 1989), and some reptiles (Bennis et al., 2006) and teleosts (Zhang et al., 2018) are ovovivipar (Kappeler, 2020). The benefit in this case is that the offspring stay longer inside the mother's body, which is a safe protected environment for the offspring. The time spent by offspring in the sensitive egg stage outside the maternal body is shorter relative to ovipary. Ovovivipary is often

characterized by higher maternal investment than ovipary. The costs in this case are that the mother invests more energy until laying the egg and she can only simultaneously harbor a given amount of eggs due to the limited space inside her body.

There are some other subtypes in the context of ovovivipary, such as larvipary and pupipary. For example, the fire salamander (*Salamandra salamandra*) retains the eggs inside the body until the aquatic larvae are born, which constitutes larvipary (Beukema et al., 2016). When the larvae pupate directly after birth, for example in the well-studied tsetse fly (Mellanby, 1937; Matetovici and Van Den Abbeele, 2018;) or in species of the family Hippoboscidae (Rahola et al., 2011), it is called pupipary (Storch and Welsch, 2012).

The third major kind of reproductive mode is **vivipary**, where the offspring are live-born after internal fertilization and embryonic development inside the mother's body. Vivipary is documented in numerous animals, for example in squamate reptiles (Gruber et al., 2018), mammals (Hayssen and Orr, 2017), fishes (Garcia and Valero, 2010), and some insects and arachnids (Shaffer et al., 1996; Jennings et al., 2019). Embryos are nutritionally provisioned during development by the mother. There are different mechanisms how this can happen. By a placenta in uterus of the therians or placenta-like organs as observed in some sharks (Kappeler, 2020). It is called pseudo-placental when the larva is nourished through glandular secretions of the mother (Meier et al., 1999). Another mechanism is oophagy. In this case, there are many eggs inside the maternal body but just some of them complete development, whereas the others are degenerating to a nutritional mush, which is fed upon by the other individuals (Storch and Welsch, 2012). Sometimes, the embryos cannibalize each other until only one is left, as observed in some sharks (Chapman et al., 2013). In some fishes, the mother individual produces a nourish fluid, which is exploited by the embryos, like in some Poeciliidae. Complex structures are sometimes built from the mother individual, or the embryo, for an optimal nutrient and oxygen assimilation (Storch and Welsch, 2012; Tomita et al., 2017).

In vivipary, costs on the side of the mother are more difficult movement and energy expenditure when having offspring inside the body. The individuals are more conspicuous for predators, and they can produce fewer offspring, because their mating opportunities and in consequence also their fertility is limited. Benefits are enhanced survival chances of offspring, because the embryos stay longer in a protected place (the mother's body), and when they are born, their development is more advanced and they can move more quickly (Kappeler, 2020).

In general, there is always a trade-off between quality and quantity of offspring produced. Are the surviving possibilities greater with a higher number of offspring and lower investment or is it a better strategy to produce fewer offspring and invest energy in parental care, to optimize the survival of the young? In the latter case, individuals usually have a longer life span than individuals who produce many offspring with low parental investment. In a competitive environment, for example, in colonizing new habitats or under unstable environmental conditions, it is a huge benefit to reproduce fast and have many offspring.

1.2 Aims of the study

In my thesis, I investigated the proximate causes of diet-related differences in egg hatching times in the plant-inhabiting predatory mite *Amblyseius swirskii.* These mites are basically oviparous but repeated observations point at occasional ovovipary, i.e. eggs that hatch extremely early after oviposition.

Several students in the laboratory of my supervisor Peter Schausberger noticed independently during their experiments with the predatory mite *Amblyseius swirskii* that eggs from pollen-fed females hatched in some cases much faster than eggs from females fed with live prey such as *T. urticae*. We discussed three possible explanations for this phenomenon:

The first one was that pollen nutrition accelerates embryonic development. Second, it could be possible that the mites respond to unfavorable environmental conditions like a dry substrate or low humidity and therefore longer retain the eggs inside their bodies. The third possible explanation put forward was that the eggs of pollen-fed individuals are smaller in size and for this reason embryonic development in the eggs happens faster.

Based on these observations and discussion of possible explanations I formulated the following research questions.

Research Question RQ1: “Are there differences in hatching time in *Amblyseius swirskii* based on maternal nutrition?”

Research Question RQ2: “Does total egg developmental time, consisting of development time in- and outside the maternal body, vary with maternal nutrition?”

Research Question RQ3: “Does maternal nutrition influence the egg size?”

To address these research questions, I conducted three experiments.

To investigate RQ1, in the first experiment I examined the hatching time of eggs produced by *Amblyseius swirskii* mothers fed on spider mites and pollen. I hypothesized that individuals fed with pollen produce eggs that have a shorter hatching time after oviposition than individuals fed with spider mites.

To study proximate aspects of varying hatching times (RQ2), in the second experiment, I compared the time of egg development in- and outside the body of *Amblyseius swirskii* with spider mite and pollen nutrition. I hypothesized that *Amblyseius swirskii* females with pollen nutrition produce eggs with a proportionally longer development time inside the body and a proportionally shorter development time outside the body compared to females with spider mite nutrition.

To exclude egg size as a possible explanation for hatching time differences, I compared the volume of eggs produced by pollen- and spider mite-fed mothers in the third experiment. My hypothesis in the third experiment was that there is no difference in the volume of eggs produced by *Amblyseius swirskii* females fed with spider mites or pollen.

1.3. Biology and ecology of the study animal *Amblyseius swirskii*

Many herbivorous insects and mites are important pests of crops and constitute a major problem in agriculture all over the world. Plant-inhabiting phytoseiid mites like *Amblyseius swirskii* (Athias-Henriot) are important natural enemies and thus potential biological control agents against herbivorous pest organisms. In some publications *Typhlodromips swirskii* is used as a synonym for *Amblyseius swirskii* (Table 1). Many phytoseiid mite species are also studied for their role as biological alternative to chemical pesticides to control plant pests (Nomikou et al., 2001, 2002; Messelink et al., 2005, 2008; Arthurs et al., 2009; Park et al., 2010; van Maanen et al., 2010; Calvo et al., 2015). Their broad prey spectrum comprises spider mites, whiteflies, and

thrips (Calvo et al., 2015) and their ability to live on alternative plant-derived food sources like pollen (Park et al., 2011), or insect eggs (Delisle, 2015), allows to release these organisms on plants already before they are infested by pests, which is a huge advantage over specialized predators.

Taxonomy of *Amblyseius swirskii* (Athias-Henriot 1962)	
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)

Table 1: Taxonomy of *Amblyseius swirskii* (Athias-Henriot 1962). Based on https://www.uniprot.org/taxonomy/ 4.12.2020.

Amblyseius swirskii has been classified as a type III generalist predator (Mc Murtry and Croft, 1997; Mc Murtry et al., 2013) and originates from sub-tropical regions (Messelink et al., 2006). The first documented observation was in Israel (Athias-Henriot, 1962). Occurrence of *Amblyseius swirskii* is reported from North, Central and South America, Southern Europe, Africa, and Asia (Demite et al., 2014). This species is found on crops like citrus, cotton, grapes, vegetables, and other fruit trees (Swirski and Amitai, 1997). Nowadays, *Amblyseius swirskii* is used as biological control agent in more than 50 countries (Calvo et al., 2015).

The five developmental stages in the life cycle of *Amblyseius swirskii* are: (i) egg, (ii) larva, (iii) protonymph, (iv) deutonymph, and (v) adult individual (Figure 1). The individuals are eyeless, but they are light sensitive (Gerson, 2003).

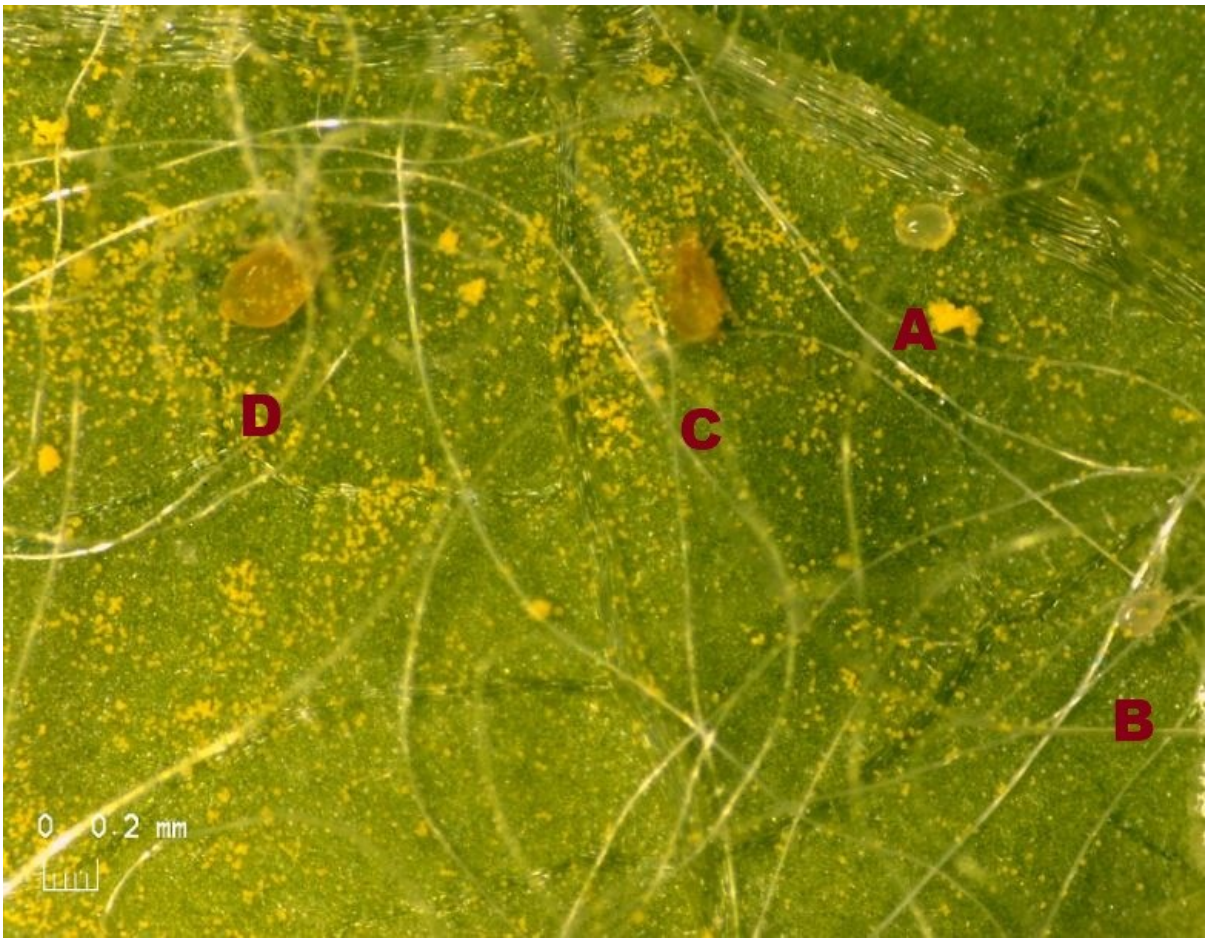

Figure 1: Different developmental stages of *Amblyseius swirskii*. (A) egg of *Amblyseius swirskii*, (B) larva stage, (C) 2nd nymphal stage and (D) adult individual of *Amblyseius swirskii*. The yellow dots on the leaf are the pollen grains used as food source for the predators. The white fibres are cotton wool threads (© Kerstin Filzmoser).

Gravid females place single oviform eggs, one by one, each with around 0.15 mm length (Doğramaci et al., 2013) on the underside of leaves on trichomes (leaf hairs). It was observed that the mother immediately starts to touch the egg with the first pair of her legs. The reason for this behaviour is unknown, a possible explanation is that she learns the smell of her egg and/or she marks the egg with scents to recognize it later again. The whole life cycle from egg to adult is finished, dependent on the

temperature (~20 to 25°C), within seven to ten days. One to three days after egg laying the six-legged whitish larva hatches. The larvae are facultative feeders, i.e. they can feed when food is available, but they also reach the next developmental stage, the protonymph, without feeding (Wimmer et al., 2008; Schausberger and Croft, 1999). All other developmental stages are eight-legged, and must feed to proceed with development (Wimmer et al., 2008). Each developmental stage is finished by molting and a diaphanous exuviae of the previous stage is left on the substrate (Figure 2).

Figure 2: Adult *Amblyseius swirskii* female immediately after the deutonymphal molting. After that molting, males were placed next to the females and they started immediately to copulate with each other. The transparent exuviae provides proof of molting and marks each developmental stage transition from larva to protonymph to deutonymph to adult (© Kerstin Filzmoser).

Adult individuals are characterized by their drop-shaped body and their partially translucent, smooth and shiny dorsal shield. Their body colour depends on their diet and ranges from whitish to yellowish and tan. After feeding on spider mites *Tetranychus urticae* their body colour darkens (Figure 3). Adult males are the smaller of the two sexes and around 0.3 mm long. Females reach a body length of around 0.5 mm. Male individuals track fertilizable females through a sex pheromone, which is already released by late female deutonymphs. Males often guard the premature females (they climb on their back or follow them) until they are mature and copulation can take place (Figure 4). An adult female deposits between 30 and 40 eggs during her lifetime (Gerson et al., 2003).

Figure 3: Adult *Amblyseius swirskii* female feeding on a spider mite. The body colour turns darker after feeding (© Kerstin Filzmoser).

Figure 4: Male guarding behaviour. Male individuals climb on the dorsal side of the female or follow her until she reaches maturity to monopolize the first mating (© Kerstin Filzmoser).

