Should I stay or should I go?
The role of dispersal and cannibalism in exploitation strategies of a predatory mite
Revynthi, A.M.

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Should I stay or should I go?

The role of dispersal and cannibalism in exploitation strategies of a predatory mite

by Alexandra M. Revynthi

The defence will take place on Wednesday December 6th, 2017 at 10.00h in the Agnietenkapel of the Universiteit van Amsterdam, Oudezijds Voorburgwal 231, Amsterdam

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# Table of Contents

<table>
<thead>
<tr>
<th>Page</th>
<th>Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>06</td>
<td>Author addresses</td>
</tr>
<tr>
<td>09</td>
<td>1 General introduction</td>
</tr>
<tr>
<td>19</td>
<td>2 Prey exploitation and dispersal strategies vary among natural populations of a predatory mite</td>
</tr>
<tr>
<td>39</td>
<td>3 Timing of aerial dispersal is a heritable trait in the predatory mite <em>Phytoseiulus persimilis</em></td>
</tr>
<tr>
<td>51</td>
<td>4 Gender-specific differences in cannibalism between a laboratory strain and a field strain of a predator</td>
</tr>
<tr>
<td>61</td>
<td>5 To cannibalize or disperse? Males cannibalize and females disperse in the predatory mite <em>Phytoseiulus persimilis</em></td>
</tr>
<tr>
<td>73</td>
<td>6 General discussion</td>
</tr>
<tr>
<td>85</td>
<td>Summary</td>
</tr>
<tr>
<td>88</td>
<td>Samenvatting</td>
</tr>
<tr>
<td>91</td>
<td>Author contributions and project funding</td>
</tr>
<tr>
<td>92</td>
<td>Acknowledgements</td>
</tr>
<tr>
<td>95</td>
<td>Curriculum vitae</td>
</tr>
</tbody>
</table>
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In memory of Maus and my grandfather George
General introduction

Overexploitation and prudent predation
The harvesting of a food source at a rate which the source cannot sustain itself, also known as overexploitation, is a phenomenon that attracts major interest nowadays from the scientific community due to its impact on biodiversity. Overexploitation occurs at all trophic levels and is called overharvesting, overhunting or overfishing when we refer to plants, terrestrial animals and fish respectively. It can lead to extinction either of a population or even of an entire species.

According to the International Union for Conservation of Nature (IUCN) Red List (2017) 24,431 species are threatened: 8,160 vertebrates, 4,553 invertebrates, 11,674 plants and 34 fungi and protists. These reports are of great concern to many people including the scientific community as well as for regulatory government agencies worldwide, and have led to adopting measures to reduce the current biodiversity loss (Pimm et al. 1995; Jones et al. 2011; Rodrigues et al. 2014). People nowadays are more informed and sensitive towards biodiversity loss because of overexploitation. An increasing percentage of the world population is adopting more prudent nutritional habits, such as becoming vegetarian or even vegan (avoid consumption of any animal product), which could lead to a reduction in overexploitation rates.

Overexploitation, however, is not a phenomenon limited to human activity, it also occurs in other herbivores and predators. A plant or prey population can be driven to extinction because of overexploitation by herbivores or predators. Prudent predators can manage prey population in a way that maximizes their density (Slobodkin 1968, 1974) by decreasing their predation rate (Gilpin 1975). In well-mixed populations, however, their exploitation strategy is not evolutionarily stable because they can be outcompeted by selfish predators (Maynard Smith 1964; Hardin 1968). The tragedy of commons describes a situation in which a selfish individual (so-called cheater) gains benefit of a shared source to maximize its individual fitness, at the expense of the productivity of the group (Hardin 1968; Rankin et al. 2007). The cheater exploits the source at its own interest leading eventually to overexploitation.

The role of dispersal in a patchy world
Food occurs in patches and overexploitation often takes place in spatially structured environments. In local predator-prey populations, selfish predators drive their prey to extinction but will eventually go extinct as a consequence. But how do such species then persist in nature? Even though local predator-prey populations go extinct, persistence is observed at a metapopulation level because of the asynchronies of the dynamics in local populations and the founding of new populations by dispersing individuals (Vandermeer 1973; Hilborn
1975; Crowley 1981; Jansen and Sabelis 1992; Janssen et al. 1997). Such dynamics occur, for example, when both predator and prey disperse at a sufficient rate to balance local extinction with recolonization (Huffaker 1958; Taylor 1990; Janssen et al. 1997; Ellner et al. 2001). Individuals dispersing from their patches at different time points create asynchronous fluctuations in local abundance, a prerequisite for the persistence of metapopulations (Holyoak and Lawler 1996).

Dispersal is a key process in population biology and ecology, not only influencing the persistence, distribution and abundance of local populations but also driving gene flow among populations (Dunley and Croft 1990; Dingle 1996; Quinn et al. 2011). The adaptive value of dispersal is determined by the balance between its costs and benefits, which are highly dependent on the life stage and condition of an individual, as well as on the contemporary local and environmental conditions (Bowler and Benton 2005, 2009; Bonte et al. 2012; Bonte and Dahirel 2016).

From the perspective that dispersal is a life-history trait (Bonte and Dahirel 2016) and that the decision to disperse from a patch is not only context-dependent but also heritable, we can consider predator dispersal as a prey exploitation strategy (van Baalen and Sabelis, 1995; Sabelis et al. 2002). In a local predator-prey population, early predator dispersal decreases predation and, as a consequence, predator offspring staying behind on the patch will have more food available, resulting in a longer interaction period between the predators and their prey. However, a longer interaction period can lead to higher risks of secondary predator invasions. These invaders can then exploit the prey population to their own interest by consuming the prey that prudent predators saved for their offspring (van Baalen and Sabelis 1995). Hence, prudent predation is not evolutionarily stable in well-connected metapopulations because prudent predators are outcompeted by invading cheaters (cf. Maynard Smith 1964; Pels et al. 2002).

**The Milker-Killer dilemma**

The consequences of predator dispersal for the population dynamics of predators and prey were modelled by van Baalen and Sabelis (1995), who defined the so-called Milker-Killer dilemma. They proposed two extreme types of dispersal strategies: the Killer strategy, in which the predators start dispersing only after prey depletion, and the Milker strategy, in which the predators disperse even though prey is abundantly available. According to this study, strategies can range from more Killer-like to more Milker-like, but overexploitation will take place in all cases. Prudent predators (i.e., Milkers) will interact longer with the local prey population and will produce higher total numbers of dispersing offspring, but they will be exposed to invasions by selfish predators (i.e., Killers), whose populations will increase faster because they do not disperse early. Consequently, Milker-like strategies will be favoured only when local predator populations are sufficiently isolated from each other, thus reducing the risk of invasions by Killers (van Baalen and Sabelis 1995; Pels and Sabelis 1999; Pels et al. 2002).

The Killer and Milker exploitation strategies differ in dispersal rate in the presence of prey. Theoretically, the predator dispersal rate during the predator-prey interaction has three consequences for the population dynamics of prey and predator. First, prey populations
increase more when exploited by Milker predators than when exploited by Killer predators, because the predation rate by Killer populations is higher than that by Milker populations. Second, as a consequence, the local interaction period between a Milker population and its prey will be longer than that of a Killer population, because a prey population will increase more when attacked by Milkers than by Killers. Third, because of the increased growth of the prey populations and of the increased interaction time, local populations of Milker predators will produce more dispersing offspring over the entire local predator-prey interaction than Killer predators.

The formulation of the Milker-Killer dilemma was inspired by predator-prey interactions among plant-inhabiting mites (van Baalen and Sabelis 1995). The herbivorous mites occur in local populations, which can be locally driven to extinction by their predators (Janssen and Sabelis 1992). These local populations are connected by predator dispersal (Janssen et al. 1997; Ellner et al. 2001) and can only persist at a metapopulation level (Sabelis et al. 2005). In this context, it seems reasonable to use an acarine predator-prey system to test the Milker-Killer dilemma, provided that the predator is not a generalist or omnivore to ascertain a tight pairwise predator-prey interaction. Nevertheless, this theory can be applied to any system that is characterized by overexploitation, such as pathogen-host interactions.

Overexploitation, cannibalism and dispersal

When local populations are driven to extinction because of overexploitation in a spatially structured environment, an alternative way to obtain food is cannibalism. Cannibalism (i.e., intraspecific predation) is a common phenomenon in nature and occurs in a wide range of animal taxa, such as birds, mammals, fish, insects, spiders and predatory mites (review in Fox 1975; Polis 1981; Schausberger 2003). Animals prey on their conspecifics to obtain food, nutrients and remove source competitors in times of scarcity (Fox 1975). Cannibalism can act as a life boat mechanism and result in species persistence, when food is scarce (van den Bosch et al. 1988). Killing and consuming a conspecific, however, may result in injuries, pathogen transmission and loss of inclusive fitness if the victim is a relative (Pfennig 1997).

Even though cannibalism and dispersal are important phenomena for the persistence of populations and can be driven by overexploitation, they hardly ever have been studied in relation to each other. So far, few theoretical studies are available (Pels 2001; Lion and van Baalen 2007; Rudolf et al. 2010), and only one experimental study (Pels 2001) addressed dispersal in relation to cannibalism. It is important to study these two phenomena together because they affect each other (e.g., cannibalism can delay dispersal of the cannibals but accelerate dispersal of individuals that stand to be cannibalized) and consequently can affect the genetic structure of a population.

There are two complementary theoretical approaches regarding the evolution of cannibalism and dispersal. The first approach treats cannibalism as a fixed trait that influences selection on dispersal, which can determine the spatial structure of natural populations (Lion and van Baalen 2007; Rudolf et al. 2010). The second approach treats cannibalism as the trait under selection due to different dispersal strategies (Pels 2001). Cannibalism can select
for dispersal when offspring try to escape from their cannibalistic parents or family members in general, thus avoiding loss of inclusive fitness (Rudolf et al. 2010). Dispersal after food depletion can select for high cannibalistic tendency (Pels 2001). Experimentally, there is lack of evidence for any of the two theoretical predictions. Using a Milker-like and a Killer-like line of a predatory mite, Pels (2001) explored whether high or low dispersal rate would result in low and high cannibalistic tendencies, respectively. His results, however, did not resolve this issue, as he did not detect differences between the two lines in either their dispersal or cannibalistic behavior. In this thesis, I investigate predator behavior in a situation, where the predators can either cannibalize or disperse or both and try to answer how dispersal affects cannibalism and vice versa.

**A generalist herbivore and its predator**

The most suitable prey-predator acarine system that we can use to study the Milker-Killer dilemma experimentally consists of the generalist phytophagous two-spotted spider mite (*Tetranychus urticae* Koch; Figure 1.1) and its predator *Phytoseiulus persimilis* Athias-Henriot (Figure 1.2). One may wonder why we chose this system besides being well-studied (Sabelis 1981; Helle and Sabelis 1985a,b), but there are other reasons why this system can be used to answer questions regarding the evolution of alternative exploitation strategies:

1. The predator is restricted to spider mites as food source. This limits the factors that can influence its exploitation and dispersal behavior in relation to prey availability.
2. In nature, *T. urticae* and *P. persimilis* live in spatially structured environments. The predatory mites drive local prey populations to extinction, after which they have to disperse in search of a new food source (Janssen and Sabelis 1992). This phenomenon makes this system the most suitable to experimentally study the Milker-Killer dilemma.

**Figure 1.1** — Adult female (centre left) and a larva (bottom centre) of the spider mite *Tetranychus urticae*. Photo: Jan van Arkel.
The first experimental study on the Milker-Killer dilemma was conducted with this prey-predator system and thus I can relate my results to earlier studies. Last but not least, both mite species play an important role in agriculture. The two-spotted spider mite is a generalist pest of many economically important plant species (Jepson et al. 1975), and the predatory mite *P. persimilis* is used as its biological control agent. The two-spotted spider mite feeds on the contents of leaf mesophyll cells, causing a characteristic symptom, consisting of small chlorotic spots. In cases where the infestation is large, the plant loses its ability to photosynthesize because of the lack of chlorophyll and the web that is produced by the mites. Consequently, the plant suffers from severe dehydration, leading to defoliation.

**Figure 1.2** — A: An adult female and B: adult male of *Phytoseiulus persimilis*. In both pictures, some strands of web, produced by the prey, can be seen. Photos: Jan van Arkel.
**Tetranychus urticae** is an arrhenotokous haplodiploid species. Virgin females produce haploid males, whereas fertilized females produce diploid females and haploid males (Helle and Sabelis 1985a). Its life cycle consists of eight stages (with the three non-feeding molting stages given in brackets): the egg, larva (protochrysalis), protonymph (deutochrysalis), deutonymph (teleiochrysalis), and adult. Its development is rapid and at high temperatures (30-35 °C) its life cycle is completed in 8-12 days (Laing 1969).

Due to the continuous applications of acaricides and its short life cycle, this pest has developed resistance to most of the active ingredients of acaricides (Van Leeuwen et al. 2010). Hence, biocontrol is an efficient approach to control this mite. The predatory mite *P. persimilis* is used as a biological control agent against the two-spotted spider mites (Sabelis 1981). It was introduced into Germany from Chile in 1958 (Dosse 1958) and was proven very efficient in controlling two-spotted spider mites on many plant species, especially on greenhouse-grown vegetables, fruits and ornamentals (Huffaker et al. 1970; Sabelis 1981; Helle and Sabelis 1985b).

This predator is a pseudo-arrhenotokous haplodiploid species. Females require insemination in order to start oviposition and both females and males start as fertilized eggs (Huffaker et al. 1970; Schulten 1985). However, the males lose the paternal set of chromosomes shortly after syngamy (Helle et al. 1978; Sabelis and Nagelkerke 1988), so only females are diploid. Its life cycle has five stages: egg, larva, protonymph, deutonymph and adult, and lacks the three quiescent stages that *T. urticae* has (Laing 1968). The egg and the larva are non-feeding stages (Laing 1968; Sabelis 1981). Adult female predators can feed on all stages of *T. urticae* (Takafuji and Chant 1976). The life cycle of this predatory mite at 30 °C can last only 5 days (Sabelis 1981). Laing (1968) showed that when *P. persimilis* is offered *T. urticae* as prey, it can develop from egg to adult in 7.45 days under a diurnal temperature cycle of 14.4 to 28.3 °C. This is approximately half the time required for the development of the two-spotted spider mites under similar conditions (Laing 1969). This is one of the reasons for this predatory mite being so efficient at controlling *T. urticae*.

**THESIS OUTLINE**

The aim of this thesis is to experimentally test the occurrence of Milker-like and Killer-like strategies in *P. persimilis* and study the evolution of these alternative exploitation strategies. Predator dispersal influences predator-prey dynamics in local patches and is likely to determine persistence of the system at a metapopulation level. Decisions of predators to disperse typically depend on local conditions such as local density of conspecifics in the same patch (Otronen and Hanski 1983), food availability (Kuussaari et al. 1996), kin recognition and kin interaction (Hamilton and May 1977) and cannibalism (Pels 2001). In this thesis, I investigate how predator-prey interactions and interactions among conspecific predators can affect predator dispersal behavior.