Due to its relevance in biological control, there are many studies about commercial mass-rearing of *Amblyseius swirskii* and about the best alternative food sources for *Amblyseius swirskii* and other phytoseiid mites (van Rijn and Tanigoshi, 1999; Xia et al., 2012; Midthassel et al., 2013). To rear *Amblyseius swirskii* in the laboratory, the temperature should be between 20 and 32 °C, higher fecundity usually occurs between 28 and 32 °C. Under 20 °C, the populations grow very slowly and under 13 °C development is halted (Lee and Gillespie, 2011). *Amblyseius swirskii* does not survive temperatures below freezing levels (Allen, 2009) and is thus also allowed to be released in central Europe in the open field. *Amblyseius swirskii* is also able to learn in the context of foraging. Adult individuals that experienced a given prey early in life could attack it faster than individuals with no such prey experience (Christiansen et al., 2016). In the context of commercial use as a biocontrol agent this might be a great benefit, as it may enhance pest suppression. Apart from the type of food, the oviposition substrate and the ambient relative humidity are also major

factors affecting oviposition. The egg stage is the developmental stage that is most vulnerable to low humidity in phytoseiid mites. A dry substrate could be a major problem. Drying-out is a danger to the egg, which is immobile and cannot change to more favorable locations. Some species are more drought sensitive than others, *Amblyseius swirskii* is considered drought-sensitive relative to other phytoseiid mite species (Ferrero et al., 2010).

2. Materials and Methods

2.1 Origin and rearing of experimental animals

I used four lines of two different populations of *Amblyseius swirskii* in the experiments. The field-collected Israel population (collected on citrus trees) and the mass-reared Koppert population. Upon receipt, each population had been split into two lines, which were separately maintained on acrylic tile arenas.

Each acrylic tile arena consisted of a 20 x 20 x 0.3 cm acrylic plate, which was placed on a cube of a water-soaked foam with the same size. A piece of moist filter paper was placed between the foam and the acrylic plate as a barrier for the mites. The arenas were housed in the middle of a tray that was half-filled with water. The edges of the plate were covered with a thin layer of tissue paper, which was hanging in the water to ensure constant water supply, and to prevent the mites from escaping. One line was fed with two-spotted spider mites (*Tetranychus urticae*) and the other line was fed with cattail pollen (*Typha latifolia*: Nutrimite®, Biobest, Westerloo, Belgium). The rearing arenas were stored in a walk-in climate chamber at a temperature of 23 ± 1 °C, a relative humidity of 40 ± 5 % and 16:8 h light:dark. The prey spider mites *Tetranychus urticae* were reared on whole common bean plants (*Phaseolus vulgaris* L.) in a greenhouse (Figure 5).

Figure 5: Common bean plants *Phaseolus vulgaris* L. They were grown in the greenhouse at a temperature of 24 ± 4 °C. Detached leaves of the plants were used to construct the leaf arenas used in experiments. The food source *Tetranychus urticae* was also reared on whole bean plants.

2.2 Rearing arenas

In both experiments 1 and 2, I used detached leaf arenas for rearing the predatory mites. Each leaf arena consisted of a water-soaked foam cube (15 x 15 x 4 cm) placed inside a plastic box (20 x 20 x 6 cm), which was half-filled with water. The foam cube was covered with a moist filter paper followed by a detached bean leaf placed abaxial side up on the filter paper. The accessible area on the leaf was delimited by moistened strips of tissue paper, to prevent escaping of the mites. Cotton wool fibres under a cover slip on the leaf surface served as shelter and oviposition sites. Predatory mites like this microhabitat and often stay on the fibres or with their dorsal side down on the underside of the cover slip (like on the abaxial leaf surface in their natural habitats).

Figure 6: Detached leaf arenas used in the experiments. On the left one the food source is pollen. On the right one the food source are spider mites. Arenas on foam cubes were placed inside a 20 x 20 x 6 cm plastic box which was half-filled with water.

2.3 Protocol of the first experiment

This first experiment aimed at investigating if the food used for rearing the two *A. swirskii* lines (subsequently dubbed rearing diet: pollen or spider mites) and/or the food received by the mothers (subsequently dubbed maternal diet: pollen or spider mites) affect the relative time of embryonic development outside the maternal body, i.e. from egg laying until hatching of larvae. To obtain eggs for experiments and estimate their hatching times I used the following protocol.

The experiment was started with the preparation of oviposition arenas (day 0). Each oviposition arena consisted of a detached bean leaf placed abaxial side up on moist filter paper resting on a water-soaked foam cube. The foam cube (5 x 5 x 5 cm) was placed inside a plastic box (10 x 10 x 6 cm), which was half-filled with water. The edges of the leaf and the rest of the filter paper were covered with a thin layer of tissue paper, which was also in contact with the water to ensure constant water supply (Figure 6). From the two spider mite lines (which were fed with either pollen (P) or spider mites (S)) of one of the two populations of *Amblyseius swirskii* (either from the field-collected – Israel – or the mass-reared – Koppert - population) similar numbers of fertilized females (approximately 5 -to 10 females based on availability), identifiable by their extended body, were randomly collected from the rearing units and placed on each of the four leaf arenas. Two of these leaf arenas featured individuals of *A. swirskii* coming from the rearing with pollen-based nutrition (P) and two arenas featured individuals coming from the rearing with spider mite-based (S) nutrition. For each of these two groups, P and S, the individuals on one leaf arena were provided with pollen and the individuals of the other leaf arena were provided with spider mites. This procedure resulted in four treatments: PP and PS – females withdrawn from the pollen rearing fed with either pollen (PP) or spider-mites (PS) on the leaf arena - as well as SP and SS – females withdrawn from the spider mite-based rearing fed with either pollen (SP) or spider mites (SS) on the leaf arenas (Figure 7). Females of all four subgroups were well provided with surplus food to exclude any inadvertent effects of food stress.

Figure 7: Illustration of the four experimental treatments of the first experiment: P for pollen, S for spider mites; first letter refers to the rearing diet, second letter refers to the maternal diet.

All eggs laid before day 4 were removed from the leaf arenas, to warrant standardized conditions for all individuals and that all individuals use the food source provided on the arenas during internal egg formation. I assumed that after about 3 days ingesting a given type of food, eggs laid from day 4 onwards were produced from those nutrients.

From day 5 to day 8, all laid eggs of each arena were collected and placed inside acrylic cages (Schausberger, 1997). Each cage consisted of a rectangular plate (8 x 3.5 x 0.3 cm) of acrylic glass. Each plate had two cells, each with 1.5 cm diameter, closed on the bottom side with a nylon mesh and on the upper side with a microscope slide. The acrylic plate and the microscope slide were held together by three foldback clips (Figure 8).

From day 5 to day 8, the leaf arenas and acrylic cages were checked twice a day, in the morning and in the afternoon (at an 8 h interval) for hatched larvae inside the cages and counting and collecting the eggs on the leaf arenas. After day 10 all larvae should have hatched.

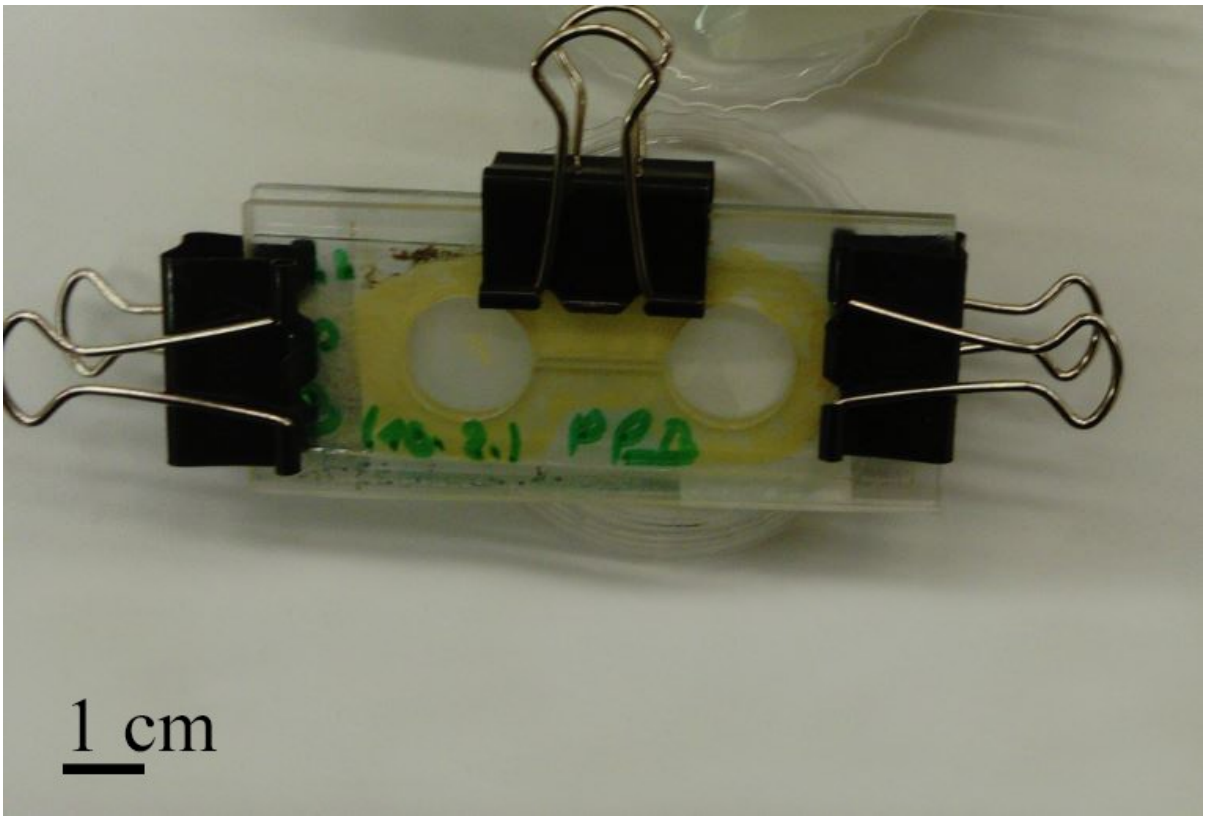

Figure 8: Acrylic cages used to observe egg hatching in the first experiment.

To calculate the numbers of hours of egg development outside the body in the first experiment I used the following method: I took the full time (h) from egg laying until the last observation time before hatching plus standardized 12 h for the time elapsed since copulation and from the observation period between observing the egg and finding the hatched larva we took half of the hours of the observation interval.

2.4 Protocol of the second experiment

The second experiment was started with the preparation of two 10 x 10 cm sized leaf arenas, one harbored pollen (P) and the other harbored spider mites (S). Ten to 20 females were randomly withdrawn from each of the two rearing lines (one fed with pollen (P) and the other one fed with spider mites (S)) from either the mass-reared (Koppert population or the field-collected Israel population) of *Amblyseius swirskii* and placed onto leaf arenas resting on top of water-soaked foam cubes inside plastic boxes (20 x 20 x 6 cm).

Eggs laid by *Amblyseius swirskii* on leaf arenas between days 1 and 4 were collected once per day, placed on separate leaf arenas and stored in the refrigerator at 8 °C, to halt their development, until 20 eggs of each line were available.

The 20 eggs of each line were split, like in the first experiment, into two subgroups (pollen nutrition (P) and spider mite nutrition (S)) on leaf arenas, which resulted in four treatments: PP, SS, PS and SP - each with 10 eggs (Figures 9, 10). PP and PS were coming from the pollen reared line; SS and SP were coming from the spider mite reared line.

Figure 9: Treatments of the predatory mites in the second experiment. (S) stands for spider mite nutrition, (P) stands for pollen nutrition; first letter refers to the rearing diet (origin of the females), second letter refers to the diet provided to mothers during development and oviposition. Sample size was 10 eggs in each of the four treatments (PP, PS, SP and SS).

From day 6 to 10, the predators were monitored once per day until they reached the deutonymphal stage. Female deutonymphs were collected and isolated on separate leaf arenas (harboring the same food as on the origin leaf arenas, i.e. with either pollen or spider mites) until reaching adulthood.

After reaching adulthood (days 11 to 12), each female was provided with a male mate and the time elapsed until the onset of copulation, i. e. the mating latency, was measured; males were removed from the arenas on the next day.

From days 12 to 20, leaf arenas were checked two times per day (at 8 h intervals) for the timing of laying the first three eggs. Food on the leaf arenas was replenished as needed. To identify the rank of a given egg in the sequence of eggs laid, their position on the leaf was marked with a water-color dot to keep track of their identity and enable recording their hatching time.

To account for dead and male individuals in the first set of 10 eggs per treatment, the above described procedure was repeated until the minimum number of 10 successfully fertilized females per treatment had been reached.

Figure 10: Detached leaf arenas to rear the mites in the four different treatments (PP, PS, SP and SS) of the second experiment. To identify the arenas, I marked them with a small piece of paper on the tissue layer.

Calculations for the second experiment were done according to the following method: To estimate the time of egg development inside the body and larval hatching times (i.e. the time between egg laying and hatching) I used two alternative procedures: (A) for the first internal fertilization model (called “simultaneous”: fertilization of all eggs occurs at time of copulation), I assumed the same internal fertilization date and time for all three eggs: (B) for the second internal fertilization model (called “delayed”), I assumed a delay in the internal fertilization time of 33% of the time between copulation and egg laying for the second egg and 67% for the third egg (Figure 11).

To calculate the proportional hatching time, I took the hours of egg development outside of the maternal body and divided them by the hours of the total egg development time (in- and outside the maternal body combined). I analyzed the data for both types of internal fertilization assumptions because internal fertilization is under female control and determining the accurate internal fertilization time is impossible for the experimental observer.