In **CHAPTER 2**, I present a comprehensive survey of predator dispersal and exploitation strategies using strains of *P. persimilis* that I collected in Turkey and Sicily. My aim was to investigate whether there were differences in dispersal behavior among the collected strains and whether these differences would result in the effects on population dynamics predicted by the Milker-Killer theory (van Baalen and Sabelis 1995). I found significant variation in
the dispersal strategies among predator populations, and I therefore tested whether there was a genetic component for timing of aerial dispersal in the presence of prey, as was suggested earlier (Pels and Sabelis 1999). In Chapter 3, I therefore ask whether it is possible to select for Milker-like and Killer-like predatory mite lines. I aimed to explore whether this artificial selection resulted in differences between the two selected lines in the dispersal rate, the interaction period and the number of dispersers that were produced.

During daily observations in the experiments of both Chapters 2 and 3, I noticed that many predators disappeared. Specifically, I often observed adult males cannibalizing larvae and this behavior had not been described before. Although it is known that cannibalism can affect dispersal behavior of individuals, there is a lack of experimental work linking these two phenomena. All these reasons led me to Chapters 4 and 5, where I focused on the cannibalistic behavior of P. persimilis and how this behavior could affect its dispersal tendency. In Chapter 4, I ask to what extent prolonged culture of predators affects the cannibalistic behavior of adult females and males. I tested two contradictory hypotheses about adult cannibalism on juvenile. The first hypothesis predicts that strains that have been in culture for a long time have a higher tendency to cannibalize than strains in the field, because laboratory strains are unintentionally selected for higher tendency to cannibalize due to space limitation. This prediction will hold especially for females because they are the first to disperse from the patch when food is scarce. In the second hypothesis I took the increase in kin-relatedness among individuals when they are cultured for long time into account. I hypothesized that strains that have been in culture for long time have lower cannibalistic rates than strains that derived recently from the field. However, the experiments of Chapter 4 showed me only the potential occurrence of cannibalism, as I conducted my trails using a closed system, where the predators did not have the option to disperse. Thus, in Chapter 5 I aimed to investigate the interaction of cannibalism with dispersal using the selected lines of Chapter 3, which employ different dispersal strategies. In this chapter I ask (1) when food is limited and given the option to disperse, will predators choose to cannibalize or not and to disperse or not? And (2) if the predators cannibalize when they have the option to disperse, which line and gender of P. persimilis is more voracious towards its conspecifics?

The chapters of this thesis raise many questions about the evolution of prudent predation and cannibalism, as well as the applied aspect of the Milker-Killer dilemma to optimize biological control of spider mites. In the general discussion (Chapter 6) I address the following questions:

1. Eventually, which is the best strategy for a predator, to milk or to kill?
2. Despite the theoretical predictions about the evolution of prudent exploitation strategy, can we still find Milkers in nature?
3. Can we assume cannibalism as a form of prudent predation?
4. When we study cannibalism and dispersal together, which is the trait under selection? Does cannibalism drive the evolution of dispersal or does dispersal drive the evolution of cannibalism?

Despite the fact that P. persimilis is an efficient biocontrol agent of T. urticae, the growers face the problem of having to release it repeatedly: because of its high dependence on the
prey, *P. persimilis* goes extinct after the prey are overexploited. Therefore I also address the following questions:

(5) Which exploitation and dispersal strategy is the best for efficient biological control, the Milker or the Killer? Is it better to release predators that allow the prey population to develop, but stay longer on the crop, i.e., Milkers, or is it better to use predators that exterminate the prey fast, but persist only for a short period on the plant, i.e., Killers?

(6) How can cannibalism affect the ability of *P. persimilis* to control spider mite infestations?

**References**


AlexandraRevynthi-ch1_Vera-ch1.qxd  20/10/2017  14:48  Page 17

General introduction


CHAPTER 1

2

Prey exploitation and dispersal strategies vary among natural populations of a predatory mite

A.M. Revynthi, M. Egas, A. Janssen & M.W. Sabelis

ABSTRACT — When predators commonly overexploit local prey populations, dispersal drives the dynamics in local patches, which together form a metapopulation. Two extremes in a continuum of dispersal strategies are distinguished: the ‘Killer’ strategy, where predators only start dispersing when all prey are eliminated and the ‘Milker’ strategy, in which predator dispersal occurs irrespective of prey availability. Theory shows that Milker strategies are not evolutionarily stable if local populations are well connected by dispersal. Using the predatory mite Phytoseiulus persimilis, we aim to investigate whether these two strategies occur in nature. We present a comprehensive survey of predator dispersal strategies using strains of P. persimilis, collected from 11 native populations from coastal areas in Turkey and Sicily. Using small wind tunnels, we measured dispersal rates and population dynamics of all populations in a system consisting of detached rose leaves, spider mites (Tetranychus urticae) as prey and P. persimilis. We found significant variation in the exploitation and dispersal strategies among predator populations. However, none of the collected strains showed the extreme Killer or Milker strategy. The results suggest that there is genetic variation for prey exploitation and dispersal strategies. Thus, different dispersal strategies in the Killer-Milker continuum may be selected for under natural conditions, which affects the predator-prey dynamics in local patches and is likely to determine persistence of the system at the metapopulation level.
INTRODUCTION

Dispersal is a key process in population biology and ecology, influencing the persistence, distribution and abundance of populations as well as driving gene flow (Dunley and Croft 1990; Dingle 1996; Quinn et al. 2011). Decisions of individuals to disperse typically depend on local conditions such as the local density of conspecifics in the same patch (Otronen and Hanski 1983), food availability (Kuussaari et al. 1996), interspecific interactions (Weisser et al. 2001), sex ratio (Lawrence 1987, 1988; Colwell and Naeem 1999), kin recognition and kin interaction (Hamilton and May 1977), inbreeding avoidance (Greenwood 1980; Pusey and Wolf 1996), cannibalism (Pels 2001), individual personality (Quinn et al. 2011), temporal and spatial heterogeneity (Holt and Barfield 2001; Wiens 2001) and patch isolation (Conradt et al. 2001).

Dispersal affects various levels of biological organization, from an individual’s fitness to population dynamics and community composition (Bowler and Benton 2005). Dispersal is particularly important when local populations are driven to extinction because of overexploitation, whereas persistence is observed at a metapopulation level due to continuous foundation of new local populations by dispersing individuals. Such dynamics occur, for example, when both predator and prey must disperse at a sufficient rate to balance local extinction with recolonization (Huffaker 1958; Taylor 1990; Janssen et al. 1997; Ellner et al. 2001). When individuals disperse from their patches at different time points, this creates asynchronous fluctuations in local abundance, which are a prerequisite for the persistence of metapopulations (Holyoak and Lawler 1996).

Whereas the role of dispersal in metapopulation persistence of systems characterized by local overexploitation received much attention, the effects of dispersal on local population dynamics are less well understood (Bowler and Benton 2009). The consequences of predator dispersal for the population dynamics of predators and prey were modelled by van Baalen and Sabelis (1995), who defined the so-called Milker-Killer dilemma, describing under which conditions these extremes of a continuum of dispersal strategies can evolve. Predators with the Killer strategy disperse only when the prey are eliminated. Under the Milker strategy, predators will disperse irrespective of prey density, thereby decreasing predation and allowing the prey population to produce more offspring. As a result, the predators’ offspring will have more food, resulting in a longer interaction period of predators and prey on the patch. In contrast to the Killer strategy, the Milker strategy is a less selfish strategy (van Baalen and Sabelis 1995), in which the predators show a more prudent exploitative behavior. The Milker strategy is not evolutionarily stable because a local population of Milkers can be invaded by Killers, which have a reproductive benefit because they exploit the prey left behind by dispersing Milkers. When there is a low probability of invasion of Milker patches by Killers, the evolution of Milkers may be favored at the metapopulation level because the total number of offspring produced by a local population of Milkers is higher than that of Killers (van Baalen and Sabelis 1995; Pels 2001).

The Killer and Milker exploitation strategies differ in dispersal rate in presence of prey. Individuals of a Killer-type predator population do not disperse until the prey population is being exterminated, whereas Milker-individuals disperse early, long before prey elimination. Depending on the dispersal rate during the predator-prey interaction, three conse-
sequences on the population dynamics of prey and predator are predicted. First, prey populations increase less when exploited by Killer predators than when exploited by Milker predators. Second, the local interaction period between a Killer predator population and its prey will be shorter than that of a Milker population. Third, local populations of Killer predators will produce less dispersing offspring over the entire local predator-prey interaction than Milker predators.

The formulation of the Milker-Killer dilemma was inspired by a study of plant-inhabiting mites, specifically the predatory mite *Phytoseiulus persimilis* Athias-Henriot and its prey, the phytophagous spider mite *Tetranychus urticae* Koch. This spider mite species occurs in local populations, which can be locally driven to extinction by their predators (Janssen and Sabelis 1992). These local populations are connected by predator dispersal (Janssen et al. 1997; Ellner et al. 2001). An experimental study investigated whether both dispersal strategies occur among predator strains of *P. persimilis* (Pels and Sabelis 1999), originally sampled from wild populations along the coast and inland on Sicily (Italy). Pels and Sabelis (1999) showed that all predator strains exterminated local prey populations, and the timing of dispersal appeared to have a genetic basis: one isofemale line derived from a coastal strain consistently showed dispersal close to or after prey elimination, whereas an isofemale line derived from an inland strain consistently dispersed long before all prey were eliminated. These behaviors were in line with Killer- and Milker-like strategies, respectively.

In their study, Pels and Sabelis (1999) did not replicate measurements of dispersal behavior of the strains. From their first measurements they chose two strains that showed the most extreme differences in dispersal behavior and created one isofemale line from each, which they used for further experiments. Thus, dispersal strategies were characterized only for two isofemale lines and hence, a thorough survey of predator dispersal behavior among natural populations is lacking. Also, quantifying the extent of variation in dispersal strategies from natural populations allows testing the predictions on the population dynamics of Killers and Milkers with their prey. We therefore returned to the Mediterranean area to collect natural populations of *P. persimilis* and measured their dispersal characteristics using local populations in a laboratory set-up, similar but more accurate than that of Pels and Sabelis (1999). We aim to quantify the extent of variation in dispersal strategies by estimating dispersal rates in the presence of prey, and to test the predicted consequences of dispersal for the population dynamics of predators and prey.

**MATERIALS AND METHODS**

*Collection of predatory mites*

*Phytoseiulus persimilis* was collected from fields in Turkey in 2013 and in Sicily in 2014. These locations were chosen because natural populations of this predator occur there and it was not introduced as a biological control agent. Upon spotting the predators in spider-mite colonies, infested leaves with prey and predators were transferred inside plastic bottles that were closed and had an air inlet covered with mite proof gauze (80 μm).

In Turkey, samples were collected from six sites in the region of Hatay (36°04.950′N, 35°56.728′E) and Erdemli (36°36.267′N, 34°15.926′E) (Figure 2.1). In Hatay, predators were collected from Samandağ, Koyunoğlu, Kuşalanı, Karaçay and Uzunbağ; in Erdemli from
Kocahasanlı. Predatory mites were found on cucumber (*Cucumis sativus*), bean (*Phaseolus vulgaris*) and eggplant (*Solanum melongena*). In Erdemli, predators were also collected from the weed *Tribulus terrestris*. All host plants were infested with spider mites (*T. urticae*).

Predators were also collected from the western part of Sicily (38°02.573’N, 12°59.747’E) at five sites: Castelvetrano, Alcamo, Palermo, Trabia and Lascari (Figure 2.1). Natural populations of *P. persimilis* occurred on spider-mite infested melon (*Curcubita pepo*) and castor bean plants (*Ricinus communis*) and on spider-mite infested weeds of *Convolvulus* sp.

All sampling sites, in both locations, where the mites were collected, were along the coast. We visited inland sites (approx. 50 km away from the coast), but we did not manage to find and collect predators.

**Laboratory cultures**

Rose plants (*Rosa* sp. var. Avalanche) were provided by Olij Rozen and were allowed to grow in a climate room (25 °C, 70% RH, 16L:8D) free of herbivores. Lima bean plants (*Phaseolus lunatus* L.) were grown from seeds in a climate room (25 °C, 60% RH, 16L:8D) free of herbivores.

The *T. urticae* strain used to feed the predators was originally collected from cucumber plants in a commercial greenhouse in May 1994 (Pallini et al. 1997), and was reared on Lima beans (*P. lunatus*) in a climate room (26 °C, 60% RH, 16L:8D).

The predatory mite strains were reared on spider-mite infested Lima bean leaves in a walk-in climate room at 25 °C, 70% RH and 16L:8D. We used the same closed rearing system as Pels and Sabelis (1999), consisting of a plastic float inside a plastic tray, which was...
filled with a 15-mm layer of water with dissolved soap. Three times per week, two bean leaflets infested with spider mites were placed on the float, which provided the predators with sufficient food. In order to allow the mites to disperse ambulatorily without drowning, the plastic float was covered with a plastic aquarium (19.5 × 13.0 × 11.5 cm) with a piece of fine-meshed gauze hanging from the ceiling, touching the float/leaflets. For ventilation, a rectangular hole was made in the ceiling of the aquarium, covered with fine meshed gauze (80 μm).

**Sequencing of COI and ITS genes**

To identify the collected strains to the species level, the mitochondrial cytochrome oxidase I (COI) gene and the internal transcribed spacer (ITS) gene were sequenced. DNA was extracted from single adult females of *P. persimilis* with the Chelex maceration method (Walsh et al. 1991). Five mites per strain were used for the DNA extraction and were introduced individually in 0.5 ml tubes containing 100 μl of 5% Chelex 100 (Biorad Chelex 100, Richmond, CA, USA). The samples were incubated at 56 °C with 5 μl of proteinase-K for 60 min and were then heated for 10 min at 95 °C. They were then stored in the freezer at -20 °C.

The mitochondrial COI region was amplified using the 5’GGTCAACAAATCATAAGATATTGG3’ (forward) and 5’TAAACTTCAGGGTGACCAAAAAATCA3’ (reverse) primers (Jørgensen et al. 2007). The primers that were used for amplifying the nuclear ITS region were 5’AGAGGAAGTAAAAGTCGTAACAAG3’ (forward) and 5’ATATGCTTAAATTCCAGGG3’ (reverse) (Navajas et al. 1999). For the PCR, we used 25 μl reaction volumes containing 13.3 μl water, 2.5 μl of 10 × Buffer (HT BioTechnology, Cambridge, UK), 0.5 μl Super Taq polymerase (5 U/μl), 2.5 μl dNTP’s, 1.2 μl BSA, 0.5 μl of each primer and 4 μl of DNA sample. For COI, samples were preheated at 94 °C for 2 min, 35 cycles of denaturation at 94 °C for 10 s, annealing at 48 °C for 30 s and amplification at 72 °C for 55 s, and a final extension step at 72 °C for 10 min (Jørgensen et al. 2007). For ITS, samples were denatured at 94 °C for 4 min and then PCR was carried out for 30 cycles of 1 min denaturation at 93 °C, 1 min annealing at 50 °C and 1 min extension at 72 °C (Navajas et al. 1999). The PCR products were visualized with UV light using a 1.5% agarose gel stained with ethidium bromide. Direct sequencing of PCR amplifications were done by Macrogen EZ-seq service, using the same primers as for the PCR. The sequences were read and compared using the CLC Genomics workbench 3 (Qiagen, CLC Bio).

**Dispersal experiments**

To measure dispersal, we used wind tunnels similar to those of Pels and Sabelis (1999). They consisted of a plastic aquarium (25.3 × 15.8 × 15.5 cm) with two holes on the short sides (11.5 cm diameter each) covered with mite-proof gauze (80 μm). The aquarium was closed with a glass lid and sealed with Parafilm. A fan was placed close to one hole, causing an air flow of 0.4 m/s inside the aquarium (Figure 2.2).

Initially, Lima bean (*P. lunatus*) was used as a host. However, the leaves wilted within 24 h after being cut. We therefore used rose leaves, which could be preserved for a long period without suffering from water stress. Rose leaves with a shoot of ca. 5 cm were cut and the shoots were inserted in a small vial (24.5 mm diameter, 40 mm height) filled with wet floral foam (Oasis) to maintain leaf turgidity. A thick layer of lanolin was applied to the base of the petiole to prevent mites from escaping. Fifteen 2-day-old adult female spider mites
were introduced on the leaf and were allowed to feed and oviposit for two days. Missing females were replaced daily during these two days. After 48 h, one 2-day-old mated adult female predatory mite was introduced on the leaf, which was then placed with the vial at the upwind side in the wind tunnel. Predatory mites that dispersed aerially from the leaf using the air flow through the wind tunnel landed somewhere inside the wind tunnel. In order to capture them, we introduced a Petri dish with a young, spider-mite infested rose leaf as a trap, located on the downwind side on the bottom of the wind tunnel. The infested rose leaf also had a ca. 3 cm shoot, which was inserted through a hole in the lid of an Eppendorf tube (1.5 ml) filled with wet floral foam, providing it with the necessary moisture. The tube was sealed with Parafilm (Figure 2.2). After introduction of the predator, the numbers of adult prey and adult predators on the leaf, on the trap, on the cover and elsewhere in the wind tunnel were assessed every 24 h. The experiment ended when there were no prey and predators left on the leaf. The experiment was repeated 5× for each strain in a climate room at 25 °C, 70% RH and 16L:8D.

Per country of origin, the strains were tested simultaneously, using six wind tunnels in one climate room. A line derived from mites of Koppert Biological Systems (Berkel en Roderijs, the Netherlands) was tested together with the Sicilian strains. This line has been maintained in our laboratory for many years and was used to get an indication of the dispersal behavior of this laboratory population.

To characterize a strain in the continuum from Milker to Killer, we analyzed three parameters: the dispersal rate of the predator during the interaction period, the interaction period of the predators and prey and the cumulative number of dispersing predators. To calculate the predator dispersal rate during the predator-prey interaction, we first calculated the proportion of dispersers of the total number of predators in the wind tunnel for each day of the interaction, and then averaged these daily proportions. Because all predators will disperse towards the end of the interaction period due to lack of prey, irrespective of their exploitation strategy, we excluded the last part of the interaction period, limiting it from the day of predator introduction until the last day, with at least three adult prey individuals on the leaf. The interaction period was taken as the time interval between predator introduction and prey elimination, i.e., the day when there were no more prey on the leaf. The cumulative number of dispersing predators was taken as the total number of predators that dispersed from the leaf during the entire interaction period and until the end of the experiment.

**Figure 2.2** — Wind tunnel, consisting of a plastic aquarium with two holes covered with mite-proof gauze and a fan connected to one of them. a: Rose leaf, where the prey and predator were released, and b: trap with spider-mite infested rose leaf. Arrows indicate the direction of the air current.
Specifically, we aimed at finding significant differences among strains with respect to the three parameters, with Milker-like strains showing a higher dispersal rate during the predator-prey interaction, having a longer interaction period with the prey and producing a higher cumulative number of dispersers than Killer-like strains.

All statistical analyses were performed with R v.3.0.1 (R Development Core Team 2012). A MANOVA was applied to the dispersal rate (arcsine transformation), the interaction period and the cumulative number of dispersing predators combined, with strain and time in culture as explanatory variables. Time in culture is the number of weeks since the start of the predator culture, and was included to verify whether culturing the predators in the laboratory resulted in changes in their exploitation strategy (see TABLE S2.1). After having found a significant difference among strains with the MANOVA, each parameter was tested separately.

Differences in the arcsine-transformed dispersal rate among strains were tested with a GLM with a Gaussian error distribution. For differences in the interaction period a GLM with Gaussian error distribution was used and differences in the cumulative number of dispersing predators among strains were assessed with a GLM with quasi-Poisson error distribution. Contrasts were made by joining the non-significant factor levels (Crawley 2007).

After the first replicate with the Turkish strains, we started keeping a detailed record of the number of predators present on the leaf through time. With these numbers and the number of dispersed individuals, we estimated the population growth rate $\gamma$ of each strain:

$$\gamma = \left[ \ln \left( \frac{P_t}{P_0} \right) / t \right] + \mu,$$

where $P_t$ is the predator population size at time $t$, $P_0$ is the initial predator density, $\mu$ is the dispersal rate (estimated as described above), $t$ is the time from the day when the predator was introduced on the leaf until the last day with at least three adult prey individuals on the leaf (Diekmann et al. 1988; van Baalen and Sabelis 1995). Differences in population growth rates among strains were tested with a GLM with a Gaussian error distribution.

**RESULTS**

**Sequencing of the COI and ITS genes**

The sequences of both COI and ITS genes showed that the mites belonged to the species *P. persimilis*. All strains, including the Koppert line, had a COI sequence identical to the KF966638 entry in GenBank and an ITS sequence identical to the HQ404818 entry (Tsolakis et al. 2012).

**Dispersal experiments**

The combination of dispersal rate during the interaction period, interaction period and cumulative number of dispersing predators varied significantly among strains as well as with the time in culture and their interaction (MANOVA, Strain: $F = 1.82$, d.f. = 90, $P = 0.01$, Time in culture: $F = 8.07$, d.f. = 28, $P < 0.001$, Strain $\times$ Time in culture: $F = 1.70$, d.f. = 90, $P = 0.03$). Dispersal rates varied significantly among strains (GLM: deviance = 0.005, d.f. = 11, $P = 0.03$), with time in culture (GLM: deviance = 0.001, d.f. = 1, $P = 0.03$), as well as with their interaction (GLM: deviance = 0.009, d.f. = 11, $P = 0.001$). The strains Palermo, Alcamo, Koppert, Uzunbağ, Trabia, Castelvetrano and Lascari, had significantly
FIGURE 2.3 — Parameters of the predator-prey (Phytoseiulus persimilis – Tetranychus urticae) population dynamics experiments used to characterize all strains in the Milker-Killer continuum. 

a: Dispersal rate during the interaction period (i.e., the time interval between predator introduction until a maximum of three adult prey were present). 
b: Interaction period (i.e., the time interval between predator introduction and prey elimination). 
c: The cumulative number of dispersing predators (i.e., the total amount of dispersers during the experiment). Boxes indicate the second and the third quartile, horizontal lines separating the boxes indicate the medians (N = 5), whiskers above and below the box indicate the 90th and 10th percentiles. Different letters indicate significant differences (contrasts after GLM, P<0.05).
lower dispersal rates than the strains Kocahasanlı, Samandağ, Koyunoğlu, Kuşalanı and Karaçay (deviance = 0.01, d.f. = 2, P<0.001; Figure 2.3a).

There was variation in the dynamics of the prey and predators among replicates and strains (Figures 2.4, S2.1 and S2.2). In all cases, predators initiated dispersal while there were still prey present on the leaf, yet the interaction period, i.e., the time between predator introduction and prey elimination, was not significantly different among strains (GLM: deviance = 51.73, d.f. = 11, P = 0.16; Figure 2.3b). The interaction period increased significantly with time in culture (GLM: deviance = 33.38, d.f. = 1, P = 0.002).

Differences in dispersal rates did not result in significant differences in the cumulative number of dispersing predators among strains (GLM: deviance = 195.0, d.f. = 11, P = 0.52; Figure 2.3c). Of all collected strains, the one from Alcamo produced the highest number of dispersers and that of Trabia the lowest (70.2 ± 16.8 and 25.8 ± 6.2 individuals on average, respectively).

**Figure 2.4** — Predator-prey (*Phytoseiulus persimilis* – *Tetranychus urticae*) population dynamics in the wind tunnel experiments. Shown are the adult prey on the leaf (dashed lines, left-hand axis) and cumulative number of dispersing predators (solid lines, right-hand axis). The presented strains demonstrate the variation in prey exploitation and cumulative number of dispersers in every replicate. a: Samandağ, b: Koyunoğlu, c: Kuşalanı, d: Uzunbağ, e: Trabia, f: Alcamo. Blue: replicate 1, green: replicate 2, red: replicate 3, orange: replicate 4 and purple: replicate 5. For the population dynamics of all strains see Figures S2.1 and S2.2.
According to the Milker-Killer concept, the interaction period should be positively related to the dispersal rate during the interaction period and the cumulative number of dispersers. We found little evidence for this (Figure 2.5). Even though there was no significant variation regarding the interaction period among strains, Alcamo, Koppert and Palermo strains showed a tendency towards the Killer strategy, whereas Kuşalanı and Karaçay showed a tendency towards the Milker strategy.

The population growth rates did not differ significantly among the strains (GLM: deviance = 0.02, d.f. = 11, P = 0.96) (see Table S2.2). On average, the growth rate was 0.39 for the Turkish strains, 0.38 for the Sicilian strains and 0.42 for the Koppert strain.

![Figure 2.5](image_url)

**Figure 2.5** — The relation between interaction period, dispersal rate during the interaction period, and cumulative number of dispersers. The size of each dot represents the average cumulative number of dispersing predators. a: Theoretical prediction based on the model proposed by van Baalen and Sabelis (1995) and b: results from the tested *Phytoseiulus persimilis* strains; different colors represent different strains. Strains with more Killer-like dispersal strategy have a low dispersal rate, a short interaction period and low cumulative numbers of dispersing predators, hence, they are expected to appear on the lower left side of the graph and have a smaller dot. Strains with a more Milker-like dispersal strategy are expected in the top right side of the graph and with a larger dot.
DISCUSSION

Earlier work showed first evidence for the existence of variation in exploitation strategies of the predatory mite *P. persimilis*, one isofemale line resembling the Killer strategy in which predators start dispersing at prey depletion, the other isofemale line resembling the Milker strategy in which predators start dispersing before prey elimination, leaving food for their offspring (Pels and Sabelis 1999). Here, we aimed to investigate whether the Killer and the Milker strategies occur in nature using strains derived from native populations. We used dispersal rate during the predator-prey interaction as a criterion to determine which exploitation strategy the predators employ. Our results showed significant variation in dispersal rate across the strains (FIGURE 2.3a). The strains Palermo, Alcamo, Koppert, Uzunbağ, Trabia, Castelvetrano and Lascari had lower dispersal rates, and therefore we conclude that they employ a more Killer-like dispersal strategy, than the strains of Kocahasanlı, Samandağ, Koyunoğlu, Kuşalanı and Karaçay that employ a more Milker-like dispersal strategy. Given the differences in the dispersal rates, we expected to observe the predicted consequences for the predator-prey dynamics and the cumulative number of dispersers; however, this was not the case. Higher dispersal rates did not result in an increase in prey population, a prolonged interaction period and a higher production of dispersers, thus we conclude that none of the strains we collected showed an extreme Killer or Milker strategy. Our results show variation in exploitation strategies ranging from a more Killer-like to a more Milker-like strategy.

Given that the variation in dispersal rates did not result in the expected consequences on the population dynamics of the prey and predator, other characteristics, such as population growth rate might also vary with strain (cf. dispersal syndromes; Stevens et al. 2013, 2014). Predator populations with high growth rate develop faster and exploit the prey patch more rapidly than populations with lower growth rate. As a result the interaction period is shorter and the cumulative number of dispersers produced over the interaction time is smaller. Therefore, we also estimated population growth rates from the data. The Turkish strains seemed to exhibit a slightly higher growth rate than the Sicilian strains (see TABLE S2.2), however, the variation in the population growth rate among the strains was not significant. This implies that the lack of population dynamical response to variation in dispersal rate is not due to variation in population growth rate.

The population dynamics may of course also vary with prey characteristics such as prey dispersal and anti-predation strategies. If the prey mites respond flexibly to predation pressure, the predator exploitation strategy might not have much net effect on the interaction period and the cumulative number of predator dispersers. In predator-prey interactions, it is to be expected that prey disperse as well, either to avoid predation or to find a better host plant. Milker-Killer-like strategies may occur in spider mites exploiting their host plant as well; spider mites also overexploit their food source, and Killer-like predator exploitation selects for higher prey dispersal (Sabelis et al. 2002). Even though dispersing prey were never observed in the wind tunnels, we cannot exclude the possibility that more prey dispersed from the arena to the trap in replicates with Killer-like predator strains, and the setup of the wind tunnels did not allow for observations on anti-predator behavior. If the interaction period is also dependent on such condition-dependent prey behavior, it is still an open question what would be the best exploitation strategy for the predators.
We tried to maintain the natural variation in dispersal behavior of the predators in the laboratory by using closed rearing units, which allowed the predators to leave the prey patch, but to which they could subsequently return. The mites were collected from the field and subsequent adaptation to the rearing conditions might have affected their dispersal behavior. Indeed, we found that the longer the predators had been kept in the laboratory, the longer became the interaction period and the lower their dispersal tendency. Mites collected from the field tend to be more active (personal observation), hence this is another reason why time in culture was chosen as an explanatory variable (see Table S2.1). Furthermore, animals are known for changing their dispersal behavior and increase their dispersal rates in response to translocation (Pettit et al. 2016).

Genetic variation among predators within each strain may also contribute to variation among replicates. In contrast to Pels and Sabelis (1999), we did not conduct the experiments with isofemale lines but tested one family in each replicate (each experiment was started with one adult female predator). The experiments of Pels and Sabelis (1999) show much less variation in interaction time among replicates than the experiments reported here, suggesting that there was genetic variation for prey exploitation within the strains studied here. The variation found among strains in this study suggests that there is a genetic component for dispersal tendency in the presence of prey, but the question remains to what extent this variation is heritable. To test this, selection should be performed for Milker and Killer lines of predators.

In the current study, we did not observe the population dynamical consequences predicted by the model of van Baalen and Sabelis (1995). We suggest that the discrepancy is due to the model assumption of a fixed dispersal probability during the interaction period; dispersal likely depends on the condition of the individual (Kisdi et al. 2012) and its environment (Clobert et al. 2009). It remains to be investigated how these factors affect dispersal behavior. In our experiment, dispersal was probably affected by predator density and food availability. All predators from all strains and in all replicates started dispersing close to, but still before prey elimination. The predators that initiated dispersal were all adults, mostly females. Females need much more food to reproduce than nymphs need to become adult (Sabelis 1981). Hence, when there is no longer enough food for adult females to reproduce, it might be more profitable for them to disperse. The small amount of food left behind is probably enough for many nymphs to become adult and disperse later. Hence, there may be a relation between the stage structure of the population and the dispersal behavior.

The Milker-Killer dilemma is broadly applicable to predator-prey systems that are characterized by local overexploitation and metapopulation dynamics, typical for many arthropod predator-prey systems (Hassell 1978). Analogies may also be found in parasitoid-host systems. We can hypothesize that parasitoids that do not kill their host immediately, i.e., koinobiont parasitoids, may have alternative exploitation strategies. By adopting a prudent exploitation strategy, the parasitoid larvae developing in the host allow the host to increase in body mass. This exploitation strategy can be evolutionary stable only when the host is parasitized by one adult. Superparasitism of the same host will select for more selfish exploitation strategies adopted by the parasitoid larvae, since co-exploitation will induce competition for food. We are not aware, however, of experimental evidence for the above
hypothesis. The hymenopterous parasitoid *Pachycrepoideus vindemmiae* can be considered as a suitable candidate to test it because female parasitoids of this species appear to vary the number of eggs deposited in a host depending on the number of conspecifics exploiting the same patch with hosts (Goubault et al. 2005). Another example of analogy is host infection by pathogens (van Baalen and Sabelis 1995; Sabelis et al. 2002). In the same way that a predator can drive local prey to extinction rapidly or slowly, a pathogen can reproduce fast in its host, thereby decreasing the host’s longevity, or it can reproduce more slowly without affecting the longevity of its host much. This results in a continuum ranging from extreme virulence to extreme avirulence (van Baalen and Sabelis, 1995; Sabelis et al., 2002).