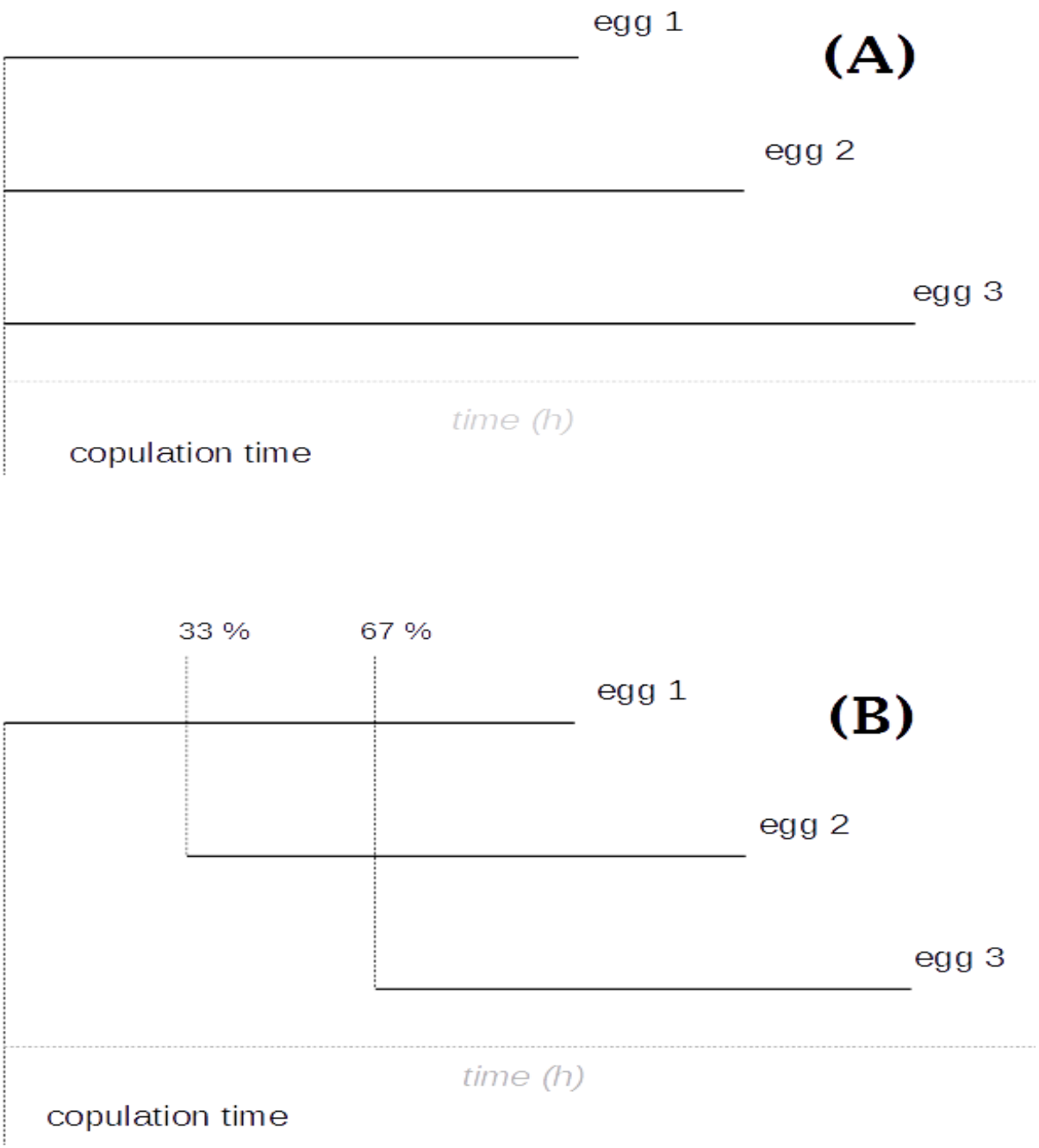

Figure 11: The two assumptions of the timing of internal fertilization in experiment 2. (A) Internal fertilization is the same for all three eggs (“simultaneous”); (B) internal fertilization of the second egg is delayed by 33% of the time between copulation and egg laying; internal fertilization of the third egg is delayed by 67% of the time between copulation and egg laying (“delayed”).

To estimate the internal egg development time (i.e. time from copulation until egg laying), I calculated the full time from copulation time until the last observation time before the laid egg was found and from the last observation until egg laying time half of the time. For the hatching time (i.e. time from egg laying until hatching), I used the same calculation from egg laying time until the last observation the full time and from observation date and time until larvae hatching date and time I took half of the time (Figure 12). The rows total simultaneous and total delay illustrate the whole developmental times for both models depending on the alternative assumptions of internal fertilization times.

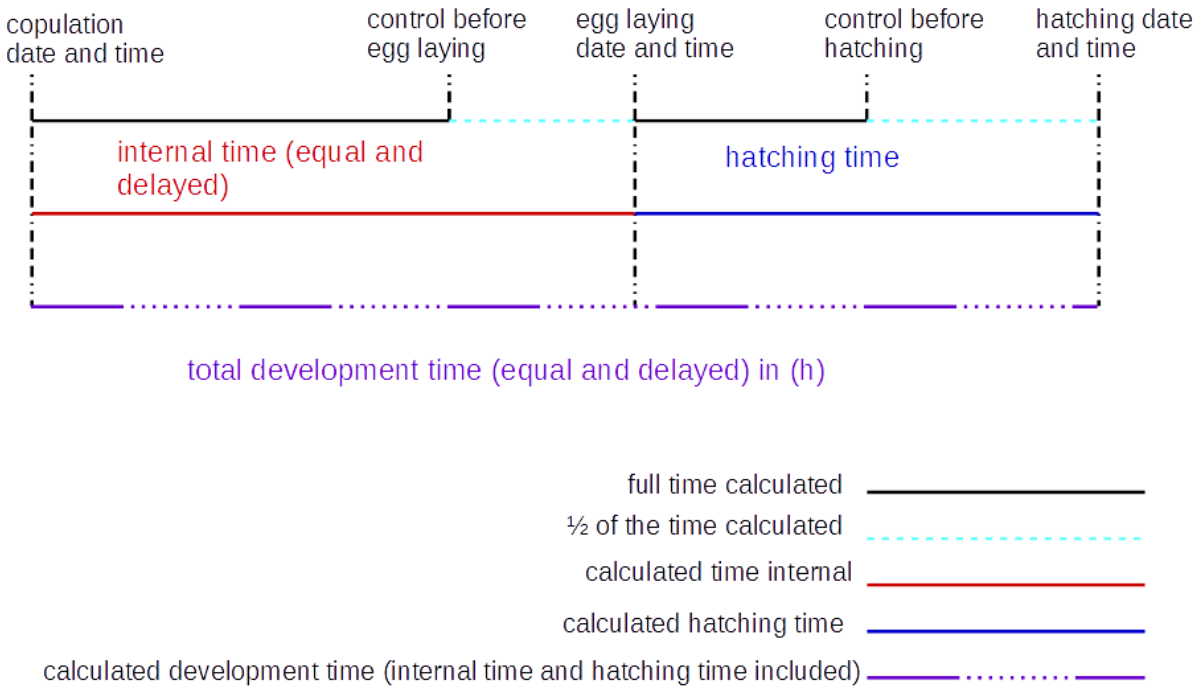

Figure 12: Explanation of the calculated time in experiment 2 (internal simultaneous, internal delay, hatching, total simultaneous, total delay).

2.5 Protocol of the third experiment

The aim of the third experiment was finding out if the maternal diet influences the size of their eggs. Several studies (Schausberger, 1992; Delisle et al., 2015; Goleva et al., 2015) showed that the type and quality of food is a decisive factor for development and reproduction of predatory mite species. We assumed that egg size could be an important co-determining factor for the proportion of egg developmental time occurring inside and outside the maternal body.

To measure egg size, ten eggs produced by females from the SS and the PP groups of the second experiment were embedded in a drop of Hoyer's medium (https://entomopraxis.com/tienda/es/montaje-de-preparaciones/272-liquido-de-hoyer-17-ml.html, last accessed 12.3.2019) on a microscope slide and the drop covered by a cover slip. After drying, the length and width of the eggs was measured using a Leica DMS1000 digital microscope (Figure 13) and the volume of the eggs calculated using the formula for a prolate spheroid (Figure 14).

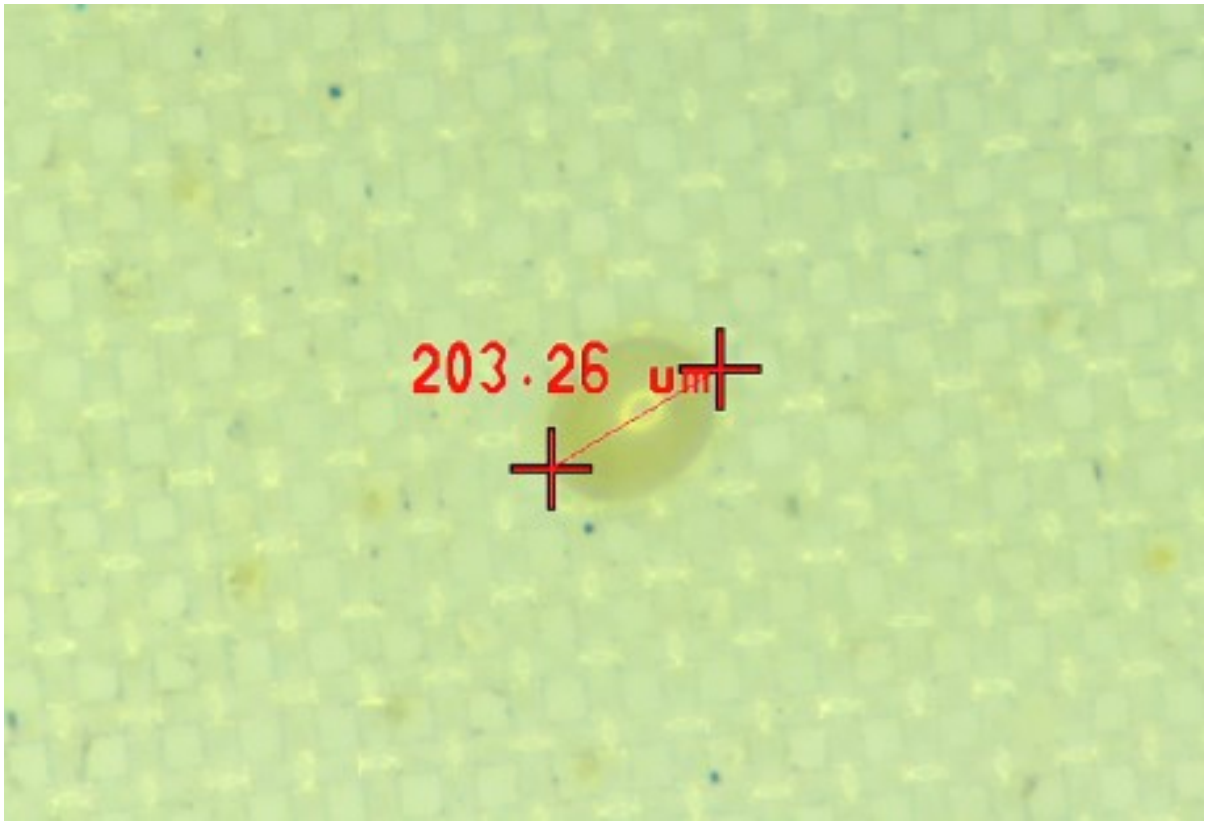

Figure 13: Length and width measurements of the eggs of the SS and PP treatments in the third experiment using a Leica DMS1000 digital microscope.

$$v = \frac{4}{3}\pi \left(\frac{w}{2}\right)^2 \frac{l}{2}$$

Figure 14: Formula to calculate the volume of a prolate spheroid. .This formula was used to calculate the volume of the predatory mite eggs in experiment 3.

2.6 Statistical analyses

Documentation and analyses of the observations were conducted with MS Excel and the free software R (www.r-project.org 15.3.2019 17:36). Statistical analyses of the data of experiments 1 and 2 were conducted using SPSS 25 (IBM, Armonk, NY, USA). Statistical analyses of the data of experiment 3 were conducted using R.

In the first experiment, I used a generalized linear model (GLM; normal distribution, identity link) to analyze the influence of rearing diet and maternal diet on hatching times. Least significant difference tests (LSD) were used for post-hoc pairwise comparisons.

In the second experiment, I used separate generalized linear models (GLM; normal distribution, identity link) to analyze the influence of treatment (combination of rearing diet and maternal diet) on egg developmental time inside the maternal body and the proportional time outside of the body. Separate generalized estimating equations (GEE; normal distribution, identity link; AR(1) correlation structure between eggs of the same female) were used to compare the internal egg developmental time and the proportional time outside of the body as affected by treatment (combination of rearing diet and maternal diet). In GEEs we assumed delayed fertilization of the second and third egg of a female. Least significant difference tests (LSD) were used for post-hoc pairwise comparisons.

In the third experiment, I used a t-test for independent samples to evaluate if there are significant differences in the egg volumes of the two treatments PP and SS.

3. Results

3.1 First experiment

GLM (normal distribution, identity link) revealed that both the rearing diet (Wald X^2_1=40.608, p<0.001) and the maternal diet during egg production (Wald X^2_1=34.878, p<0.001) and the interaction of these two factors (Wald X^2_1=24.031, p<0.001) had a significant effect on the larval hatching times (Figures 15, 16). Larvae produced by mothers reared on pollen hatched faster than larvae produced by mothers reared on spider mites. However, the interaction shows that pollen had only an effect in the combination of rearing diet and maternal diet. Pairwise comparisons showed that larvae of the PP treatment hatched significantly faster (p<0.05) than larvae of the PS, SP and SS treatments, which did not differ among each other.

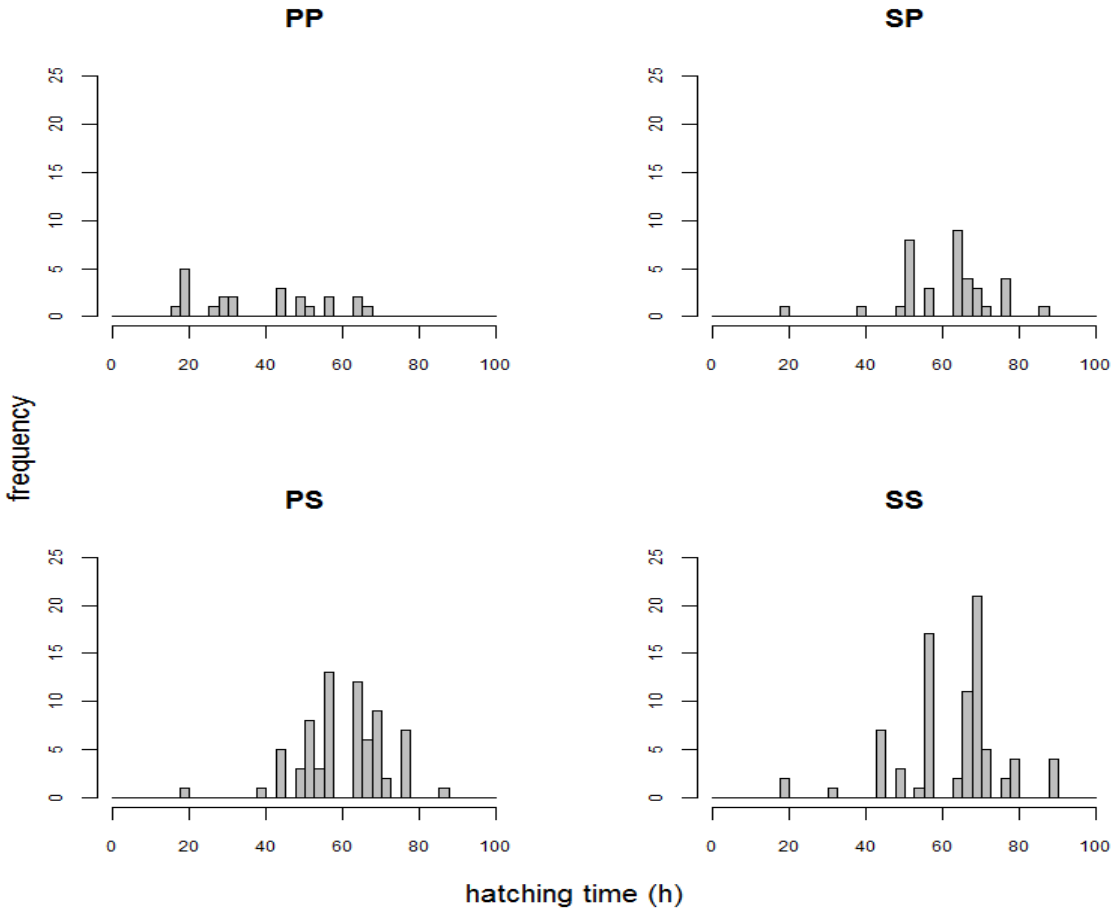

Figure 15: Histogram of the larval hatching times (i.e. the time from egg laying until larvae hatch) in the first experiment. On the y-axis the frequency of the eggs is plotted on the x-axis the hours are depicted.

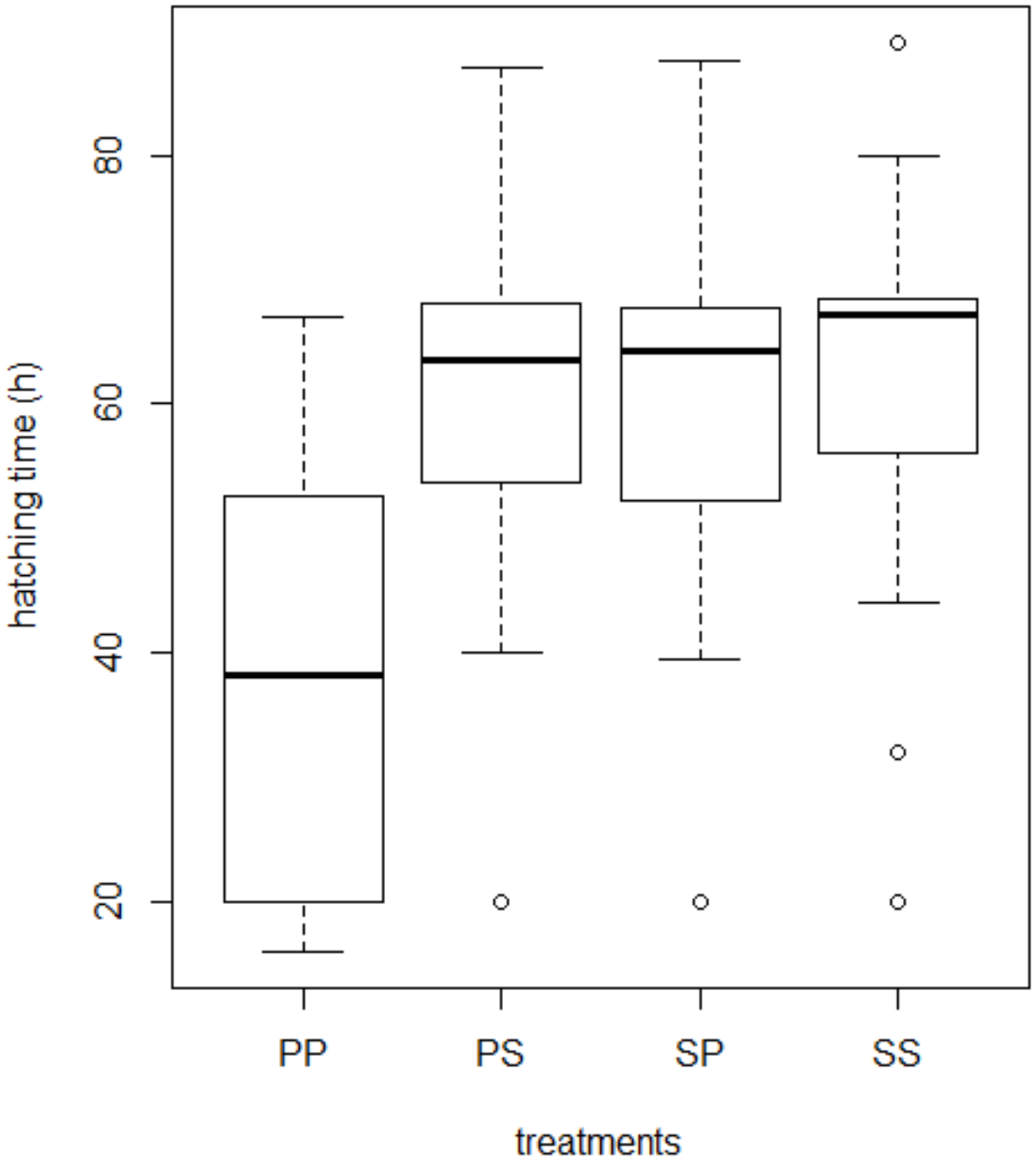

Figure 16: Box plot of larval hatching times (egg laying until hatching) in the first experiment, outliers are included (dots). The boxes indicate the space between the first and the third quartile (Q3-Q1). The median, the thick line in the box, marks half of the observations that are half over and half under this line. The whiskers indicate the minimum and the maximum of the observations. The dots depict the outliers.

3.2 Second experiment

Generalized linear models (GLM; normal distribution, identity link) revealed a significant difference among treatments in internal developmental times of the first egg (Wald X^2_3=14.762, p=0.002) (Figures 17 and 18) and larval hatching times (i.e. proportional developmental time of the first egg outside the body - proportional hatching time) (GLM; Wald X^2_3=8.821, p=0.032) (Figures 19, 20).

Pairwise comparisons showed that development of the first egg in the treatment PP differed significantly from the other three treatments ($p<0.05$). The other three treatments SS, PS, SP did not significantly differ from each other ($p>0.05$ for every pairwise comparison). The first eggs of the PP treatment had a significantly longer developmental time than the first eggs of the SS, PS and SP treatments.

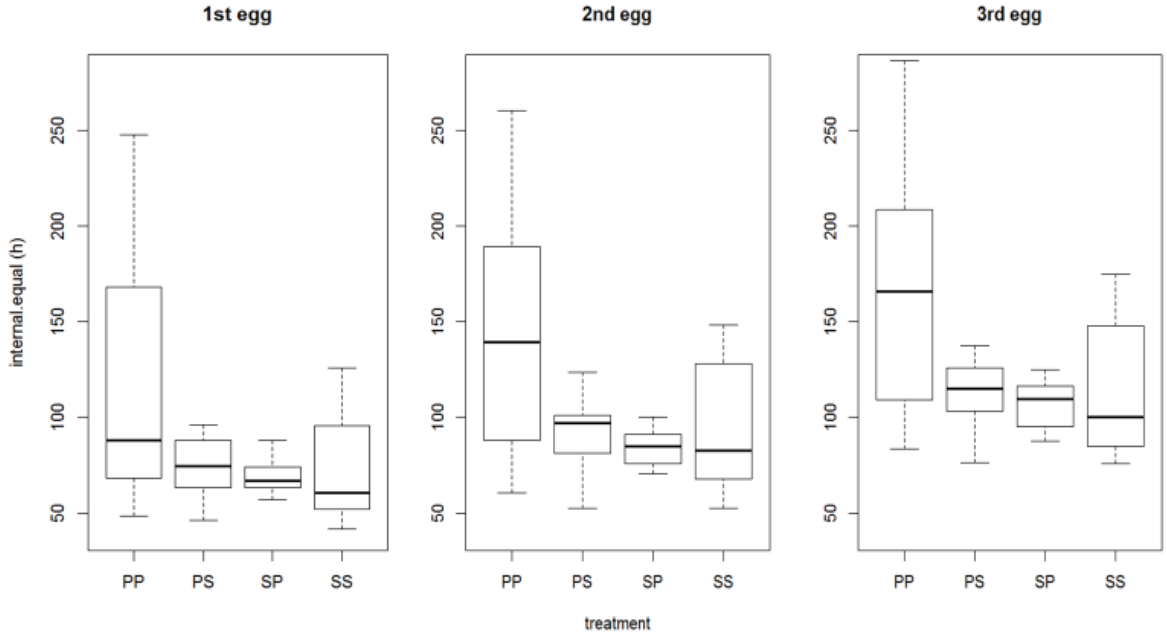

Figure 17: Time from copulation until egg laying in the second experiment under the assumption of simultaneous fertilization time for all three eggs. The boxes indicate the space between the first and the third quartile (Q3-Q1). The median, the thick line in the box, marks half of the observations that are half over and half under this line. The whiskers indicate the minimum and the maximum of the observations. The dots depict the outliers. Sample sizes were between 10 and 14 eggs per treatment.

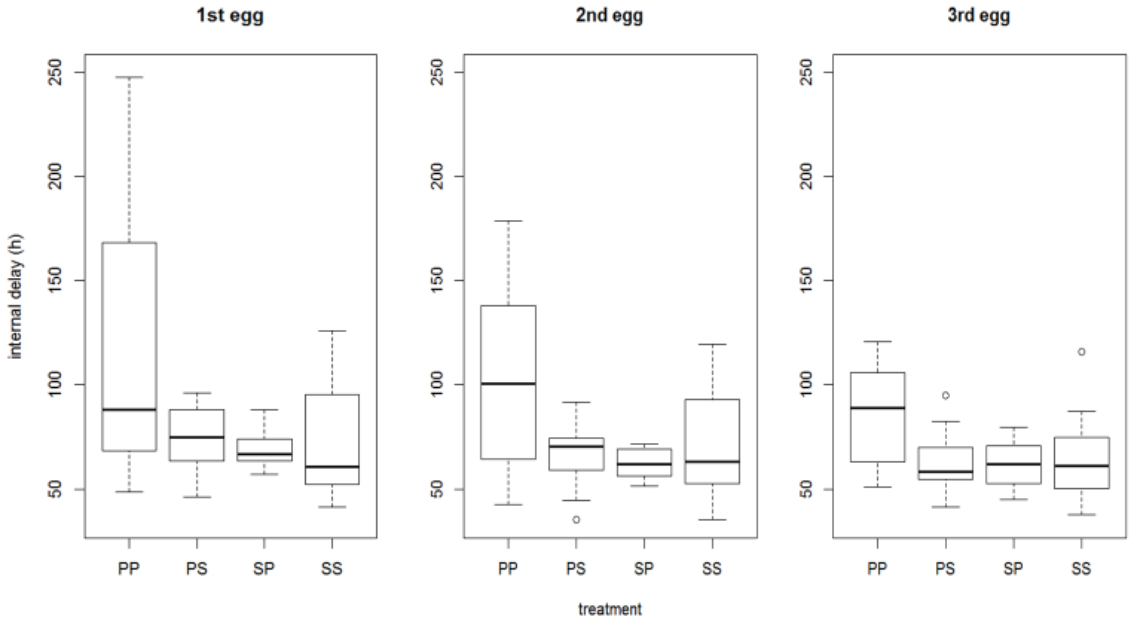

Figure 18: Time from copulation until egg laying in the second experiment under the assumption of delayed internal fertilization time (33% for the second and 67% for the third egg). The boxes indicate the space between the first and the third quartile (Q3-Q1). The median, the thick line in the box, marks half of the observations that are half over and half under this line. The whiskers indicate the minimum and the maximum of the observations. The dots depict the outliers. Sample sizes were between 10 and 14 eggs per treatment.