Dispersal is generally treated as a phenomenon that is either only genetically determined and linked to life-history traits (e.g., Stevens et al. 2013, 2014) or plastic and depends on context (e.g., Clobert et al. 2009; Bitume et al. 2013, 2014). However, for a comprehensive understanding of dispersal, both perspectives need to be brought together (Bonte and Dahirel 2016). As explained above, dispersal behavior in our strains of *P. persimilis* also appears to depend on the individual state and its environment, but the significant variation in dispersal rate among strains encountered here suggests a genetic component (see also Jia et al. 2002). This in turn suggests that it is possible to select for lines with more Killer-like or more Milker-like strategies. Creating such lines would provide an important tool for further studies of the evolution and maintenance of variation in prey exploitation strategies and their effects on local and global population dynamics.

Acknowledgements — This paper is dedicated to the memory of MW Sabelis, who passed away on the 7th of January 2015. We thank C Kazak, I Doker, S Ragusa and H Tsolakis for helping with the sampling in Turkey and Sicily. We also thank CA Villarroel and JM Alba for helping with the analysis of the sequences and JA Pascual, JM Campo for helping design the map, F Faraji for help with the identification of the predatory mites and P de Ruiter for constructive comments on the manuscript.

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Pels B (2001) Evolutionary Dynamics of Dispersal in Predatory Mites. PhD dissertation, Faculty of Science, University of Amsterdam, Amsterdam, the Netherlands.


Genetic variation for prey exploitation and dispersal strategies


**Supplementary material**

**Table S2.1** — Timetable of when each strain of *Phytoseiulus persimilis* was collected, cultured and tested

<table>
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<tr>
<th>Origin</th>
<th>Strain</th>
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<th>Date of culture</th>
<th>Replicate</th>
<th>Date of testing</th>
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Genetic variation for prey exploitation and dispersal strategies

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**TABLE S2.2** — Average population growth rate ($\gamma$) per strain of *Phytoseiulus persimilis*

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CHAPTER 2

FIGURE S2.1 — Population dynamics of adult prey (*Tetranychus urticae*) on the leaf (dashed lines, left-hand vertical axis) and cumulative number of dispersing predators (*Phytoseiulus persimilis*) (solid lines, right-hand vertical axis). Different color corresponds to different replicate. Blue: replicate 1, Green: replicate 2, Red: replicate 3, Orange: replicate 4 and Purple: replicate 5. a: Samandağ, b: Koyunoğlu, c: Kuşalanı, d: Karaçay, e: Uzunbağ, f: Kocahanlı.
Genetic variation for prey exploitation and dispersal strategies

**Figure S2.2** — Population dynamics of adult prey (*Tetranychus urticae*) on the leaf (dashed lines, left-hand vertical axis) and cumulative number of dispersing predators (*Phytoseiulus persimilis*) (solid lines, right-hand vertical axis). Different color corresponds to different replicate. Blue: replicate 1, Green: replicate 2, Red: replicate 3, Orange: replicate 4 and Purple: replicate 5. a: Castelvetrano, b: Trabia, c: Alcamo, d: Lascari, e: Palermo, f: Koppert.
Timing of aerial dispersal is a heritable trait in the predatory mite *Phytoseiulus persimilis*

A.M. Revynthi, D. Verkleij, A. Janssen & M. Egas

**ABSTRACT** — Dispersal is a main determinant of the dynamics and persistence of predator-prey metapopulations. Theoretical studies predict the existence of two types of dispersal strategies: the Milker, an early dispersal strategy, and the Killer, a late dispersal strategy. In the predatory mite *Phytoseiulus persimilis*, empirical studies have suggested a genetic component for timing of aerial dispersal in the presence of prey. We tested this by selecting for early and late dispersers from rose leaves with two-spotted spider mites as prey, showing that 6 rounds of selection for early or late dispersal resulted in predator lines displaying earlier or later dispersal. In a population dynamics experiment, we furthermore show that selection for timing of dispersal also results in predicted differences in the local interaction time with the prey and in the cumulative number of dispersers. We conclude that timing of dispersal is a heritable trait that can be selected for and results in lines with quantitative differences in local predator-prey dynamics. This opens ways to experimentally investigate the evolution of alternative prey exploitation strategies.
INTRODUCTION

Dispersal is a key process in the persistence of predator-prey metapopulations and has major consequences for individual fitness and for population dynamics (Janssen et al. 1997; Zemek and Nachman 1998, 1999; Ellner et al. 2001; Revilla et al. 2004; Bowler and Benton 2005, 2009; Clobert et al. 2009). Even when local predator-prey populations go extinct, persistence is observed at a metapopulation level because of the asynchronies of the dynamics in these local populations and the founding of new populations by dispersing individuals (Vandermeer 1973; Hilborn 1975; Crowley 1981; Jansen and Sabelis 1992; Janssen et al. 1997). The adaptive value of dispersal is determined by the balance between its costs and benefits, which are highly dependent on the life stage and condition of an individual, as well as on the local and environmental conditions (Bowler and Benton 2005, 2009; Bonte and Dahirel 2016).

From the perspective that dispersal is a life-history trait (Bonte and Dahirel 2016) and that the decision to disperse from a patch is not only context-dependent but also heritable, we can consider predator dispersal as a prey exploitation strategy (van Baalen and Sabelis 1995; Sabelis et al. 2002). Generally, the argument runs as follows: In a local predator-prey population with a limited interaction period, early predator dispersal decreases predation, resulting in a longer interaction period between the predators and their prey. As a consequence, the predator offspring will have more food available, and this prudent predation will therefore result in the production of more dispersing offspring during the total interaction period. However, a longer interaction period can lead to higher risk of secondary predator invasions. These invaders may not disperse early and exploit the prey population to their own interest by consuming the prey that prudent predators saved for their offspring (van Baalen and Sabelis 1995). Hence, a local population of prudent predators can be invaded by selfish predators that will benefit from them, so prudent predation is not evolutionary stable in well-connected metapopulations because prudent predators are outcompeted by cheaters (cf. Maynard Smith 1964). In an earlier theoretical paper, van Baalen and Sabelis (1995) named the selfish strategy Killer and the prudent Milker. Theory predicts that Milker-like strategies will be favored only when local predator populations are sufficiently isolated from each other, thus reducing the risk of invasions of Killers (van Baalen and Sabelis 1995; Pels and Sabelis 1999; Pels et al. 2002).

Empirical evidence supporting these theoretical predictions is scarce and incomplete. Pels and Sabelis (1999) investigated the dispersal strategies of several field populations of the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae), and found that an isofemale line of predators from a coastline population, where subpopulations are well-connected by dispersal, only dispersed when prey patches were depleted. In contrast, another isofemale line of predators from an inland population, where subpopulations are isolated from each other, dispersed before the prey patch was entirely depleted. In addition, dispersal before prey depletion was associated with a longer interaction period and, in some cases, increases of prey numbers during this period (Pels and Sabelis 1999). In addition, Revynthi et al. (CHAPTER 2) collected predatory mites of the same species from six sites along the coast of Turkey and from five sites in Sicily, and investigated their dispersal behavior and population dynamics in a set-up similar to that of Pels and Sabelis (1999). The
collected strains showed large variation in prey exploitation strategies and significant differences in dispersal rate.

Together, these studies show that there is variation among lines and populations in behavior regarding prey exploitation and timing of aerial dispersal, and suggest that there is a genetic component for dispersal tendency. However, evidence of genetically determined alternative dispersal strategies remains absent. Taking into account that previous research regarding alternative dispersal strategies assumed that aerial dispersal rates have a genetic component (Pels and Sabelis 1999; Revynthi et al., CHAPTER 2), we investigated whether it is possible to select for Milker-like and Killer-like predatory mite lines in a bidirectional artificial selection experiment, i.e., one line selected for early aerial dispersal, the other for late dispersal. The timing of dispersal in these selection lines was compared to control (unselected) lines. Furthermore, we confirmed these differences in dispersal behavior in a population dynamics experiment, which also served to test the theoretical predictions that such differences should result in differences in the interaction period and the total number of dispersers from these patches.

**MATERIALS AND METHODS**

**Roses**
Young rose plants (Olij Rozen, De Kwakel, the Netherlands) were transferred to a climate room, where they were hydroponically grown on rock wool. Conditions in the climate room were 25 °C, 70% RH and 16L:8D. The rose plants were watered 2× per week and fertilized (20-10-20 N-P-K) once per week.

**Spider mites**
Two-spotted spider mites (*Tetranychus urticae* Koch) were originally collected from cucumber plants in a commercial greenhouse in May 1994 (Pallini et al. 1997). The spider mite culture was kept on Lima bean plants (*Phaseolus lunatus* L.) in a climate room at 26 °C, 50% HR and 16L:8D.

Cohorts were created to obtain adult females of approximately 2 days into adulthood. One hundred and fifty adult female spider mites were divided over the two primary leaves of a Lima bean plant. The leaves were placed on a bed of water-saturated cotton wool in a plastic tray, which kept them turgid, and prevented the spider mites from dispersing because spider mites cannot walk over wet cotton wool. The females were left on the bean leaves to oviposit for 48 h, after which they were removed and their offspring were allowed to develop. The cohorts were kept in a climate room at 25 °C, 65% RH and 16L:8D, for 17 days.

**Predatory mites**
To ensure sufficient genetic variation for selection to act on, a base population was created by mixing predatory mites from six strains collected in Turkey in 2013 and five strains from Sicily in 2014, which were shown to harbor significant variation in dispersal rate (Revynthi et al., CHAPTER 2). Two gravid females of each of the 11 strains were haphazardly selected and placed on Lima bean leaflets (*P. lunatus*) infested with two-spotted spider mites (*T. urticae*). Cultures of predatory mites were kept as described in Pels and Sabelis (1999). In short, the bean leaflets were isolated on a plastic float, which was placed in a plastic tray,
which was filled with a 15-mm layer of water with dissolved soap. In order to reduce selection for mites with higher dispersal tendency the plastic float was covered with a plastic aquarium (19.5 × 13.0 × 11.5 cm) with a piece of fine-meshed flexible gauze (80 μm) hanging from the ceiling that connected to the float/leaflets. In this way the mites had the opportunity to leave the leaves with prey and consequently return without drowning. This method ensured that the predators that left the prey patch did not disappear; hence, it reduced the selection for predators that did not disperse in the presence of prey (i.e., Killers).

Rectangular holes were made in the ceiling of the aquaria and were covered with fine mesh (80 μm) for ventilation. Individual plastic trays were placed in a fine mesh (80 μm) cage. The cultures were fed 3× per week by adding two spider mite-infested Lima bean leaflets to the floats and were kept in a climate room at 25 ºC, 70% RH and 16L:8D.

To obtain sufficient numbers of gravid females of the same age (2 days old) for selection and experiments, cohorts were created as follows. Ten gravid female predatory mites from the base population were placed on a spider mite-infested bean leaf on a bed of water-saturated cotton wool in a Petri dish (14 cm diameter, 2 cm height). In this way, the leaves remained turgid for at least 10 days. The gravid females were allowed to oviposit for 48 h, after which they were removed and only their eggs and prey were left on the leaves. The cohorts were kept in a climate room at 25 ºC, 70% RH and 16L:8D, for 10 days.

**Selection procedure**

For the experimental set-up of the selection procedure, 9 wind tunnels were prepared as in Revynthi et al. (Chapter 2) for each round of selection. Each wind tunnel consisted of a plastic aquarium (25.3 × 15.8 × 15.5 cm) with holes (11.5 cm diameter) on both sides, covered with a fine mesh (80 μm). A fan was placed in front of the mesh on one side and created a constant air flow inside the wind tunnel, which was kept at approximately 0.4 m/s during the selection. The shoots of two rose leaves, each with five leaflets, were inserted in a plastic vial (24.5 mm diameter, 40 mm height) filled with water-saturated Oasis floral foam and the vial was placed at the upwind end of the wind tunnel. The width and length of the leaves ranged from 5-7 cm and 8-10 cm, respectively. Each of the rose leaves was infested with 10 adult female spider mites, 24 h prior to the selection procedure. At the downwind side of the wind tunnel, a trap was placed in order to capture the aerially dispersing predatory mites. The trap consisted of a Petri dish containing the three top leaflets of a rose leaf with spider mites, with the shoot (ca. 3 cm) inserted through a hole in the lid of an Eppendorf tube (1.5 ml) filled with wet Oasis floral foam.

**Early dispersal line**

In the first round of selection for early dispersal, 60 gravid predator females were transferred from the cohorts to upwind rose leaves in each of six wind tunnels. At 2, 4, 6, 8 and 24 h, the trap leaves were replaced with new ones, the dispersed mites on the trap leaves were counted and the first 20-25 dispersers of each of the six wind tunnels were used to set up a culture of the early-dispersal line. The remaining 210-240 individuals were discarded. Simultaneously, another 60 gravid predator females were transferred to upwind rose leaves in three other wind tunnels. Using the same time intervals, all dispersed predatory mites were collected, transferred into a common cage and 120 of them were randomly chosen at
the end of the 24 h and used to start a control line. Both lines were kept in rearing units as described above. Subsequently, cohorts were created using gravid females from the selection line and control line 7 days after the selection, and the adults from these cohorts were subjected to a new round of selection. The total procedure was repeated for six rounds.

**Late dispersal line**

Instead of selecting for predators that did not disperse and running the risk that this would include sick or otherwise disabled mites, we selected for late dispersers in a similar manner as above, except that the 20-25 predators that had dispersed last (within 24 h) were used to set up this line. A separate control line was started simultaneously in three separate wind tunnels as above. This selection procedure was also repeated for six rounds.

For logistic reasons, selection of the two lines could not be run simultaneously. Moreover, a period of 14 days between selection rounds of each line was required to obtain a new generation of adult offspring. Therefore, selection of the early-dispersal line and its control were alternated with the late-dispersal line and its control.

**Selection response experiment**

After six rounds of selection, the dispersal rates of the selected lines and control lines were measured in a set-up similar to that of the selection procedure. To standardize the quality of the prey patch from which the predators dispersed, these only contained spider mite web, prey eggs and no other prey stages. Furthermore, the patch consisted of one rose leaf only, but was otherwise similar to those in the selection procedure. Prior to the experiment, 15 2-day-old adult female spider mites were allowed to oviposit for 48 h on this leaf, and then were removed and the number of spider mite eggs was reduced to 80 per leaf, which was subsequently used for the experiment. A trap was placed at the downwind side of the wind tunnel, as described above (**Selection procedure**).

At the start of the experiment, 30 gravid 2-day-old female predators from the selection line or its respective control line were placed on the prey patch. The mites that had dispersed to the traps were counted during 8 h with 2-h intervals. At the end, the predators remaining on the prey patch were counted. The response experiment was performed in three blocks of four replicates of each selection line and its respective control line. The response to early-dispersal selection and late-dispersal selection were measured in separate blocks.