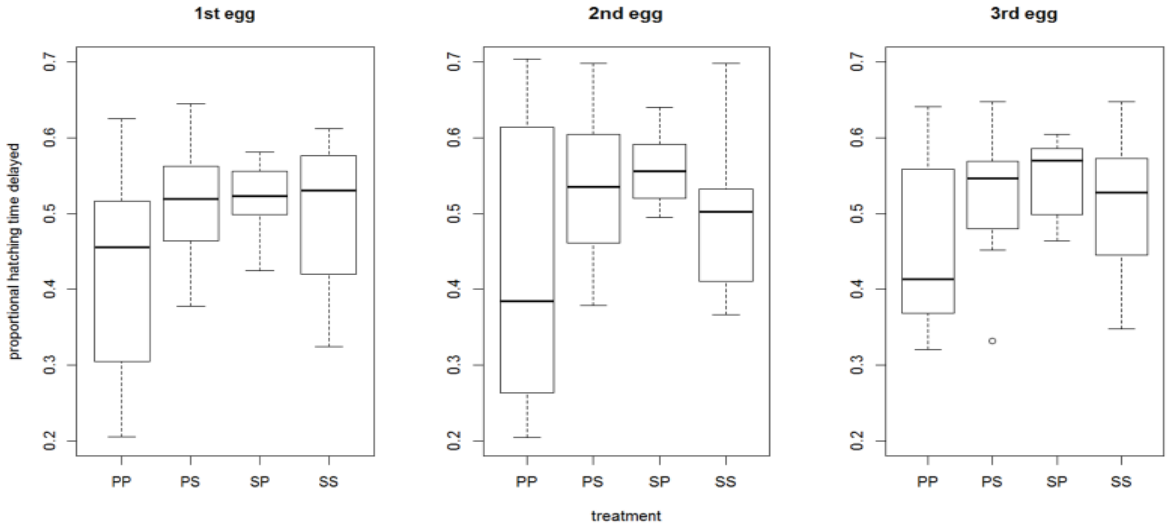

Figure 19: Proportional hatching time (egg laying until hatching) under the assumption of simultaneous fertilization time for all three eggs. The boxes indicate the space between the first and the third quartile (Q3-Q1). The median, the thick line in the box, marks half of the observations that are half over and half under this line. The whiskers indicate the minimum and the maximum of the observations. The dots depict the outliers.

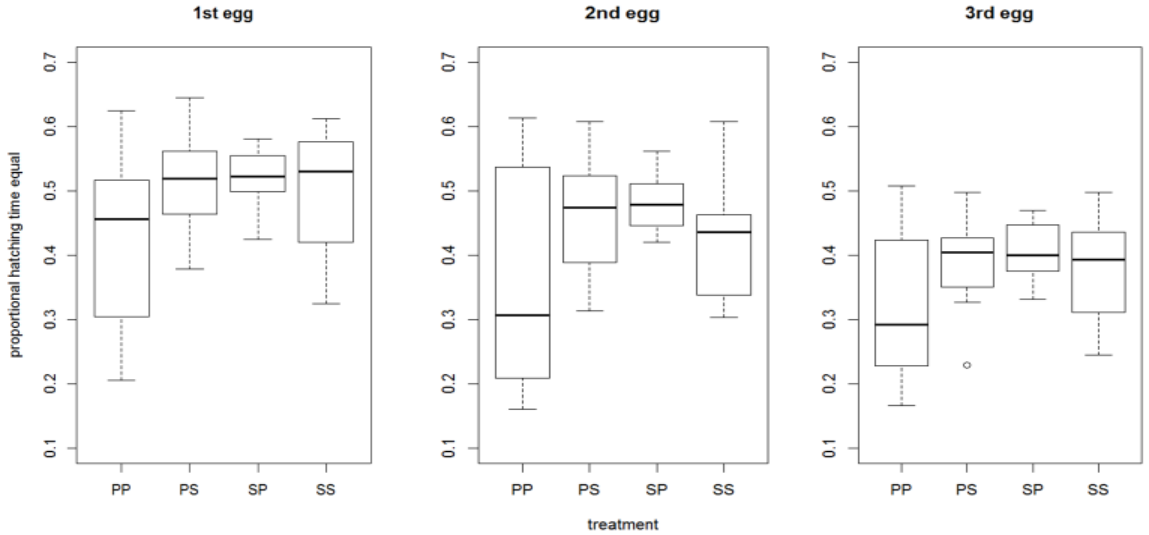

Figure 20: Proportional hatching time (egg laying until hatching) under the assumption of delayed internal fertilization (33% for the second and 67% for the third egg). The boxes indicate the space between the first and the third quartile (Q3-Q1). The median, the thick line in the box, marks half of the observations that are half over and half under this line. The whiskers indicate the minimum and the maximum of the observations. The dots depict the outliers.

Generalized estimating equations (GEE; normal distribution, identity link) revealed significant differences among treatments in the developmental times inside the body for all three eggs (Wald X^2_3=16.405, p=0.001) (Figures 17, 18). Results are calculated under the assumption of delayed fertilization. Similarly, GEE showed a significant difference among treatments in the proportional hatching times of all three eggs (Wald X^2_3=9.919, p=0.019) (Figures 19, 20).

Pairwise comparisons showed that the treatment PP was significantly different from the other three groups (p<0.05). The other three groups SS, PS, SP did not differ from each other (p>0.05 for every pairwise comparison). The significance levels did not change when assuming simultaneous fertilization of the three eggs of a female (not shown here), i.e. the results were basically the same no matter whether I assumed that all three eggs were internally fertilized at copulation time or whether I adjusted the fertilization time by the sequence of eggs (33% later for the 2nd egg, 67% later for the 3rd egg) (Figures 21 and 22).

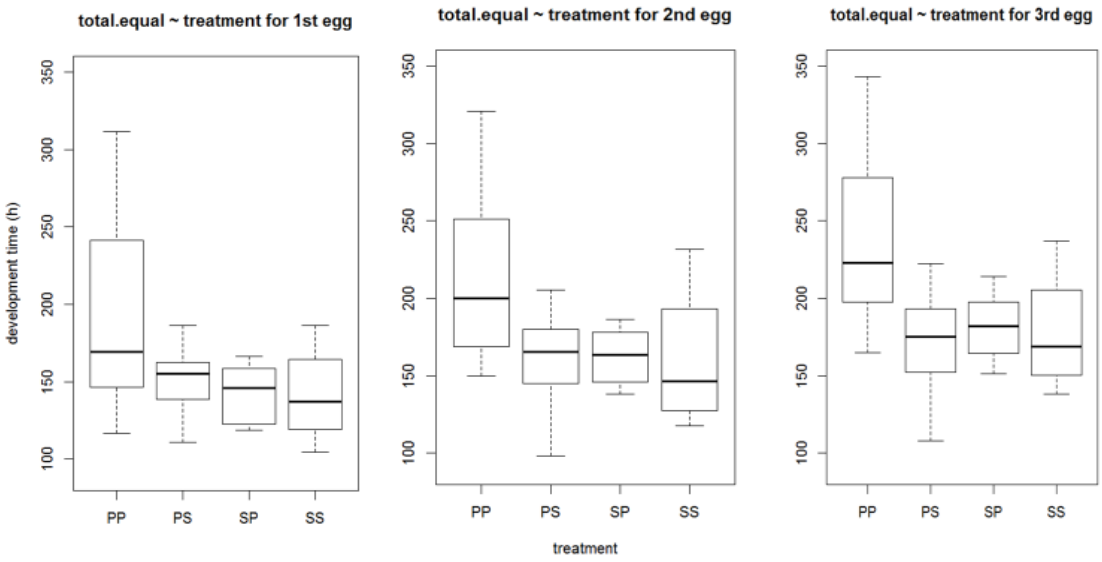

Figure 21: Time from copulation until hatching for all three eggs under the assumption of the same internal fertilization time (assumption “simultaneous”) in the second experiment. The boxes indicate the space between the first and the third quartile (Q3-Q1). The median, the thick line in the box, marks half of the observations that are half over and half under this line. The whiskers indicate the minimum and the maximum of the observations. The dots depict the outliers.

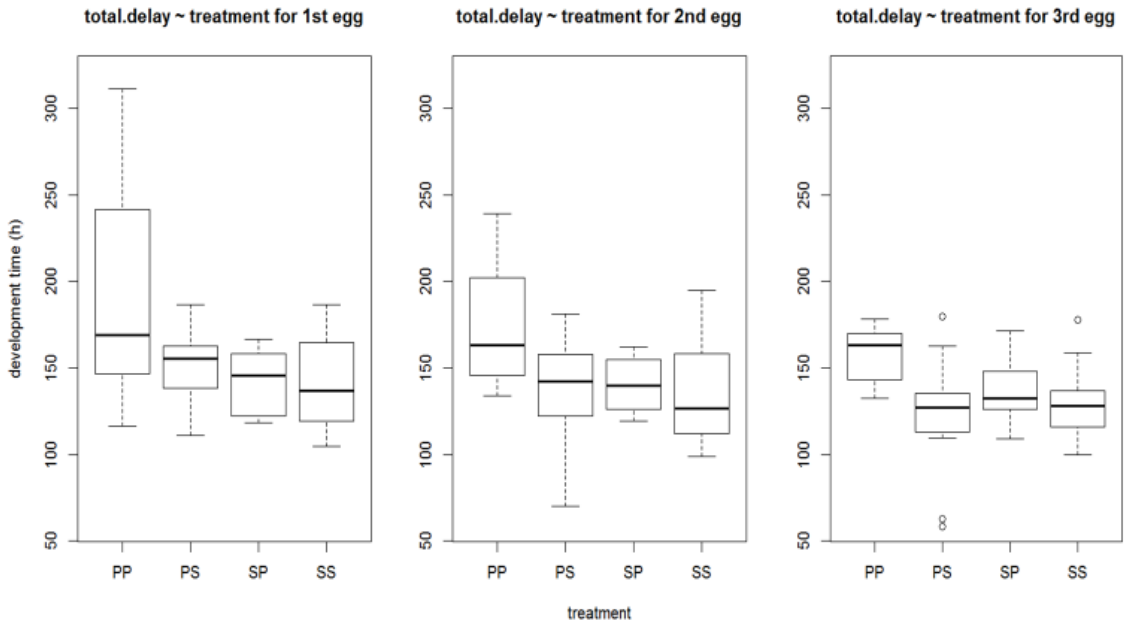

Figure 22: Time from copulation until hatching (h) for all three eggs under the assumption of delayed internal fertilization time (33% for the second and 67% for the third egg assumption “delayed”) in the second experiment. The boxes indicate the space between the first and the third quartile (Q3-Q1). The median, the thick line in the box, marks half of the observations that are half over and half under this line. The whiskers indicate the minimum and the maximum of the observations. The dots depict the outliers.

3.3 Third experiment

To obtain information about a possible impact of the diet during rearing and oviposition on egg size, the results of egg measurements with the Leica DMS1000 digital microscope are depicted in Figures 23 and 24. Eggs of the PP and SS *Amblyseius swirskii* treatments of experiment 2 were measured. I used the formula of a prolate spheroid to calculate the volume of the eggs (Figure 14).

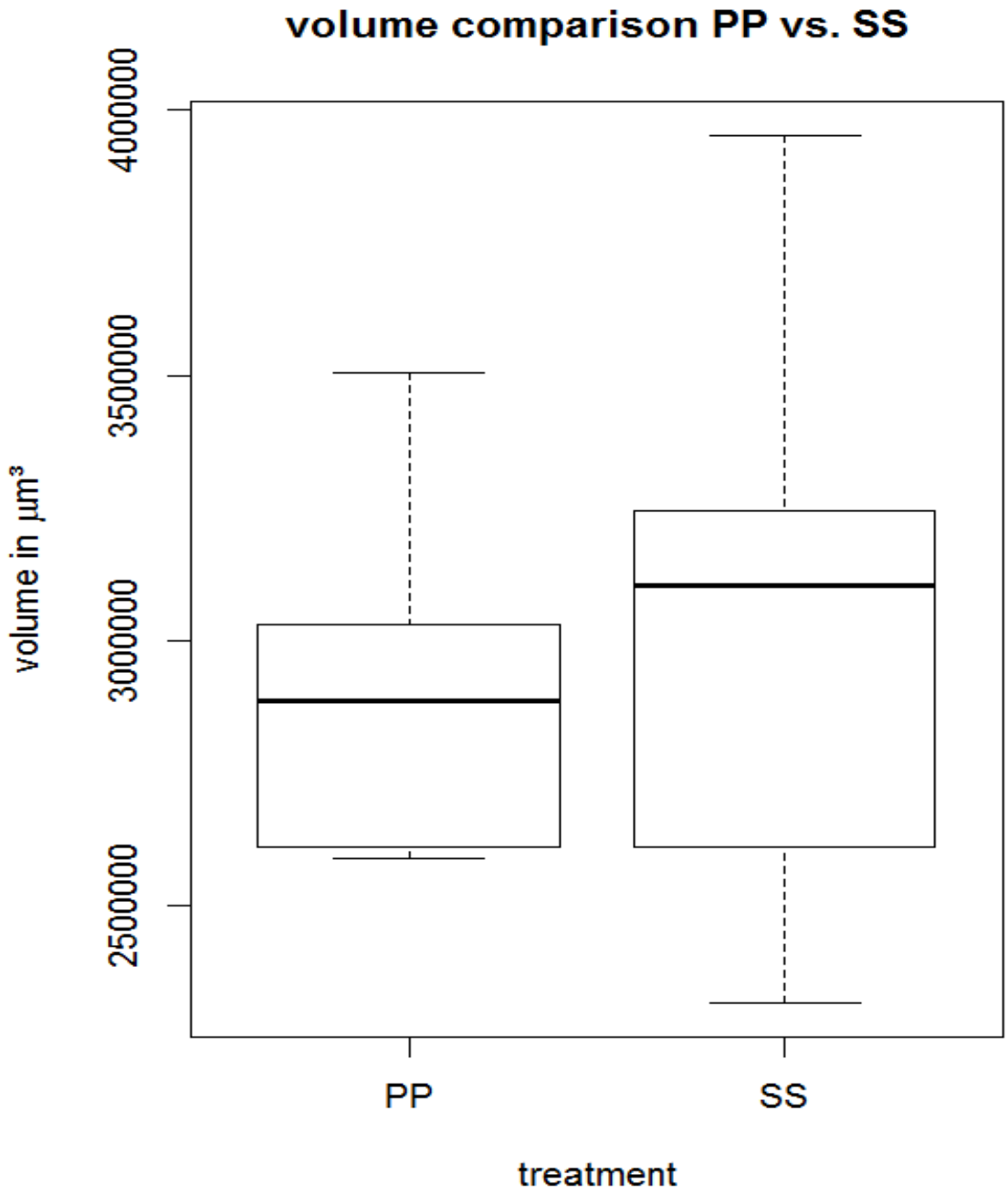

Figure 23: Calculated volume of the eggs in μm^3 on the y-axis of the two different treatments (SS and PP on the x-axis) of the measured eggs in experiment 3. The boxes indicate the space between the first and the third quartile (Q3-Q1). The median, the thick line in the box, marks half of the observations that are half over and half under this line. The whiskers indicate the minimum and the maximum of the observations.

A t-test for independent samples was used to analyze if there is a difference in egg size between the two treatments PP and SS. The t-test was not significant (t=0.646, p=0.528). Eggs of both treatments have nearly the same size (Figure 24).

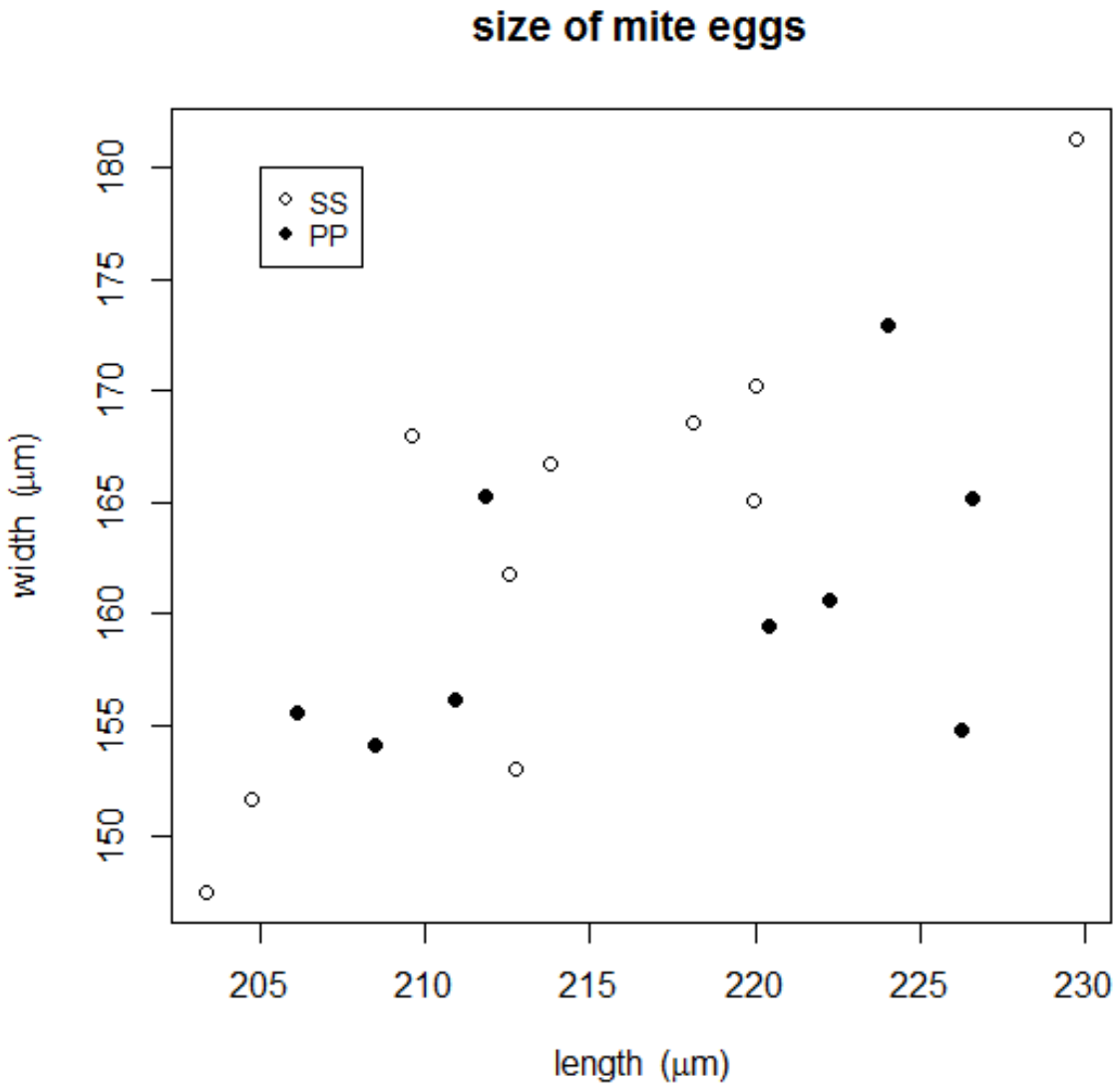

Figure 24: Plot of the measured egg sizes in experiment 3. Eggs of the SS treatment are represented by white diamonds. Eggs of the PP treatment are represented by black diamonds. The x-axis depicts the length of the observed eggs in µm the y-axis their width in µm.

4. Discussion

The aim of my study was to investigate the effects of different types of nutrition combined with the oviposition substrate, on egg development and hatching times of the predatory mite *Amblyseius swirskii*. I assumed, on one hand - based on observations in previous studies - that pollen nutrition accelerates the embryonic development compared to spider mite nutrition, and on the other hand, that the mother mites are sensitive to the oviposition environment and respond to adverse conditions by egg retention. To investigate these assumptions, three experiments were conducted and possible effects of host plants and substrate, as well as the influence of nutrition and the climatic impact on egg laying behaviour, are discussed.

4.1 Effects of host plants and substrate

Host plants play a major role in the performance and oviposition behaviour of predatory mite species. Plants have different defense strategies, chemical ones (Becerra, 2015) and mechanical ones, like thorns, spines and prickles (Farmer, 2014; Bagella et al., 2019), sometimes combined with mimicry (Lev-Yadun, 2003), to cope with attacking herbivore species. *Tetranychus urticae* is able to live and reproduce on more than 1100 host plant species (Dermauw et al., 2013). *Amblyseius swirskii* and other biocontrol agents have to cope with these conditions. Additional to the defense strategies of the plants, other components like the thickness of the leaves, number, types and density of trichomes on the leaves, architectural structure and the texture (for example a waxy surface) of the leaves are very important components for the performance of mites. For example, Buitenhuis et al. (2014) observed that a high density of trichomes negatively influences the walking speed and the dispersal rate of *Amblyseius swirskii*. Trichomes are favourable egg laying sites, and in certain configurations advantageous for the reproductive behaviour. The bean plant *Phaseolus vulgaris*, which I used in the experiments, was perfectly suitable for the mites, their life history performance and oviposition behaviour were favourable. Also, the walking speed and predation rate of *A. swirskii* were balanced. Predator

individuals on the arenas with spider mites developed normally and were in good condition. Clotuche et al. (2013) made similar observations in the spider mite *Tetranychus urticae*. They used different substrates/surfaces (living and non-living), and with and without spider mite silk, to check the optimal egg laying sites. When plastic substrate was used, the walking speed of predatory mites was higher (Buitenhuis et al., 2014), but the substrate was dryer and therefore the risk that eggs dry out was higher. In my experiments, I used two different nourishments (spider mites and pollen) on the leaf arenas. *Amblyseius swirskii* was able to move on leaves with both types of food without problems.

4.2 The influences of nutrition and environment on egg production

Nutrition is the most important factor when producing eggs and very energy consuming. In my thesis, I asked whether the differences between the two food sources spider mites and pollen are big enough to have an impact on egg production and egg development, particularly hatching times.

There are different studies on development, body weight, oviposition rate and survival of phytoseiid mites with pollen only diet of various plants (Kolokytha et al., 2011; Goleva and Zebitz, 2013; Kishimoto et al., 2014) and mixed diet as a food source (Delisle et al., 2015; Nguyen et al., 2014; Goleva et al., 2015; Riahi et al., 2016; Fadaei et al., 2018). Not every pollen is useable for the mites. They have to open each grain separately to suck the nutrients out of the pollen grain. In this context the pollen surface is important. For example, grains with bumps or other pronounced surface structures are difficult to break. The nutrients inside the pollen grains play a major role. Some of them may even be toxic for *Amblyseius swirskii* (Goleva and Zebitz, 2013). The pollen used in my study, *Typha latifolia,* is well useable by *Amblyseius swirskii* and its survival, development and oviposition rate with this diet are favorable (Park et al., 2011).

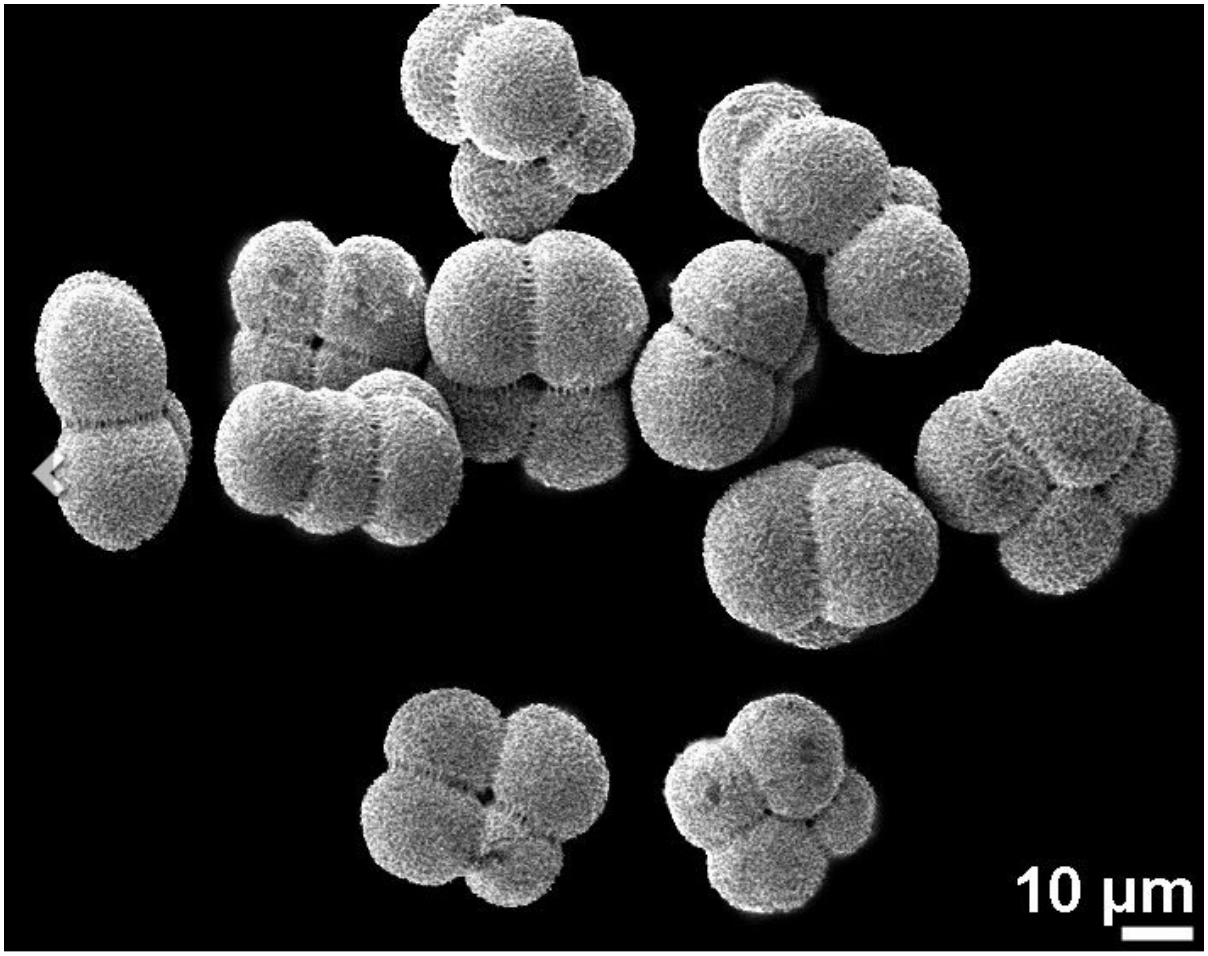

Figure 25: Pollen grains of *Typha latifolia*, the pollen source used in my experiments. The picture was taken with an electron microscope (Halbritter, 2005).

Each animal is vulnerable to external stressors during its life cycle. There is always the risk of predation, sickness and death. Ghazy et al. (2016) reported different causes of environmental stress in phytoseiid mites. In mites and insects in general the early developmental stages are the most vulnerable ones. This complex of problems is well studied (Montserrat et al., 2007; Walzer et al., 2007). The life cycle of *A. swirskii* is dependent on temperature and ambient humidity. San et al. (2021) pointed out in their study that the accessibility of drinking water and elevated humidity are highly important factors for reproduction, development and survival of *Amblyseius swirskii*. When free water is available, immature stages can also develop into adults at lower relative humidity. With a higher temperature, between 28° and 32° C, the development proceeds faster (Lee and Gillespie, 2011). Extremes are always a

problem. Is the humidity to high, further development is disturbed, which may results in the degeneration of the eggs. To stay competitive, individuals may adapt to changing environmental conditions with a developmental switch in the offspring or in egg production (Fischer et al., 2003; Montserrat et al., 2007). Le Hasran et al. (2020) observed in their study about the predatory mite *Phytoseiulus persimilis,* that females that live under constant low relative humidity conditions of around 65 % for a period of 102 hours produce eggs that are more drought resistant than individuals, which live under high relative humidity conditions of around 95 %.