**Population dynamics experiment**

To measure dispersal of the selected lines in a population dynamics context, eight wind tunnels were prepared as in Revynthi et al. (Chapter 2) (see also **Selection procedure**). The experimental procedure followed was the same as in Revynthi et al. (Chapter 2): eight rose leaves were infested with 15 2-day-old adult female spider mites, each placed in a wind tunnel and one gravid 2-day-old adult female predatory mite was released on each leaf 48 h later. From this day on, adult prey and predators on the leaf and dispersers in the wind tunnel were counted every 24 h until there were no more mites (either prey or predators) present on the leaf. This experiment was performed in two blocks of four replicates for each selection line.
Statistical analysis
We tested for differences in time to dispersal between the various lines with a time-to-event analysis with a Cox proportional hazard model. Censoring was applied to predators that did not disperse during 8 h. The packages survival and coxme of the open source program R v.3.2.2 (R Development Core Team 2015) were used. Selection regime was used as a fixed factor and block (replicate of the experiment) as random factor. Contrasts were assessed through joining of non-significant factor levels (Crawley 2007).

To investigate whether the selected lines showed a Milker-like or a Killer-like strategy we analyzed the dispersal rate of the predator during the interaction with its prey. Furthermore, theory predicts that the interaction period of the prey with the predator and the cumulative number of dispersing predators should differ as a consequence of these different dispersal strategies (van Baalen and Sabelis 1995). We therefore tested differences in these three parameters between selected lines and their controls. First, a MANOVA was applied to the dispersal rate (arcsine square root transformed), the interaction period and the cumulative number of dispersing predators combined, with selection regime and block as explanatory variables. After having found a significant difference between selected lines with the MANOVA, each parameter was tested separately.

Differences in the transformed (arcsine square root transformation) dispersal rate and the interaction period between lines were tested with a GLM with a Gaussian error distribution. To detect differences in the cumulative number of dispersing predators between lines, a GLM with a quasi-Poisson error distribution was applied.

RESULTS
Selection response experiment
The predators from the early-dispersal line dispersed significantly earlier than the predators from their control line ($\chi^2 = 7.5$, d.f. = 1, $P = 0.006$; FIGURE 3.1A). Similarly, the predators from the late-dispersal line stayed significantly longer on the leaf than the predators from their control line ($\chi^2 = 8.65$, d.f. = 1, $P = 0.003$; FIGURE 3.1B). These findings show that there is indeed a genetic component in the dispersal behavior of *P. persimilis*.
The two selection procedures were not performed exactly at the same time but in alternating weeks (see selection procedure in Materials and methods). Nevertheless, they were done under identical conditions in the same period, so we are convinced that the results were not affected by this slight asynchrony, hence, we also compared the two selection lines and the two control lines. The predators from the early-dispersal selection line dispersed significantly earlier than the late-dispersal selection line ($\chi^2 = 17.17$, d.f. = 1, $P<<0.001$). The two control lines did not differ from each other ($\chi^2 = 1.51$, d.f. = 1, $P = 0.22$).

**Population dynamics experiment**

We subsequently measured predator dispersal in a population-dynamical context (Figure 3.2). The two lines exploited the prey population in a different way: the early-dispersal line exploited the adult prey population much more slowly than the late-dispersal line and, as expected, initiated dispersal when there were still many adult prey on the leaf in comparison with the late-dispersal line, which initiated dispersal when the prey population was close to elimination (Figure 3.2).

**Figure 3.2** — Population dynamics of adult prey on the experimental leaf (dashed lines, left-hand vertical axis) and cumulative number of dispersing predators (solid lines, right-hand vertical axis). A: Early-dispersal selection line; B: Late-dispersal selection line. Different colors correspond to different replicates.
The combination of dispersal rate during the interaction period, interaction period and cumulative number of dispersing predators varied significantly between the selected lines as well as between blocks (MANOVA, Line: F = 15.71, d.f. = 1, P<0.001, Block: F = 7.01, d.f. = 1, P = 0.007). Dispersal rate varied significantly between the selected lines (GLM: χ² = 0.02, d.f. = 1, P = 0.02) and blocks (GLM: χ² = 0.04, d.f. = 1, P = 0.003). The early-dispersal line had a significantly higher dispersal rate during the interaction period than the late-dispersal line (FIGURE 3.3A). In addition, the early-dispersal line interacted significantly longer with the prey (GLM: χ² = 25.00, d.f. = 1, P<<0.001; FIGURE 3.3B) and produced significantly more dispersers than the late-dispersal line (GLM: χ² = 48.62, d.f. = 1, P = 0.04; FIGURE 3.3C). These results show that it is possible to select for Milker-like and Killer-like predatory mite lines.

**FIGURE 3.3** — Parameters from the two selected lines in the population dynamics experiment. A: the dispersal rate during prey exploitation (i.e., the time interval between predator introduction to the leaf and last day with at least three adult prey individuals on the leaf), B: the interaction period (i.e., the time interval between predator introduction to the leaf and prey elimination) and C: the cumulative number of dispersing predators (i.e., the total number of dispersers during the experiment). Boxes indicate the second and the third quartile; horizontal lines indicate the medians, whiskers above and below the box indicate the 90th and 10th percentiles. Different letters indicate significant differences (N = 8 for each line).
DISCUSSION

We found that it was possible to select for early and late aerial dispersal of the predatory mite *P. persimilis*, which supports the hypothesis that there is a genetic basis for the tendency to disperse aerially in *P. persimilis*. The population dynamics experiment showed that dispersal rates are intimately connected to patch exploitation strategies, with early dispersal (i.e., the so-called Milker strategy) from a prey patch resulting in prolonged predator-prey interactions on the patch and a higher total number of dispersing predators over the entire interaction period, and late dispersal (i.e., the so-called Killer strategy) resulting in a shorter interaction period and a lower number of dispersing predators. Hence, we selected for dispersal and show that this results in the predicted differences in dynamics on the prey patch.

Several studies show that dispersal behavior in *P. persimilis* and closely related phytoseiid species is, to some extent, genetically determined and heritable (Jia et al. 2002; Maeda 2005; Nachappa et al. 2009). However, these studies focused on ambulatory dispersal, rather than dispersal by means of air currents. These modes of dispersal are fundamentally different in both the mechanisms that trigger them and their consequences: ambulatory dispersal is triggered by cues that indicate the nearby presence of prey (Mayland et al. 2000), whereas such cues suppress aerial dispersal (Sabelis and Afman 1994). Moreover, ambulatory dispersal always leaves the possibility to return to the point of departure, since it takes place within local populations (Sabelis et al. 2005). Aerial dispersal, in contrast, is passive, takes place among local populations at a metapopulation level (Sabelis et al. 2005), and it is virtually impossible for the predators to return to the departure point after dispersing because the distance that they have covered may be large and the wind direction should change exactly 180° between departure and return. In our study, the predators could not perceive the cues of the infested rose leaf, which served as a trap, because it was placed downwind from the prey patch, and they could not leave the leaf by walking because of the lanoline barrier at the bottom of the petiole. Hence the predators could only disperse by using the airflow in the wind tunnel and they could not return to the prey patch after they had departed. Our study thereby adds to the literature, showing that aerial dispersal in this species has a genetic basis.

The initiation of aerial dispersal might be stimulated or suppressed by a combination of cues, including the availability of prey and the density of competitors within a prey patch. However, other information may also play a role. For instance, if co-invasions by unrelated predators are not very frequent, kin-recognition (the ability to identify relatives) or social familiarity (the ability to identify individuals with which there was a prior association) may affect dispersal tendency. Several studies have reported that *P. persimilis* and other phytoseiid species are able to distinguish related from non-related conspecifics (Faraji et al. 2000; Schausberger and Croft 2001) and socially familiar and unfamiliar conspecifics (Schausberger 2005, 2007; Zach et al. 2012; Muleta and Schausberger 2013) and adjust their behavior accordingly. In our study we used related (to some extent) and familiar individuals for the selection process, which are factors that can stimulate dispersal and can affect their dispersal tendency (Zach et al. 2012). Possibly there is genetic variation for the degree to which predatory mites adjust their dispersal tendency according to the presence of related conspecifics. Likewise, there may be genetic variation for the degree to which predatory
mites adjust their dispersal tendency according to chemical cues relating to the presence of prey in close proximity.

Better understanding of genetic heritability of traits relating to dispersal tendency and prey exploitation behavior in *P. persimilis* can contribute to our understanding of the evolution of alternative predator exploitation strategies. Furthermore, it can provide a basis for breeding programs to create strains with desirable traits for effective biological control. Because *P. persimilis* is commercially available as biological control agent of two-spotted spider mites, selecting for strains with desirable traits could improve the efficiency of managing spider mite pests. For instance, for the purpose of biological control of spider mites in ornamental crops, it is essential that predatory mites do not disperse before prey elimination. Hence, knowledge regarding the genetic heritability of traits related to dispersal behavior could potentially provide a basis for optimization of biological control programs.

Local population dynamics in metapopulations are often unstable and dispersal is therefore necessary for the global persistence of such predator-prey systems. Dispersal can also determine the exploitation strategy of predators: by dispersing earlier, predators can save prey for their offspring. When the local prey population grows, this offspring can produce more offspring, eventually resulting in a higher total number of dispersers from the patch. Yet, this prudent strategy can be invaded by predators with other, less prudent strategies, which consume the prey that was saved. Given that local populations may be more or less isolated, so the risk of invasions of prey patches by multiple predators varies, it is expected that there exists a continuum of strategies, ranging from prudent, milker strategies to selfish, killer ones. We show that it is possible to select for these strategies, confirming that such selection can also occur in nature. This is an important step in investigating the evolution of alternative prey exploitation strategies because it empirically demonstrates genetic heritability of the innate tendency to disperse aerially in *P. persimilis*, and it opens ways to experimentally investigate the consequences of these exploitation strategies, either alone or when played against each other.

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**References**


Selection on early and late timing of dispersal


Gender-specific differences in cannibalism between a laboratory strain and a field strain of a predator

A.M. Revynthi, A. Janssen & M. Egas

ABSTRACT — Cannibalism – i.e., intraspecific predation – is a common phenomenon that often occurs when food is limited. Many phytoseiid species, including *Phytoseiulus persimilis*, are known to engage in cannibalism when food is scarce and when there is no possibility to disperse. Under natural conditions, however, especially the females of these predatory mites are known to disperse when food is scarce. Males, in contrast, are expected to stay and wait for potential mates to mature. While staying, they could then obtain food by cannibalizing. Because conditions under which these mites are usually cultured prevent dispersal, it has been suggested that prolonged culturing may affect cannibalistic behavior. We hypothesize that it should especially affect cannibalism by adult females, because they consume by far most food. We tested this by comparing two strains, one of which had been in culture for over 20 years, whereas the other was recently collected in the field. Because it is known that this predator can discriminate between kin and non-kin and prefers cannibalizing the latter, we also started isofemale lines of these two original strains, with the idea to construct lines with high kin-relatedness. We subsequently tested to what extent the adult females and males of the original strains and the isofemale lines fed on conspecifics from the same culture. In a closed system, we observed the behavior of adult predators that were offered conspecific larvae as prey. Males engaged more often in cannibalism than females, and females of the strain with the long culture history engaged more in cannibalism than those of the recently collected strain, both in agreement with our ideas. The original strains and the isofemale lines did not differ in cannibalistic behavior, suggesting that there was no large difference in kin-relatedness within the original strains and the isofemale lines.
INTRODUCTION

Cannibalism, the act of killing and consuming an individual of the same species, is a common phenomenon across the animal kingdom. It occurs in various species such as birds (Cain et al. 1984), fish (Okuda and Yanagisawa 1996), frogs (Ringler et al. 2017), salamanders (Takatsu and Kishida 2015), spiders (Bilde and Lubin 2001), insects (Tschinkel 1981) and mites (Yao and Chant 1989). Animals prey on their conspecifics mainly as a response to low food densities (Fox 1975). Cannibalism, however, can also be affected by stress, kin competition or mate competition and prey vulnerability (Fox 1975; Polis 1981; Pfennig 1997). In times of food scarcity, cannibalism can be very beneficial to the cannibal, because under certain circumstances it can be the only way of obtaining food and nutrients (Fox 1975; van den Bosch et al. 1988). Nevertheless, cannibalism can result in injuries, pathogen transmission and, in case the victim is a relative, in loss of inclusive fitness (Pfennig 1997).

Many mite species of the Phytoseiidae family are known for their cannibalistic behavior (Schausberger 2003), and the cannibalistic stage commonly used in experiments is the gravid adult female feeding on juveniles (Schausberger 2003). Many of the experiments on cannibalism in phytoseiids are done in closed arenas, where cannibal or victim cannot escape. Hence, these experiments test for the possibility for cannibalism to occur rather than assessing their importance under more natural conditions where individuals can disperse. For phytoseiids, this is essential because typical populations of predators and prey are short-lived, and end either by the host plant of the prey being overexploited or by the prey being exterminated by the predators (Janssen and Sabelis 1992; Pels and Sabelis 1999). At the end of this interaction period, the predators disperse in search of new prey patches. Adult females disperse earlier than adult males and juvenile stages (Pels 2001; Revynthi et al., CHAPTER 2); once inseminated, females need food to reproduce, and juveniles need to become mature and inseminated before they can successfully disperse. Consequently, males are better off not dispersing, but waiting for new mates to develop. In order to survive, males and juveniles can engage in cannibalism. In previous experiments with predatory mites, we indeed observed much cannibalism by adult males on larvae under conditions of low prey densities (Revynthi et al., CHAPTER 2). Hence, we suggest that males may have a higher tendency to cannibalize than females.

It is generally accepted that culturing organisms may result in changes in behavior, life history and genetic variation (Mackauer 1976; Hopper et al. 1993), and these changes may affect cannibalism (Dennehey et al. 2001). When rearing phytoseiids, dispersal is often prevented, for obvious reasons. However, this can result in strong selection against dispersal behavior because individuals that try to disperse either end up dead in some barrier, or loose time and possibly energy when attempting to escape. It has been suggested that under conditions of laboratory cultures, higher cannibalism tendency is unintentionally selected for: due to space limitation, predators cannot disperse in search of more food when the prey are temporarily eliminated (Elliot et al. 2002). This would mean that laboratory strains would have a higher tendency to cannibalize than strains in the field, and this would hold especially for females.

However, rearing for longer periods may also increase the kin-relatedness among individuals, and it is known that phytoseiids tend to avoid kin-cannibalism (Schausberger and Croft 2001). This would result in lower rates of cannibalism in strains that have been in cul-
ture for a longer period. In the present study we have tested whether a recently collected strain of the predatory mite *Phytoseiulus persimilis* is more or less cannibalistic than a strain that has been in culture for more than 20 years. Several studies investigated the cannibalistic behavior of adult females of this species (Yao and Chant 1989; Walzer and Schausberger 1999; Schausberger and Croft 2001; Schausberger 2007; Schausberger and Hoffmann 2008), but not cannibalism by adult males. We investigated cannibalism in both males and females and tested whether males and females of both strains differed in the tendency to cannibalize in relation to the time that had been in culture.

**MATERIAL AND METHODS**

**Plant and prey cultures**

Lima beans (*Phaseolus lunatus*) were used as a host and were grown from seeds in a climate room (25 °C, 60% RH, 16L:8D) free of herbivores. The spider mites (*Tetranychus urticae*) that were used as food for *P. persimilis* were originally collected from cucumber plants in a commercial greenhouse in May 1994 (Pallini et al. 1997). They were reared on Lima bean plants in a walk-in climate room (26 °C, 60% RH, 16L:8D).