Based on species-specific requirements, I maintained the predatory mites during my experiments at favorable conditions, i.e. in a climate chamber with a temperature of 23 ± 1° C and a relative humidity of 40 ± 5 %. With the use of detached leaf arenas I ensured constant water supply for the mites.

Many animals who lay their eggs on terrestrial grounds take care of their eggs and some produce eggs with one or more amnions to protect the eggs from adverse abiotic factors, for example from drying out or from an unsuitable substrate. Different strategies evolved to take care of the offspring when they are outside the maternal body. Nest building is a wide-spread strategy, for example in birds (Collias and Collias, 2014; Alambiaga et al., 2020), in frogs (Hödl, 1990), or in some lizards in Australia, which lay their eggs in clutches in secure sites, like termite nests or they built nest holes in compost. Sometimes they glue them to hard surfaces, where they can develop safely and without parental care (Wilson, 2012). Many insects and mites also deposit their eggs in clusters (Agarwala and Dixon, 1993; Faraji et al., 2002).

Some species can switch between reproductive cycles to optimize the survival chances of offspring. For example, many aphids evolved highly complex life cycles. They reproduce most of the time by thelytokous parthenogenesis and switch to vivipary when the environmental conditions are favorable. The members of the colony are in this case genetically almost identical. Unfavorable environmental

conditions lead to a switch in the reproductive cycle to a single sexual generation and ovipary (winter eggs).

The life cycle of the predatory mite *Amblyseius swirskii* includes five developmental stages (see Figure 1) until they reach maturity and start reproduction after mating. Juvenile development is finished within seven to ten days, depending on ambient humidity and temperature. Based on this knowledge, I positioned an adult male close to the female after the second nymphal molt and the couple started immediately to copulate. In my experiments, male individuals remained for 24 hours on the arenas with the females but just one single successful sperm transfer from the male individual to the female is sufficient for egg production in *A. swirskii*. Females of *Amblyseius swirskii* store sperm in spermathecas and can adjust the times when their eggs are internally fertilized. The exact mechanisms determining the point of time when fertilization occurs are unclear. Around three days after mating, females started to lay the eggs on the arenas. During the act of oviposition, when the egg slides out of the abdomen, the mother often rolled the egg with her first pair of legs and touched it several times, before she finally positioned it on the substrate. To my knowledge, this behaviour has not yet been rigorously explored but it could be that she marks the eggs with special odors and/or she learns the smell of her eggs to recognize them later again.

All observed eggs developed normally and no egg degeneration occurred. Collection of the eggs by using a moistened brush and placing them in the acrylic cages worked very well. I used the same type of acrylic cages for observing the process of egg hatching as Schausberger (1997). The eggs were not damaged and the embryonic development proceeded normally. Storing the eggs in the refrigerator at a temperature of 8° C to halt development, until a sufficient number of eggs needed for my experiments was reached, was also without any problems. After taking the eggs out of the fridge, the development of the eggs continued normally.

4.3 Egg retention

Egg retention (delaying oviposition by retaining the eggs inside the body) is well known from different animals, for example reptiles (DeMarco, 1992; Buhlmann et al., 1995; Rodriguez-Diaz and Braña, 2012), amphibians (Kupfer et al., 2006), some insects (Akhund-Zade et al., 2017) and also mites (Sanderson and McMurtry, 1984; Montserrat et al., 2007; Toyoshima and Amano, 2012). Depending on various factors, for example under low food supply, in presence of a predator or at low quality of the habitat, mites can delay oviposition until the conditions for their offspring become more favorable (Sanderson and McMurtry, 1984; Montserrat et al., 2007). Toyoshima and Amano (2012) observed delayed oviposition, when they studied the loss of paternal genomes in the phytoseiid mite *Neoseiulus womersleyi* during embryonic development. They observed that some larvae already hatched inside the maternal body (Figure 26).

Figure 26: Larva of the predatory phytoseiid mite *Neoseiulus womersleyi* hatching inside the mother's body as an extreme example for delayed oviposition by egg retention (Toyoshima and Amano, 2012).

Mother individuals try to get the best conditions for their offspring. Delayed oviposition can be considered a form of parental care. In this context, I assumed that hatching time is not fixed in *Amblyseius swirskii* but can be adjusted to internal and/or external factors It is a complex system in which different influences play major roles (Warkentin, 2011).

A major factor in the case of egg retention is the risk of predation, which is especially high for juvenile stages. For example, Montserrat et al. (2007) observed egg retention in the phytoseiid mite *Neoseiulus cucumeris* in presence of the intraguild predator *Iphiseius degenerans*. Eggs laid on patches with a predator hatched earlier than those laid on predator-free patches. These authors could observe that the embryonic development inside the retained eggs proceeded normally and at the time of egg laying their development was more advanced so that hatching happened faster. In my experiments, the arenas were all free of predators; the only influences were ancestral diest and the prevailing diet/oviposition subtsrate experienced by the mothers.

Timing of biological events is an important factor to success in competition within and between species. Shorter developmental times can be a huge advantage in the struggle for survival. For example, in the fruit fly *Drosophila melanogaster*, individuals with shorter embryogenesis showed a relatively shorter developmental time outside the body from egg to adult and a higher viability, as well as a higher reproductive rate, in a competitive environment than those taking longer time (Horváth et al., 2016; Horváth and Kalinka, 2018). Genetic variation in egg retention behavior must also be mentioned. Hormones, cell-signaling factors and cell cycle components are molecular mechanisms that influence egg developmental times (Moss, 2007). Horváth et al. (2016) observed, when they measured the embryonic development in the fruit fly *Drosophila melanogaster*, a broad range of genetic variants.

An ephemeral environment during reproduction could also induce egg retention behavior (Mueller and Bitner, 2015). Individuals with advanced development are in a

better position when food is limited. Just a few hours head start in the larval development of *Drosophila* spp. could change the outcome of competition (Bakker, 1962). A very important factor for the successful hatching and survival of larvae is the constant threat of drying out because of UV radiation. Quality and availability of food is another important factor in the case of egg retention. In competition for the same food source, for example with other mites or insects, individuals of some species can retain their eggs to search for more appropriate egg laying sites. For example, Markow et al. (2009) observed that egg retention is common in fruit flies when the available egg laying sites are unfavorable. In the predatory mite *Phytoseius hawaiiensis*, Sanderson and McMurtry (1984) observed that the mites delay oviposition in some cases to ovoviviparity when the substrate is unfavorable for egg laying. They compared different arenas consisting of bean leaves, avocado leaves or artificial tiles. On the leaf arenas oviposition was favored because there were trichomes and veins where the eggs could be placed. Tile arenas had a smooth surface without any favorable egg laying structures. Mites therefore delayed the oviposition on tiles until embryonic development inside the eggs was more advanced (Figure 27).

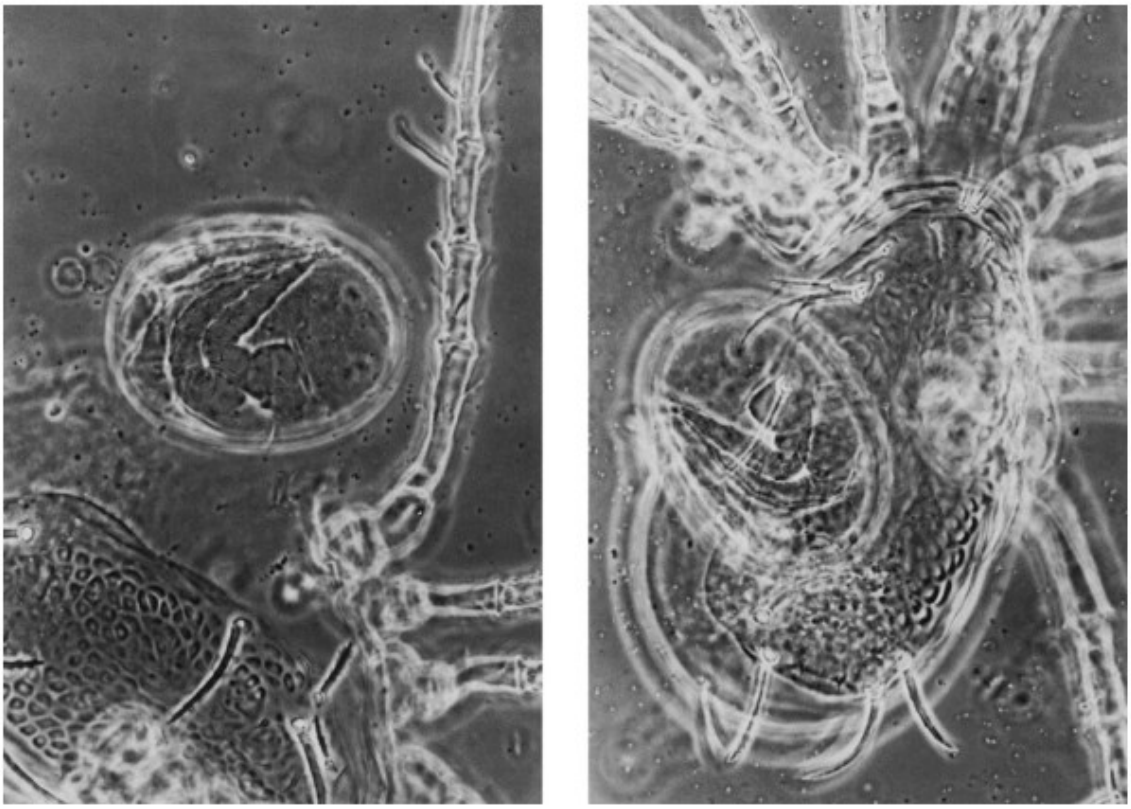

Fig. 1. Well-developed *Phytoseius hawaiiensis* larva inside egg which has been extruded (left) or partially extruded (right) from female parent during slide preparation

Figure 27: An example for delayed oviposition in the mite *Phytoseius hawaiiensis*. Development of the embryo was more advanced. Larva hatched within a short time after egg laying (Sanderson and McMurtry, 1984).

In my experimental setup, I limited the options for the mothers to use alternative strategies in taking care of the offspring. The mother individuals could not leave the leaf arenas to search for more appropriate egg laying sites. The only possible strategy was to keep the eggs for longer time inside their body until the conditions for the offspring improved, resulting in an advanced developmental state of the eggs when being laid (egg retention).

4.4 Egg size and potential influences on hatching times

Body size is a very important fitness determinant in insects and other arthropods. There are different parameters like genetic predisposition, nutrition and other environmental factors, which may affect the body size of individuals. Beukeboom (2018) pointed out that in insects larger individuals often have a longer life span and a higher reproductive success than smaller individuals.

The size of eggs is also often influenced by the mother's age. There are different studies about the decreasing size of the eggs with increasing age of mothers in insects (Murphy et al., 1983; Yuma, 1984; Boggs, 1986). In my experiments, all experimental females were nearly of the same age when they started to reproduce.

Benton et al. (2008) observed in their study about the mite *Sancassania berlesei* that the developmental time of the individuals was not influenced by offspring sex, but individuals that hatched from larger eggs had a shorter developmental time than individuals that hatched from smaller eggs.

Steigenga (2008) investigated in his thesis about the tropical butterfly *Bicyclus anynana* the influence of temperature on oviposition and development. Depending on the temperature during development there was an influence on the oviposition behaviour of the next generation. Higher temperatures lead to smaller eggs and shorter developmental times, and the number of laid eggs was higher. Lower temperatures increased the size of the eggs, and decreased the number of laid eggs.

Genetically based geographical differences could also be a factor for different sizes between individuals of the same species. Meister et al. (2018) studied the developmental stages of two populations, a southern and a northern one, of the moth *Ematurga atomaria*. To investigate the genetic influence, they used a controlled garden experiment to measure the size of the individuals. Eggs and newly hatched

larvae from the southern population were significantly larger than eggs and larvae of the northern population.

Nutrition is another major factor that may influence the size of the eggs. For example, Toyoshima and Amano (1998) observed in their study about two different predatory mite species, that eggs produced in times of low prey density were smaller than eggs produced in times of high prey availability. I ensured in my experiments a constant food availability on all of the arenas to prevent any influence of food shortage on egg production. In my experiments, egg size did not vary with ancestral and maternal diet (pollen or spider mites).

4.5 Conclusions

Several students in the laboratory of Peter Schausberger observed in their studies that individuals of *Amblyseius swirskii* fed with pollen hatched faster than individuals fed with other diets. Nutrition in general, and in this context the available type of diet, is a very important factor for producing eggs, additionally to temperature and humidity. There are lots of studies were different food sources such as pollen (Kutuk and Yigit 2011; Park et al., 2011; Kumar et al., 2014), whiteflies (Nomikou et al., 2001; Calvo et al., 2008), as well as other mites (van Maanen et al., 2010; Onzo et al., 2012) and thrips (Wimmer et al., 2008; Arthurs et al., 2009; Chow et al., 2010) were tested as food for *Amblyseius swirskii*. Survival and reproduction were possible with many of them.

Massaro et al. (2016) observed in their study about three potential biocontrol agents (*Amblyseius tomatavensis*, *Euseius concordis* and *Neoseiulus anonymus),* the egg laying behaviour of these three species under specific diets. They were fed with 15 astigmatid mite species, pollen from five different plants and with one nematode species. The highest oviposition rates were observed in *A. tomatavensis* fed with mites, in *Neoseiulus anonymus* ovposition was similar on all diets and in *Euseius concordis* fed with pollen. It is difficult to determine the perfect diet (pollen), because

each plant needs nutrients in a unique mix and therefore ingredients of the pollen are also different from plant to plant and may change over time. I have chosen *Typha latifolia* L. pollen as one food source in my experiments. The pollen grains have a smooth surface and they are known to be a favorable food source for *Amblyseius swirskii* (Park et al., 2011; Nguyen et al., 2013).