**Predatory mites**

Two strains of *P. persimilis* were used. One strain derived from Koppert Biological Systems (Berkel en Roderijs, the Netherlands) and one from Alcamo in Sicily (see Revynthi et al., **CHAPTER 2**). These two strains were chosen because we have information about their dispersal behavior (Pels and Sabelis 1999; Revynthi et al., **CHAPTER 2**) and because we were interested in observing whether there are differences in the cannibalistic behavior between a laboratory strain – i.e., Koppert – and a strain recently collected from the field – i.e., Alcamo. The predators were kept in closed rearing cages, which allowed the predators to leave and subsequently return to the prey patch (as described in Pels and Sabelis 1999) inside a climate room at 25 °C, 70% RH and 16L:8D. To test for an effect of kinship, an isofemale line of each strain was created by isolating a gravid female of *P. persimilis* from the culture and introducing it individually in a separate rearing unit with prey (*T. urticae*). The female was allowed to oviposit and create her own family. The strains and isofemale lines were fed 3× per week by introducing two bean (*P. lunatus*) leaves infested with spider mites (*T. urticae*).

To obtain sufficient numbers of gravid females and males of the same age (2-day-old adults), as well as larvae, cohorts were created as follows. Ten gravid female predatory mites from each of the two strains and the two isofemale lines were placed on a spider mite-infested bean leaf on a bed of water-saturated cotton wool in a Petri dish (14 cm diameter, 2 cm height). In this way, the leaves remained turgid for at least 10 days. The gravid females were allowed to oviposit for 48 h, after which they were removed and only their eggs and prey were left on the leaves. The cohorts were kept in the same climate room as the cultures.

**Evaluation of cannibalistic behavior**

To measure the cannibalistic tendency of the predatory mites in a closed system that did not allow for dispersal, small plastic cups (2.8 cm diameter, 2.2 cm height) were used. Each cup had a lid with a hole (12 mm diameter) covered with mite-proof gauze (80 μm diameter) for ventilation. Forty-eight h prior to the start of the experiment, males and gravid females from
the cohorts described above were individually isolated in a cup, which contained a bean leaf disc (24 mm diameter) on water-saturated cotton wool. The predators did not receive any food during this period (48 h in total).

At the start of the experiment, three young predator larvae from the same culture as the adult and from a cohort started three days earlier were transferred to a cup similar to those used for the starvation process. Subsequently, a starved adult predatory male or female was released in the same cup; hence, adults and larvae originated from the same culture, but were produced in separate cohorts. The predator was observed 5 min after its release and after that every 15 min for a total period of 1 h. Every time the number of alive and consumed larvae was recorded. Cannibalized larvae were recognized by the carcass from which the haemolymph was removed (Yao and Chant 1989). Replicates where a larva had molted to protonymph or died from natural causes were excluded. There were eight treatments, each with 30 replicates. In the ‘non-kin’ treatment, predators and larvae came from different cohorts of the same main culture; in the ‘kin’ treatment, predators and larvae were from different cohorts of the same isofemale line. The experiment was conducted in 12 blocks (days) and each block contained all the treatments.

Statistical analysis
To estimate which of the two genders and/or strains engaged more often in cannibalism and whether kinship affected their behavior a generalized linear mixed effect model with binomial distributions was used (glmer of the lme4 package; Bates et al. 2015). The response variable was the occurrence of cannibalism (a binomial variable) during the entire observational period, gender, strain and kinship were the fixed factors and block was used as a random factor. The analysis was performed using R v.3.0.1 (R Development Core Team 2015).

RESULTS
We only analyzed the data at the end of the observation period, i.e., after 1 h since the release of the adult predator in the cup, because we did not observe changes of cannibalism rate in the course of the experiment since within an hour on average one cannibalistic event occurred. Male predators engaged in cannibalism more often than females (GLMER: $\chi^2 = 25.5$, d.f. = 1, P $< 0.001$; FIGURE 4.1). The Koppert strain had significantly more cannibals than the Alcamo strain (GLMER: $\chi^2 = 8.93$, d.f. = 1, P = 0.003; FIGURE 4.1). This was because females of the Koppert strain cannibalized significantly more than females of the Alcamo strain (GLMER: $\chi^2 = 6.71$, d.f. = 1, P = 0.0096; FIGURE 4.1), but cannibalism by males was not significantly different ($\chi^2 = 2.24$, d.f. = 1, P = 0.135). Kinship did not affect cannibalistic behavior (GLMER: $\chi^2 = 0.032$, d.f. = 1, P = 0.86; FIGURE 4.1).

DISCUSSION
We used one laboratory strain and one that was recently collected in the field (Revynthi et al., CHAPTER 2) and found that the laboratory strain (Koppert) had a higher tendency to cannibalize than the field strain (Alcamo). It has been suggested that under conditions of laboratory cultures, higher cannibalism tendency is unintentionally selected for (Denehy et al. 2001; Elliot et al. 2002). Our results, however, show high levels of cannibalism in a field strain; the difference in cannibalistic tendency between the strains was small.
Earlier studies have focused on the voraciousness of cannibalistic females of *P. persimilis* (Walzer and Schausberger 1999) and their ability to discriminate between kin and non-kin (Schausberger and Croft 2001). To the best of our knowledge, this is the first time that the cannibalistic behavior of male predators of *P. persimilis* is investigated. Our experiments show that the males of two strains, one that has been in culture for a long time, and one that was recently collected, were more prone to cannibalize than gravid females, regardless of relatedness with the victims.

**Figure 4.1** — The proportion of adult predators that cannibalized. Shown are proportions of males (black bars) and females (grey bars) of two strains (Alcamo and Koppert) that cannibalized on related larvae (kin, panel A) or unrelated larvae (non-kin, B). Asterisks indicate significant differences. N = 30 for each bar. Whiskers indicate the standard errors of the proportions.
Predatory mites that had been in culture for a long period had a significantly higher tendency to cannibalize than a recently collected strain of the same species. However, cannibalism rates were high for both strains. Interestingly, males cannibalized significantly more than females. This difference is probably caused by differences in the behavior of males and females: whereas females search for prey, males search for conspecifics to mate. However, there is an alternative explanation for the differences in the cannibalistic behavior of males and females, which is based on asymmetries in relatedness with offspring. Inclusive fitness theory (Hamilton 1964a,b; Gardner et al. 2011) suggests that evolution will favor the individuals that are able to recognize relatives and avoid feeding on them, regardless the gender (Pfennig 1997). In haplodiploid systems, however, differences are expected between the genders due to the difference in average degree of relatedness with the victim between male and female cannibals. Phytoseiid mites are pseudo-arrhenotokous (Schulten 1985): males and females both derive from fertilized eggs but only the females remain diploid and carry both maternal and paternal chromosomes (McMurtry et al. 1970). The males loose the paternal set of chromosomes shortly after syngamy (Helle et al. 1978; Sabelis and Nagelkerke 1988). Hence, in these predators, the adult females are expected to suffer from greater inclusive fitness loss than males, because they have more offspring than males, which only contribute genetically to daughters, and do not have sons. Thus it can be hypothesized that gravid adult females are less prone to cannibalism than males, despite higher energy requirements. We expect that males would specifically cannibalize other, immature males, because this reduces future competition for mates, and by letting female immatures live, they increase the chance on a future mate. This remains to be tested.

We found no effect of kinship on cannibalism, suggesting that under no-choice conditions, the decision to cannibalize is not affected by the degree of relatedness with the potential victims they encounter. Hence, this suggests that no inbreeding effects occurred in the strain that had a long history of being cultured or that inbreeding did not affect the tendency to cannibalize. To our best knowledge, inbreeding effects in *P. persimilis* are absent unless long periods of strong inbreeding are invoked (Poe and Enns 1970). This is common for haplodiploid species, where selection against recessive alleles always acts on the haploid males and the frequency of such alleles therefore remains low, except for genes that specifically code for female traits such as egg production (Tien et al. 2015). We also did not observe population declines or any other adverse effects of inbreeding in either of the two isofemale lines. The isofemale lines were started with one female of each strain. This female was therefore potentially not representative for the entire population. For example, it could have had a higher genetic tendency for cannibalism. This would then have resulted in less variation in the cannibalistic behavior in the isofemale lines than in the original lines, since those mites are genetically fixed whereas the original lines were genetically more diverse. However, we found no such difference in variation in the cannibalistic behavior between strains and lines.

Several studies have focused on kin recognition in phytoseiid mites (Faraji et al. 2000; Schausberger and Croft 2001), but also in other animal taxa (Pfennig 1997; Bilde and Lubin 2001; Parsons et al. 2013; Bayoumy and Michaud 2015; Ringler et al. 2017). Schausberger and Croft (2001) show that *P. persimilis* is able to discriminate between kin and non-kin and preferred to cannibalize the latter. Even though our study was not focused on kin discrimi-
nation of *P. persimilis*, we explored whether the level of relatedness with the prey could have affected cannibalistic behavior. The lack of variation between the kin and non-kin treatment does not contradict earlier reports of kin discrimination in this species (Schausberger and Croft 2001; Schausberger 2004) because we did not offer the cannibals a choice between kin and non-kin victims.

In natural settings, the predators can opt out of cannibalizing by dispersing away from the patch without prey and search for a new prey patch. For the two strains used here, we have information about their dispersal behavior (Pels and Sabelis 1999; Revynthi et al., Chapter 2). In wind tunnel experiments, both strains showed a tendency to disperse only after heterospecific prey were depleted, showing the so-called Killer strategy of prey exploitation (van Baalen and Sabelis 1995). Theoretical work on the evolution of cannibalism and predator dispersal predicts that predators with the Killer strategy are selected for higher cannibalistic tendency (Pels 2001). Given the variation for this prey exploitation behavior that was found in previous experimental work (Pels and Sabelis 1999; Revynthi et al., Chapter 2), we argue that there may also be variation among natural populations of *P. persimilis* in cannibalistic tendency. Future research should explore whether the two genders will show similar behavior as observed in this study when they have the option to disperse.

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**References**


Cannibalistic behavior of females and males


To cannibalize or disperse?
Males cannibalize and females disperse in the predatory mite *Phytoseiulus persimilis*

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**ABSTRACT** — Cannibalism is a widespread phenomenon in nature, often occurring when food is scarce, for example among predators that have over-exploited a local prey population. Instead of cannibalizing, predators can disperse, thereby avoiding being cannibalized or cannibalizing kin-related conspecifics, resulting in inclusive fitness loss. Theory on prey exploitation in ephemeral predator-prey systems predicts that predators may be selected to display prudent predation (‘Milker’ predators) by dispersing early, thus saving food for their remaining offspring. This is especially advantageous when average relatedness in the local population is high. Less prudent predators refrain from dispersing until all prey are exterminated. These prey exploitation strategies may also have repercussions for cannibalism, especially when it is driven by food shortage. We therefore investigated to what extent adult females and males of lines with different dispersal strategies cannibalize or disperse when prey are exterminated. We used two lines of the haplodiploid predatory mite *Phytoseiulus persimilis* that were selected for early and late dispersal. Using wind tunnels, we observed the behavior of adult predators of these lines, offering conspecific larvae as victims. Our results show that both selection lines behaved similarly but that male predators stayed significantly longer in the patch and engaged more often in cannibalism than females. This is in agreement with differences in their behavior and in their kin-relatedness to the other individuals on the patch. We therefore propose that males and females of haplodiploid species may have different tendencies to cannibalize.
INTRODUCTION

Cannibalism (i.e., intraspecific predation) is a common phenomenon in nature and occurs in a wide range of animal taxa, such as birds, mammals, fish, insects, spiders and mites (review in Fox 1975; Polis 1981; Schausberger 2003). Animals prey on their conspecifics to obtain food, nutrients and remove resource competitors in times of scarcity (Fox 1975). Killing and consuming a conspecific, however, may result in injuries, pathogen transmission and loss of inclusive fitness if the victim is a relative (Pfennig 1997). Nevertheless, cannibalism occurs often when food is scarce.

When a local prey population is overexploited by a local predator population, the predators eventually need to disperse to find a new prey patch. Such dispersal promotes persistence at a metapopulation level due to the foundation of new local populations by dispersing individuals (Huffaker 1958; Taylor 1990; Janssen et al. 1997; Ellner et al. 2001). After exterminating the prey, predators have the option to stay or disperse and to cannibalize or not. Staying without cannibalizing will result in starvation, and dispersing means losing the possibility to cannibalize, hence, different dispersal strategies can result in different levels of cannibalism. A similar choice between staying or dispersing and cannibalizing or not is found in solitary predators that have a transient gregarious life stage, such as hatching spiderlings (Lesne et al. 2016), preying mantids (Fea et al. 2014), toads (Child et al. 2008) and coccinellids (Michaud and Grant 2004). Hence, dispersal and cannibalism are behaviors that can often occur under the same circumstances, and selection on one type of behavior can therefore affect the other (Rudolf et al. 2010).

There is a continuum of strategies for the exploitation of ephemeral prey patches by predators. Early dispersal of adult predators, before the prey are exterminated, reduces predation and the prey population can therefore persist longer. As a result, the offspring of the dispersed predators will have more food and, hence, they can interact with their prey for a longer, but finite, period of time, and this will result in a higher total number of dispersing predators over the entire local predator-prey interaction period (van Baalen and Sabelis 1995). Because the number of dispersers is an appropriate stand-in measure for fitness in metapopulations (Gyllenberg and Metz 2001), such prudent predation (the so-called ‘Milker strategy; van Baalen and Sabelis 1995) through increased early dispersal results in higher overall fitness for the predators. Early dispersal will not only decrease predation of the prey, but also cannibalism for the same reason. Late predator dispersal in contrast, drives the local prey population to extinction faster, resulting in a shorter interaction period between the predator and its prey and a lower fitness. It also offers more opportunities for cannibalism once the local prey density is low. However, the early dispersal strategy can be invaded by the late dispersal strategy (the so-called ‘Killer’ strategy; van Baalen and Sabelis 1995), because the latter consume the prey saved by the prudent predators faster than the prudent offspring.

In theoretical studies, cannibalism has been treated either as a selective process in the evolution of dispersal, which can determine the spatial structure of natural populations (Lion and van Baalen 2007; Rudolf et al. 2010), or as the trait under selection due to different dispersal strategies (Pels 2001). In both cases the evolution of cannibalism is affected by kin selection (Hamilton 1964a,b). Rudolf et al. (2010) showed that cannibalism selects for dispersal because offspring try to escape from their cannibalistic parents, avoiding this way loss
of inclusive fitness. Alternatively, Pels (2001) suggested that late dispersal can select for high cannibalistic tendency among juveniles. We hypothesize that predators that disperse only after their local prey population is depleted (Killers) are more prone to cannibalize than predators that disperse before prey depletion (Milkers), but run a higher risk of substantial loss in inclusive fitness because they might consume relatives.