To investigate the effects of food (rearing diet and the food received by mothers) on the embryonic developmental time outside the maternal body, i.e. from egg laying until larvae hatching, I designed the first experiment. I hypothesized that pollen nutrition accelerates the embryonic development in the first experiment. The results of the first experiment showed a significantly shorter hatching time of eggs in the PP treatment (both rearing diet and maternal diet were pollen). My first research question (Are there differences in hatching time in *Amblyseius swirskii* based on nutrition?) could be definitely affirmed. As hypothesized, pollen nutrition caused significantly earlier larval hatching than spider mite nutrition. However, this was just the case in the PP treatment (rearing diet pollen, mother diet pollen) but not in the SP group (rearing diet spider mites, mother diet pollen). I conclude that the time between the switch from ancestor spider mite diets during rearing to the maternal pollen diet was too short to impact the mites in their oviposition behaviour.

In the second experiment, the time spent inside the maternal body, time spent outside the body and total egg developmental time (from fertilization to hatching; sum of the previous two periods) were compared among four groups of *Amblyseius swirskii* with different nutrition during ancestor rearing and egg production (pollen and spider mites) to provide insights into the effects of the oviposition environment and the diet on egg developmental times. I observed that individuals of the PP treatment (rearing diet pollen, maternal diet pollen) had on the one hand a significantly longer egg developmental time and on the other hand a significantly shorter proportional egg hatching time outside the maternal body. This held true for the first egg laid as well as for the second and third eggs, no matter how the time of fertilization was mathematically modelled (simultaneous or staged). My first hypothesis that pollen

nutrition accelerates embryonic development could not be confirmed. The internal egg developmental time was longer in the PP treatment than in the three other treatments (SS, SP, PS), which could have been due to the nutrient-poorer pollen diet. My second hypothesis that mites respond to the experienced conditions such as diet and the oviposition substrate by egg retention was supported.

In comparison, the results of the first and second experiment seem to point at different mechanisms, but it is clear that the mites also responded to the environmental conditions in the first experiment. To which extent, internal factors (poor nutrition during egg production) or external factors (egg retention due to nutrient-poor food availability in the oviposition site) or rather the interaction of these factors caused the significantly longer developmental time inside the maternal body is not yet ascertainable. The absolute hatching times did not differ among treatments in the second experiment but the proportional hatching time (proportional time outside the maternal body) was significantly shorter in the PP treatment, as predicted.

To exclude egg size as a possible explanation for varying hatching times, I compared in the third experiment the volume of the eggs of *A. swirskii* coming from lines fed with either pollen or spider mites and fed themselves either pollen or spider mites. These egg size measurements revealed no significant differences in egg size or volume of the two treatments PP and SS. My hypothesis - that there is no difference in egg size or volume - could be accepted.

In summary, in my thesis I tried to find out why the eggs of *Amblyseius swirskii* sometimes hatch much faster when their mothers were fed with pollen. My experiments revealed that the combination of the rearing diet and the immediate diet of the mother, as well as the oviposition environment, where the eggs are placed, and the interaction of these determinants, are influencing the timing of oviposition after internal fertilization and egg retention by *Amblyseius swirskii*.

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Appendix

Table 2: Length, width and volume of the measured eggs in experiment 3.

type	number	length (µm)	width (µm)	volume (µm³)
SS	1	203.37	147.56	2318583.84
SS	2	209.62	167.99	3097406.46
SS	3	212.56	161.79	2913288.63
SS	4	229.75	181.24	3951502.39
SS	5	220.04	170.17	3336308.92
SS	6	218.09	168.56	3244467.44
SS	7	213.81	166.69	3110611.18
SS	8	212.75	153.09	2610729.26
SS	9	219.95	165.06	3137662.81
SS	10	204.7	151.69	2466211.78
PP	1	224.05	172.89	3506576.51
PP	2	222.24	160.6	3001317.45
PP	3	226.6	165.13	3235269.69
PP	4	206.09	155.56	2611267.78
PP	5	208.47	154.07	2591065.27
PP	6	220.4	159.48	2935098.41
PP	7	226.28	154.82	2839873.00
PP	8	210.92	156.11	2691397.42
PP	9	211.8	165.29	3029826.34
PP	10	201.73	156.77	2595942.25
mean SS				3018677.271
mean PP				2903763.413

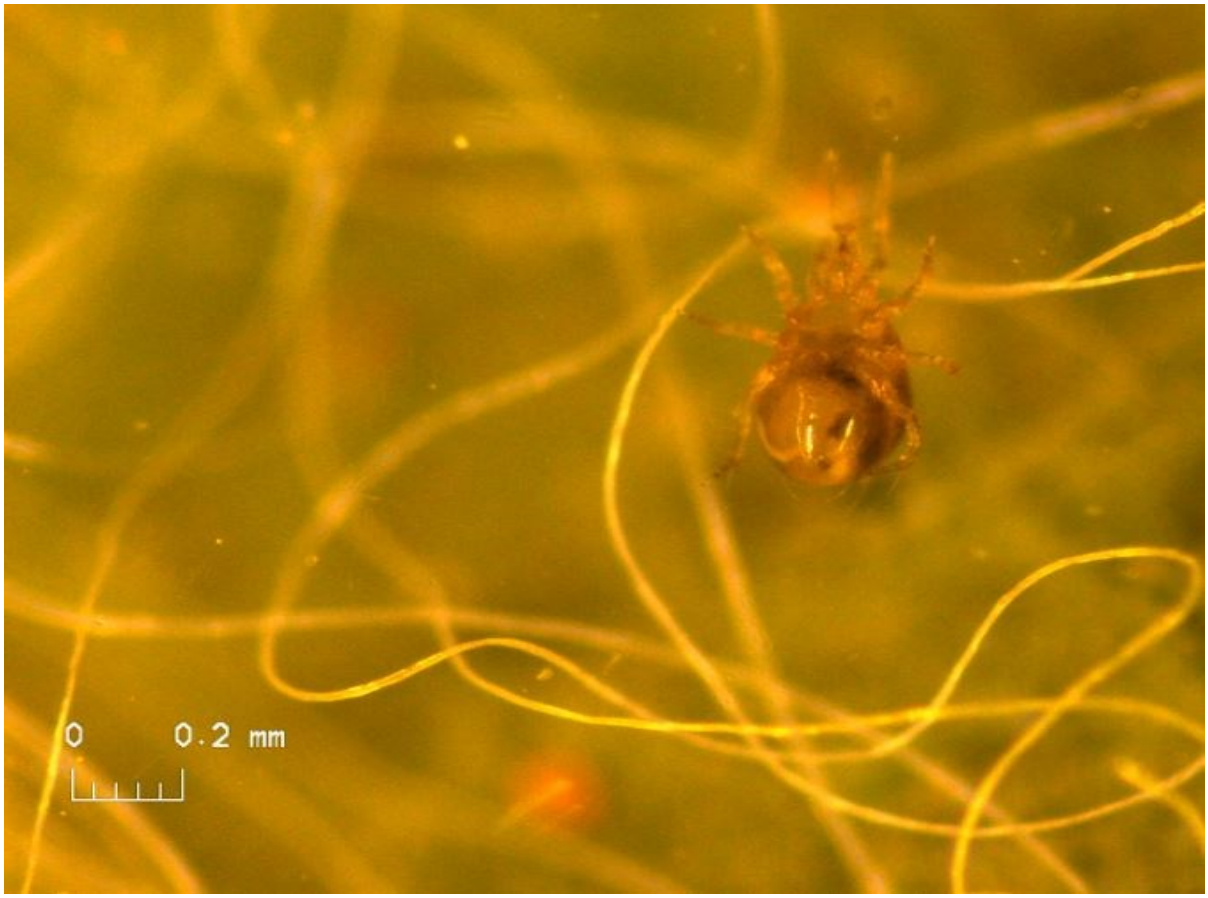

Figure 28: Gravid female of *Amblyseius swirskii*. The fertilized egg is visible inside the mite body. Females usually lay one to two eggs per day (© Kerstin Filzmoser).

Figure 29: Eggs of *Amblyseius swirskii* in the middle of the picture (ovate). Females often lay the eggs near to each other. The orange mites are *Amblyseius swirskii* individuals, the black ones are individuals of *Tetranychus urticae,* which was used as food for the predatory mites (© Kerstin Filzmoser).

Abstract

In my thesis, I investigated the egg laying behaviour of the predatory mite *Amblyseius swirskii* as affected by different diets and oviposition environments. My objective was finding out why some eggs hatch much faster than other ones, possibly in dependence of maternal diet. For my experiments I used mites of two different populations, the mass-reared Koppert population and the field-collected Israel population. The stock populations of the predators were reared over many generations either with two-spotted spider mites *Tetranychus urticae* (**S**) or with cattail pollen *Typha latifolia* (**P**). To investigate the influence of rearing diet and maternal diet/oviposition substrate on the egg developmental time of *Amblyseius swirskii*, I splitted the two rearing diet lines (**S** and **P**) again in two groups, one with spider mite diet (**S**) and the other one with pollen diet (**P**). This led to four treatments (**SS**, **SP**, **PS** and **PP**) with the first letter referring to the rearing diet, and the second letter referring to the diet provided to mothers during development and oviposition. In the first experiment I analyzed the egg development time outside the maternal body, i.e. from egg laying until larvae hatching. In the second experiment, I investigated the total egg developmental time inside the maternal body (time from fertilization to egg laying) and outside of the body (time from egg laying until larva hatching). Focus in both experiments was assessing the influence of diet and oviposition substrate on the egg developmental times. To exclude egg size as a possible influence on varying hatching times, I also measured the volume of the eggs of the treatments **SS** and **PP**. The first experiment revealed a significant lower hatching time of larvae in the **PP** treatment. In the second experiment the proportional developmental time of eggs of the **PP** treatment outside the maternal body differed from the other three treatments. Mites responded to the oviposition substrate and the diet when both the rearing diet was pollen and the mothers were fed with pollen. Proportionally, eggs were kept significantly longer inside the maternal body (egg retention) and shorter outside of the body than eggs produced by mothers in the other treatments. The total egg developmental time was longer in the PP treatment in experiment 2. There were no significant differences in egg size between the treatments **SS** and **PP**. Overall, my

thesis suggests that females of the predatory mite *A. swirskii* can adjust egg laying times and egg retention in response to ancestral and prevailing diets.

Zusammenfassung

In dieser Studie wurden das Ovipositionsverhalten sowie die Entwicklung der Eier der Raubmilbenart *Amblyseius swirskii* in Abhängigkeit verschiedener Nahrung und damit verbundener unterschiedlicher Eiablagesubstrate untersucht. Ziel war herauszufinden, warum manche Eier viel rascher schlüpfen als andere, in Abhängigkeit der Nahrung der Mutter. Zu diesem Zweck wurden Individuen aus zwei verschiedenen Populationen, der kommerziell gezüchteten Koppert Population sowie der in der Natur auf Zitrusbäumen gesammelten Israel Population, in den Experimenten verwendet. Die Tiere wurden über mehrere Generationen entweder mit Spinnmilben (**S**) der Art *Tetranychus urticae* oder mit Pollen (**P**) von Rohrkolben *Typha latifolia* gefüttert. Um den Einfluss unterschiedlicher Nahrung auf die Tiere zu überprüfen, wurden aus diesen Gruppen jeweils wieder zwei Untergruppen mit der Nahrung Spinnmilben **S** oder Pollen **P** gebildet. Insgesamt ergab dies vier experimentelle Gruppen (Abkürzungen **PP, PS, SP, SS).** Der erste Buchstabe der Gruppenbezeichnungen bezieht sich auf die Nahrung der Zuchttiere, der zweite auf die Nahrung, welche die Muttertiere während der Entwicklung und Eiablage bekommen haben. Im ersten Experiment wurde die Entwicklung außerhalb des mütterlichen Körpers, vom Zeitpunkt der Eiablage bis zum Schlüpfen der Larven, untersucht. Im zweiten Experiment wurde die gesamte Entwicklungszeit der Eier, vom Zeitpunkt der Befruchtung bis zum Schlüpfen der Larven, innerhalb und ausserhalb des mütterlichen Körpers, untersucht. Abschließend wurden die Eier aus den Gruppen **PP** und **SS** vermessen, um eventuelle Größenunterschiede der Eier, und in diesem Zusammenhang Einflüsse auf die Entwicklungszeit der Larven, zu eruieren. Im ersten Experiment konnte ein rascheres Schlüpfen der Larven aus den Eiern der **PP** Gruppen beobachtet werden. Im zweiten Experiment, auf die gesamte Eientwicklungszeit der Tiere bezogen, konnte eine eindeutige Verschiebung bezüglich der proportionalen Entwicklungszeit innerhalb des Körpers (Befruchtung bis zur Eiablage) zur proportionalen Entwicklungszeit außerhalb des Körpers (Eiablage bis zum Schlüpfen der Larve) in der **PP** Gruppe beobachtet werden. Die Eier der **PP** Gruppe verbrachten proportional eine signifikant längere Zeit innerhalb

des Körpers der Mutter bzw. eine kürzere Zeit außerhalb des Körpers als Eier der **PS**, **SS**, und **SP** Gruppen. Bei beiden Experimenten lag der Fokus auf dem Einfluss der Nahrung und des Eiablagesubstrates auf das Ovipositionsverhalten und die Eientwicklungszeit der Milben. Eine Messung der Eigröße der Gruppen **PP** und **SS** ergaben keine signifikanten Unterschiede. Insgesamt zeigt meine Arbeit, dass die Raubmilbe *Amblyseius swirskii* den Eiablagezeitpunkt je nach Nahrung bzw. Zustand des Ovipositionssubstrates anpassen kann. Diese Fähigkeit erlaubt es den Müttern, die befruchteten Eier bei ungünstigen Bedingungen länger geschützt im Körper zu behalten.