A model organism to study cannibalism in relation to dispersal and to test the above hypothesis is the haplodiploid predatory mite *Phytoseiulus persimilis*. It is a specialist predator that feeds on tetranychid mites and lives in spatially structured environments (Nachman 1981; Ellner et al. 2001), where it drives local prey populations to extinction (Janssen and Sabelis 1992; Janssen et al. 1997; Pels and Sabelis 1999). Subsequently, the predators may disperse in search of food or remain in the patch and obtain food via cannibalism. The cannibalistic behavior of females of this predator has been repeatedly studied in closed environments without the option to disperse (Yao and Chant 1989; Walzer and Schausberger 1999; Schausberger and Croft 2001; Schausberger 2007; Schausberger and Hoffmann 2008; Revynthi et al., CHAPTER 4). We are aware of only one study that measured dispersal behavior of juvenile *P. persimilis* in absence of prey (Pels 2001), however, the author focused on quantifying the number of dispersed individuals and did not quantify cannibalism.

It is important to distinguish among the different life-history stages of the predators because they face different consequences of dispersal. In predatory mites, adult reproducing females consume by far the most prey, and convert a large part of the ingested food into eggs (Sabelis 1981). Females that stop feeding can survive for long periods without food as long as there is water available (Sabelis 1981). Because the predatory mites disperse passively on air currents (Sabelis and Dicke 1985), the possibility that they will find a new prey patch is low, and the possibility to find a prey patch with conspecifics is consequently even lower. This means that the best stage for dispersal is the adult, mated female, which is the only stage that can start reproducing when arriving on a new prey patch without conspecifics. To mature, juveniles, either male or female, need much less food than reproducing adult females (Sabelis 1981), and they may therefore develop into adult by consuming the odd prey left at the end of the local interaction between predators and prey. Adult males also need little food, and for them it is actually better to stay on the patch and mate with newly developed adult females rather than dispersing to find mates. We therefore expect that mated adult females will disperse more readily than adult males, and that adult males may consequently cannibalize more than adult females. Indeed, we recently found that adult male *P. persimilis* cannibalize more than adult females (Revynthi et al., CHAPTER 4).

In a recent study, Revynthi et al. (CHAPTER 3) selected for predators that depart early (i.e., ‘Milker’-like) and late (‘Killer’-like) from a patch with spider mite prey. Although we have information about the dispersal and exploitation strategies of these two selected lines, little is known about their cannibalistic tendencies. Here we aim to investigate to what extent these selected lines that employ different dispersal and exploitation strategies cannibalize and we ask: (1) When food is limited and given the option to disperse, will predators choose to cannibalize or to disperse? (2) If predators cannibalize when they have the option to disperse, which line and/or gender of *P. persimilis* is more voracious towards its conspecifics?
MATERIAL AND METHODS

Roses
Young rose plants (Rosa sp. var Avalanche) were obtained from Dummen Orange (De Lier, the Netherlands) and transferred to a climate room, where they were hydroponically grown on rock wool. Conditions in the climate room were 25 °C, 70% RH and 16L:8D. The rose plants were watered 2× per week and fertilized (20-10-20 N-P-K) once per week.

Spider mites
Two-spotted spider mites (T. urticae) were originally collected from cucumber plants in a commercial greenhouse in May 1994 (Pallini et al. 1997). The spider mite culture was kept on Lima bean plants (Phaseolus lunatus L.) in a climate room (26 °C, 60% RH and 16L:8D) and was used to maintain the predator cultures described below.

Predatory mites
In the present study, we used two lines of P. persimilis that were selected for early and late dispersal (the so-called Early- and Late-dispersal line; Revynthi et al., 2016). The predators were kept on floating platforms in trays that contained water with dissolved soap inside a mite-proof cage (80 μm diameter). Previous experiments showed no effect of kinship on the cannibalistic behavior of females and males of this species (Revynthi et al., 2016). Hence, based on this finding we conducted the experiments using only kin predators. Therefore, isofemale lines were created by isolating a gravid female predator from each selection line and individually putting it in a separate cage with prey, where it could establish its own family. The isofemale lines were fed 3× per week by introducing two Lima bean leaves (P. lunatus) infested with spider mites (T. urticae), and were kept in a climate room at 25 °C, 70% RH and 16L:8D.

To obtain sufficient numbers of gravid females and males of the same age (2 days into adulthood), as well as larvae, cohorts were created as follows. Ten gravid female predatory mites from each of the two isofemale lines were placed on a spider mite-infested bean leaf on a bed of water-saturated cotton wool in a Petri dish (14 cm diameter, 2 cm height). In this way, the leaves remained turgid for at least 10 days. The gravid females were allowed to oviposit for 48 h, after which they were removed and only their eggs and prey were left on the leaves. The cohorts were kept in the same climate room as the lines.

Experimental procedure
To observe the cannibalistic behavior of females and males when they had the option to disperse aerially, eight wind tunnels were used as described in Revynthi et al. (2016). Each wind tunnel consisted of a plastic aquarium (25.3 × 15.8 × 15.5 cm) with holes (11.5 cm diameter) on both sides, covered with a fine mesh (80 μm). The aquarium was closed with a glass lid and sealed with Parafilm. The combination of a fan on one side and gauze on the other created a constant air flow inside the wind tunnel, which was kept at approximately 0.4 m/s during the experiments. The stem of a rose leaf, with five leaflets, was inserted in a plastic vial (24.5 mm diameter, 40 mm height) filled with water-saturated Oasis floral foam and a thick layer of lanolin was applied to the base of the petiole to prevent mites
from escaping. Then the vial was placed at the upwind end of the wind tunnel. At the downwind side, a trap was placed in order to capture the aerially dispersing predatory mites. The trap consisted of a Petri dish containing the three top leaflets of a rose leaf infested with spider mites, with the stem (ca. 3 cm) inserted through a hole in the lid of an Eppendorf tube (1.5 ml) filled with wet Oasis floral foam.

Adult males and gravid females from the cohorts were individually isolated in a cup (2.8 cm diameter, 2.2 cm height), 48 h prior to the start of the experiment. The cup contained a rose leaf disc (24 mm diameter) on water-saturated cotton wool and was sealed by a lid with a hole (12 mm diameter) covered with mite proof gauze (80 μm diameter). We starved the predators until the start of the experiment, i.e., in the 48 h they did not receive any prey.

At the start of the experiment, seven young predatory mite larvae were transferred onto the first leaflet of a rose leaf. Larvae were chosen as prey, since this stage is the most vulnerable to cannibalism and does not feed (Amano and Chant 1977). After the introduction of the larvae, a starved adult male or female predator from the same isofemale line as the larvae was released next to the larvae. Observations started 2 h after the initiation of the experiment, as pilot experiments revealed that no predators dispersed within an hour. Every hour, the trap was replaced with a new one and the old one was checked for dispersers. The experiment stopped as soon as the adult had dispersed or after 6 h and the time of dispersal was recorded. The experiment ran for a maximum of 6 h to prevent having larvae molting to protonymphs. The protonymph stage is the first feeding stage and they cannibalize when there is lack of heterospecific prey, thus they could feed on the remaining larvae. At the end of the experiment, each rose leaf was inspected to determine whether the adult predatory mite had engaged in cannibalism by counting the consumed and alive larvae. Only corpses from which the hemolymph was removed were counted as consumed larvae. We did not observe natural mortality in the larvae (dead larvae that were not eaten and their hemolymph had not been removed). If the number of consumed and alive larvae did not add up to seven, the remaining larvae were recorded as missing. In this way, we quantified cannibalism and dispersal tendencies of both genders from both selection lines (Early- and Late-dispersal). As a control for each treatment, predators were individually put on the rose leaf without conspecific larvae and only the time of dispersal was recorded by making the same hourly observations as described above. The experiment was performed in 20 blocks in a climate room (25 °C, 70% RH and 16L:8D), each block consisting of one replicate of all treatments.

**Statistical analysis**

To determine whether there was a difference in the cannibalistic behavior of the lines and genders, a χ² test was used on the numbers of cannibalistic males and females, crossed with the numbers of males and females that dispersed.

In order to detect possible differences in the timing of dispersal between the treatments, a time-to-event analysis with a Cox proportional hazard model was used. Censoring was applied to predators that did not disperse during the 6 h of the experiment. The fixed explanatory variables were the line (Early or Late), treatment (cannibalism or control) and the gender (male or female) and their interactions. Block was included as a random factor. Non-significant interactions and factors were removed to find the minimum adequate model.
To determine which of the two genders was more voracious towards its conspecifics and to detect possible differences between the two lines, two generalized linear mixed effect models with a binomial error distribution were used (glmer of the lme4 package; Bates et al. 2015). In the first model, we used all predators, because we were interested in the effect of gender and line on the average predator voraciousness. In the second model, we analyzed differences in voraciousness among cannibals. In both models the number of alive and consumed larvae combined in a vector was used as a response variable, predator line and the gender were the fixed factors, and block was a random factor. During the experiments, one male and one female from the Early-dispersal line died. Hence, for those two treatments, N = 19 instead of N = 20. All analyses were performed using R v.3.0.1 (R Development Core Team 2015).

RESULTS
The choice of the predators, to either cannibalize or not, did not differ between the selection lines ($\chi^2 = 0.46$, d.f. = 1, $P = 0.50$), but males cannibaled significantly more frequently than females ($\chi^2 = 4.06$, d.f. = 1, $P = 0.04$). Most males cannibaled, whereas ca. 50% of the females did not (FIGURE 5.1).

Predators from both lines did not differ statistically significant in timing of dispersal (Cox proportional hazard: $\chi^2 = 1.07$, d.f. = 1, $P = 0.30$; FIGURE 5.2) and there was no statistically significant difference in dispersal with or without the presence of conspecific larvae (Cox proportional hazard: $\chi^2 = 0.029$, d.f. = 1, $P = 0.87$; FIGURE 5.2). Females, however, dispersed significantly earlier than males (Cox proportional hazard: $\chi^2 = 4.56$, d.f. = 1, $P = 0.033$).

The number of larvae that the predators consumed did not vary statistically significant with line either for overall consumption (GLMER: $\chi^2 = 0.02$, d.f. = 1, $P = 0.88$; FIGURE 5.3a) or for consumption per cannibal (GLMER: $\chi^2 = 0.58$, d.f. = 1, $P = 0.45$; FIGURE 5.3b). Males of both lines consumed significantly more larvae than the females (GLMER: $\chi^2 = 3.9$, d.f. = 1, $P = 0.048$; FIGURE 5.3a). There was no statistically significant difference between

![Figure 5.1](image-url)
the genders, however, in the number of larvae consumed per cannibal (GLMER: $\chi^2 = 0.003$, d.f. = 1, P = 0.96; Figure 5.3b), hence, the differences in overall cannibalism between genders (Figure 5.3a) were caused by a higher proportion of males cannibalizing (Figure 5.1). On average 1.18 larvae out of seven were missing.

To cannibalize or disperse?

**Figure 5.2** — Timing of male and female predatory mite dispersal with or without (control) the presence of conspecific prey. Panels show cumulative proportions of dispersers over time. A: Early-dispersal isofemale line; B: Late-dispersal isofemale line. Black lines with rhombus are females, grey lines with triangles are males, solid lines are treatments with conspecifics (cannibalism), interrupted lines are control (no cannibalism).

**Figure 5.3** — Numbers of larvae consumed a): per female or male predator and b): per female or male cannibal of Early (ED) and Late (LD) dispersal line. Boxes indicate the second and the third quartile, horizontal lines separating the boxes indicate the medians, whiskers above and below the box indicate the 90th and 10th percentiles. Different letters indicate significant differences (GLMER, P<0.05).
DISCUSSION
Cannibalism and dispersal are two important phenomena that affect the population-genetic structure and are dependent on densities of the cannibals and victims, but also of their food (Fox 1975; Polis 1981; Otroen and Hanski 1983; Ellner et al. 2001). Although many studies focus on how kin recognition and kin interaction can affect decisions of individuals to cannibalize (Pfennig 1997; Faraji et al. 2000; Schausberger and Croft 2001) or to disperse (Hamilton and May 1977; Lambin et al. 2001), to the best of our knowledge, this is the first experiment in which these two behaviors are studied together. Contrary to expectations, our results did not show any statistically significant differences in cannibalism or dispersal behavior between the Early-dispersal and the Late-dispersal line, therefore do not support our hypothesis that a Killer line would be more prone to cannibalize than a Milker line.

Male predators were more prone to cannibalize (FIGURE 5.1), whereas females engaged less in cannibalism by dispersing earlier from the leaf (FIGURE 5.2). One explanation for the observed differences between the genders might be differences in the behavior of males and females. *Phytoseiulus persimilis* reproduces by pseudo-arrhenotoky, which means that females need to be inseminated before they can produce eggs (Helle et al. 1978; Sabelis and Nagelkerke 1988). Furthermore, gravid female predators cannot oviposit when cannibalizing (Yao and Chant 1989). Adult males, in contrast, do not need much food (Sabelis 1981), and do not search for food but for mates. Thus, males search for conspecifics; and mated females search for food, i.e., heterospecific prey, which they must find in other patches. When the local prey population is depleted, males can wait for immature females to mature and subsequently mate with them. While waiting, males can cannibalize to survive, preferably on immature males.

An alternative explanation for the observed differences in the cannibalistic behavior of males and females is based on the existing asymmetries in the relatedness of male and female parents with juveniles. In haplodiploid species such as *P. persimilis*, the cost of cannibalism regarding inclusive fitness loss is not balanced between the two genders. Because the females are diploid and the males haploid (only carrying genetic material from their mothers), there are asymmetries in relatedness with juveniles (Hamilton 1964a,b). Given that a local population consists of the offspring of one female and one male, the females will have more offspring than males, because the latter only contribute genes to daughters. Thus, females will suffer from greater inclusive fitness loss if they engage in cannibalism than do males. Hence we hypothesize that under food limitation, adult females are less prone to cannibalize than adult males and will more often choose to disperse in order to refrain from cannibalism. We expect that males would specifically cannibalize other, immature males, because this reduces future mate competition, and by letting female immatures live, they increase the chance on a future mate. This hypothesis, however, remains to be tested.

When cannibalizing, both genders appeared to be equally voracious (FIGURE 5.3b), but fewer females than males engaged in cannibalism (FIGURE 5.2) because they dispersed earlier. Hence, males dispersed later than females thus had more time to cannibalize. To assess whether males and females were equally voracious, we analyzed the proportion of larvae eaten, correcting for the time that the individuals remained on the patch, where they could cannibalize. This was done by including time until dispersal and gender as fixed factors and
their interaction into a mixed effects model. This analysis showed that gender had no significant effect (GLMER: $\chi^2 = 0.65$, d.f. = 1, $P = 0.42$), but that the proportion of larvae cannibalized increased significantly with the time that individuals spend on the patch before dispersing. This suggests that males and females were equally voracious when cannibalizing, and that differences in the numbers of larvae cannibalized were caused by differences in the amount of time spent on the patch. An interesting hypothesis that can be tested in haplodiploid species is that, given the asymmetries between the two genders in the relatedness with their potential victims, whether males cannibalize more often after having encountered a victim.

Besides finding no difference in cannibalism between the two lines, we also did not find statistically significant differences in dispersal behavior. At first sight, this is strange because the lines were selected for different dispersal tendencies and had different prey exploitation strategies (Revynthi et al., Chapter 3). However, this selection took place in the presence of heterospecific prey, and dispersal in the current experiments was measured without prey. In fact, both Milkers and Killers are expected to disperse when the prey population is depleted (Pels and Sabelis 1999), and Pels (2001) also found no differences in dispersal behavior between a Milker-like and a Killer-like line in the absence of prey.

Theoretical studies that focus on the evolution of dispersal and cannibalism (Pels 2001; Lion and van Baalen 2007; Rudolf et al. 2010) predict that selection on dispersal results in a genetically correlated effect on cannibalism. Our results, however, do not support this prediction at least with respect to our selected lines. The current experimental study on dispersal and cannibalism gives new insight into the cannibalistic behavior of the two genders and we therefore propose that future theoretical studies should take these differences into account.

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REFERENCES
CHAPTER 5


General discussion

This thesis extends our knowledge of exploitation and dispersal strategies in predator-prey interactions, using an acarine predator-prey system. The novelty of my study lies in three elements. First, I show that it is possible to select for differences in dispersal strategies, i.e., the Milker and Killer strategy, and this offers the opportunity to study the evolution of alternative exploitation strategies. Second, I found that prolonged culturing of predatory mites can affect their cannibalistic behavior especially in females. Third, I experimentally linked cannibalism and dispersal and investigated how these phenomena affect each other.

Prudent predation, exploitation and dispersal strategies

The exploitation of a growing food source by a consumer population at a rate that maximizes the long term yield is defined as prudent predation (Slobodkin 1968). A predator population can show prudent exploitative behavior either by decreased feeding on its prey or by part of the population dispersing away from it. Both strategies lead to a decrease in the predation rate and allow the local prey population to increase. However, prudent predation is evolutionary unstable (Maynard Smith 1964) because prudent predators are outcompeted by selfish predators. Therefore, prudent predation is only a viable strategy when the prudent predators are kin-related to the other individuals in the patch. Thus, although the fitness of the prudent individuals could be increased by being less prudent, their inclusive fitness increases by being prudent. In this thesis I focus on the second form of prudent predation, i.e., dispersal of part of the predator population before prey depletion. When a predator finds a prey patch, it is faced with a dilemma: which strategy is the best for the predator as well as the predator offspring? Is it better to adopt a selfish strategy by exploiting the prey population fast and disperse only after prey extermination, or is it more profitable to adopt a prudent strategy by dispersing before prey depletion, thereby decreasing the predation rate on the local prey population and allowing this population to grow? This dilemma is known as the Milker-Killer dilemma (van Baalen and Sabelis 1995a).

The predator’s decision regarding which exploitation and dispersal strategy to adopt is affected by external factors such as prey density, the dynamics of the prey population, seasonality, connectivity of local populations, intraspecific interactions; as well as by group and kin selection. Here, I discuss how ephemeral factors, group and kin selection can affect predator exploitation and dispersal strategies. In doing so, I address the six questions that were presented in the general introduction (CHAPTER 1).

1. Which is the best exploitation strategy for a predator, to milk or to kill?

The short answer is that it depends on the probability of invasion by predators with a different exploitation strategy and on the initial predator-prey ratio in a prey patch. The Milker
strategy is a prudent exploitation strategy. Theoretical predictions show that this strategy cannot be evolutionary stable within a local population when the invasion probability by Killer predators is high (van Baalen and Sabelis 1995a). When Killer predators invade a patch inhabited by Milkers, they disperse only after they have consumed all the prey that the Milkers have set aside for future use by their kin. Hence, the Milker strategy can only be evolutionarily stable at a metapopulation level when local prey populations are sufficiently isolated from each other (van Baalen and Sabelis 1995a; Pels and Sabelis 1999; Pels et al. 2002).

Initial prey and predator densities in a local patch can also affect predator exploitation strategies. Van Baalen and Sabelis (1995a) calculated for which combinations of initial predator-prey ratios in the patch, predator and prey growth rate and predation rate the Milker strategy can evolve. Their simulations showed that the Killer strategy is generally favored by selection, but there is also space for the Milker strategy to evolve. The authors found that the Milker strategy can evolve when the probability of co-infestation of a prey patch by another female is low. When a predator population is founded by a single female, selection will favor prudent predation because the predator population will be only consisted of relatives. When the probability of subsequent invasions by other females is high, however, selection will favor predators that rapidly exploit the local prey patch, i.e., Killers.

Predator exploitation strategies can be affected by prey density, but prey density is, in turn, also affected by their own exploitation strategy. The Milker-Killer dilemma also occurs among prey exploiting a food source. Prey can exploit their food in a prudent (Milker) way, allowing the food to grow, resulting in a longer interaction – or in a selfish (Killer) way, causing fast overexploitation of the food. The best strategy for prey as well as for predators is a result of the interaction between the prey and predator strategies and it is not clear which of the two species gains the upper hand.

2. Can we find Milkers in nature?
Theory provides some evidence for the evolution of prudent exploitation strategies, such as the Milker strategy (van Baalen and Sabelis 1995a; Pels et al. 2002). But the question whether we can find this exploitation strategy in nature still remains. Pels and Sabelis (1999) collected nine populations of *P. persimilis* along the coast and one inland population on Sicily (Italy). They show that all predator strains exterminated local prey populations, and the timing of dispersal appeared to have a genetic basis: one isofemale line derived from a coastal strain consistently showed dispersal close to or after prey elimination, whereas individuals of an isofemale line derived from the inland strain consistently dispersed long before all prey were eliminated. Even though these two isofemale lines differed in their dispersal rate and their interaction period with the prey, they did not show the predicted differences in the cumulative number of dispersers: early dispersal did not result in higher overall numbers of dispersers. In Chapter 2, I presented a more thorough survey of predator dispersal behavior in natural populations and quantified the extent of variation in dispersal strategies from these populations. The collected strains differed in their dispersal rate, but the differences in the dispersal rate did not result in differences in the interaction period between the prey and the predators or in the cumulative number of dispersers, as predicted by van Baalen and Sabelis (1995). One may argue that the predictions of the Milker-Killer dilemma are not
accurate due to the simplifying assumptions of the model, such as a constant predator dispersal rate. The results of Chapter 3, however, revealed that selected lines for different timing of dispersal do show the population dynamical consequences predicted by theory. What then can we conclude about the existence of the Milker strategy in nature?

The experiments described in Chapters 2 and 3 were conducted in a climate room under stable environmental conditions and controlled interspecific interactions. In nature, however, abiotic and biotic conditions are involved. Seasonality can have a large impact on the abundance of predator-prey populations and as a result, also on the presence of Milkers and Killers. The Mediterranean region is characterized by wet mild winters and dry hot summers. Temperature and humidity are known to affect *P. persimilis* (Stenseth 1979), which requires high temperature and high relative humidity for its development. *Tetranychus urticae* is known to develop better at moderate temperatures, i.e., 25-30 °C (Jeppson et al. 1975; Sabelis 1981). Unfavorable environmental conditions due to seasonality may affect predator abundance and, as a consequence, also initial predator-prey ratios in a local patch and connectivity among local populations. During winter and summer, the environmental conditions are not favorable for the predators or prey. Thus, we can expect that during spring months there are higher chances to find Milkers in nature because prey densities are relatively higher than in winter and summer and connectivity among local populations is not yet strong. Pels and Sabelis (1999) collected predatory mites in Sicily during May, whereas in the current study the predators that derived from Sicily were collected early June. One month may not seem a large period, but for a predator that has a life cycle of 7 days at 25 °C (Laing 1968; Sabelis 1981) a month results in four generations. Based on the theoretical predictions we expect to find Killers at the center of the geographic distribution, where predator densities are high; and Milkers at the boarders, where predator densities are low (van Baalen and Sabelis 1995a; Pels and Sabelis 1999). In practice, for *P. persimilis* it is still unknown which is its geographic distribution, thus it is very difficult to accurately estimate where we can expect to find Milkers and Killers. Pels and Sabelis in 1995 managed to collect predators from an inland isolated site 50 km away from the coast in Enna. This collected strain was the one dispersing before prey elimination, supporting the theoretical predictions about the evolution of the Milker strategy in isolated sites. The authors, however, in their article stated that this finding may be ‘sheerly coincidental’ and pointed out the need for further studies. I visited this site in 2014 but I did not find predators. Hence one may speculate that the presence of Milker-like strains is ephemeral.

Seasonality also affects the occurrence of other species that can be food competitors or intraguild predators. *Phytoseiulus persimilis* is not the only predator feeding on *T. urticae* in the Mediterranean region. Other natural enemies that feed on spider mites are phytoseiid mites such as *Neoseiulus californicus* (Escudero and Ferragut 2005) and *Iphiseius degenerans* (McMurtry 1977), and mirid bugs, such as *Macrolophus pygmaeus* (Perdikis et al. 2011). In addition, several of these predators can prey on *P. persimilis* (Walzer and Schausberger 1999, 2011; X. Zhang, pers. obs.). How predator exploitation strategies are affected by intraguild predation, i.e., when predation also occurs among predators that share a food source (Polis et al. 1989), is an open question. Which exploitation strategy is the best under these conditions? The predators are faced with the exact same dilemma as the with-
in-species Milker-Killer dilemma, including attacking and consuming their competitors (cannibalism vs. intraguild predation), but then among species.

3. Can cannibalism be assumed as a form of prudent predation?
When an individual decides to prey on its conspecifics instead of its primary food source, it decreases predation on the prey in two ways, resulting in more prudent predation. First, the cannibal feeds less on the food source and second, the cannibal removes other consumers. So, can cannibalism really be seen as another form of prudent predation? No, it cannot. Because prudent predation is favored when a patch is exploited by kin-related individuals, cannibalism in these patches will occur among kin, resulting in loss of inclusive fitness for the cannibal. Hence, prudent predation is favored by selection exactly when cannibalism is not. Several studies have shown that phytoseiids can discriminate between kin and non-kin and prefer to feed on the latter (Faraji et al. 2000; Schausberger and Croft 2001).

Although cannibalism does not appear to be a form of prudent predation, it might be used to avoid secondary invasions. When a local predator-prey patch is being exploited by a Milker population and is invaded by a single Killer predator, the Milkers could easily control the invasion by cannibalizing the offspring of the invader. Due to the higher resident predator population, this strategy can control the invasion and maintain the high relatedness among the individuals, which is necessary for the evolution of the Milker strategy.

4. Cannibalism and dispersal: which drives which?
There are two perspectives of the same situation – cannibalism is the selective process and dispersal the trait under selection, or vice versa. In both cases, kin selection will affect the outcome. Kin selection theory predicts that individuals that disperse away from their relatives to avoid feeding on them or by them in times of food scarcity will be favored by natural selection; by dispersing, they will not suffer from (inclusive) fitness loss (Hamilton 1964a,b). They may, however, suffer from fitness loss because dispersal is risky. Dispersal only after the prey extermination, can select for increased cannibalism; the predators due to lack of food may engage in cannibalism to prolong their life or acquire energy to disperse. In this case, kin selection theory predicts that individuals that are able to discriminate between kin and non-kin and feed on the latter will be favored by natural selection (Hamilton 1964a,b).

Pels (2001) assumes that cannibalism is the trait under selection while keeping dispersal fixed, or conversely, assumed that dispersal evolved while cannibalism is fixed. Pels’ model predictions show that predators that employ the Killer strategy are selected for a higher cannibalistic tendency (Pels 2001). The results of Chapter 5, however, do not support these predictions: the Milker and the Killer line that were used for these experiments did not differ in their cannibalistic behavior. The model of Pels (2001) wrongly assumes that only juveniles can cannibalize, whereas it is known that adult *P. persimilis* are the primary cannibals (Dosse 1958; Sabelis 1981). An extension of the model that includes the adult stage as cannibal might give more accurate predictions about the evolution of cannibalism in relation to different dispersal strategies. Furthermore, the differences between the two genders regarding their cannibalistic tendencies in Chapter 4 indicate that theoretical predictions should also account for different behavior depending on the sex. Reproduction in adult, mated females is through oviposition and requires food, whereas reproduction in adult males...
is through mating, thus we should not expect that the genders behave in a similar way under the same conditions. Moreover, in haplodiploid species, there are asymmetries in the relatedness between individuals depending on the gender. Despite the results of Chapter 5, I cannot conclude that dispersal and cannibalism are genetically uncoupled, because they might be correlated but not always expressed to the same extend. Depending on the local patch conditions that the predators face, they can engage more or less in cannibalism. Studying cannibalism under different conditions, i.e., with heterospecific prey present, might have given me a clearer answer.

5. Milkers and Killers in biocontrol?

The Milker-Killer dilemma can be important for biological control. In the current thesis, I did not present experiments directly linked to biological control, nevertheless, I will discuss how we can use the current knowledge to optimize the use of *P. persimilis* in greenhouses. The first question that comes to mind when we link the Milker-Killer dilemma to biocontrol is which strategy is the most suitable for efficient biological control. The answer, however, is not straightforward; it depends on many factors, which I will discuss further.

At first, one may think that since the local predator-prey populations are unstable the desired strategy for efficient biological control of spider mites is that of the Killer, because these predators eradicate the local prey population rapidly and do not allow the prey population to increase in size. However, due to their low dispersal rate, they will not be efficient in providing control on other attacked plants that are further away from the point of release. In contrast, predators that employ the Milker strategy by dispersing more during the local interaction with the prey will find and colonize more prey patches, thus providing control that extends over a larger part of the greenhouse. Milker predators will not control locally the spider mite infestation as fast as Killer predators, but over time they will occupy more other infestations. Based on this, it becomes clear that the method of release and the extent of the infestation will affect the decision to use Milkers or Killers to achieve good control.

If predators are released everywhere in the greenhouse, using Killers can result in efficient control. If, however, predators are released locally, where symptoms of the infestation are present, then it is better to use Milkers, because these predators can disperse further away from the point of release, persist longer in the greenhouse, and find other prey patches.

*Phytoseiulus persimilis* was adopted as a biocontrol agent by growers due to its ability to control small as well as large infestations within a reasonable time. Depending on the crop, but also the scale of the infestation, the growers decide when and how many predators they release to achieve the desirable results (Parrella et al. 1999). The pest density at which action should be taken to prevent reaching the economic injury level depends on the host plant. Vegetable crops have a higher economic threshold than ornamentals and can be sold even when they have been lightly infested (Parrella et al. 1999). Ornamentals, such as the roses used in the current study, cannot be sold if they show damage. Thus, a mass release of a Killer line of *P. persimilis* throughout the crop can be efficient for controlling the two-spotted spider mites in ornamentals, because it will eradicate the prey rapidly. In contrast, a spider mite infestation on a vegetable crop, such as cucumber, does not necessarily require the use of a Killer line. Because some increase in prey densities on cucumber plants can be tol-
erated, a Milker line may control local prey populations sufficiently and can persist longer in the crop, since the predators will start dispersing before prey elimination. There are examples where the release of *P. persimilis* takes place only after the prey are present (the so-called ‘Pest-in-First’ method) or even sometimes predators and prey are introduced simultaneously on the crop in order to enhance predator establishment (Parrella et al. 1999).

Although *P. persimilis* has covetable traits as biocontrol agent of *T. urticae*, such as its ability to cope with the spider mite web, the growers do face a problem. Because of its high dependence on the prey, *P. persimilis* goes extinct after the prey are overexploited. Thus, farmers need to repeatedly release the predators to prevent spider mite outbreaks. These releases increase costs and growers take this seriously into account before they decide to use this predator as a biological control agent. Predatory mites that employ the Milker strategy may offer a solution to this problem. Milker predators can persist longer than Killer predators and therefore have higher chances to be present when another spider-mite outbreak appears. Furthermore, due to their high dispersal tendency they can be released on a local infestation, but they will disperse to the surrounding plants as well. The growers may not notice prey patches in an initial phase, but dispersing predator females would find them and therefore provide better biological control. Of course, Milker predators can only be used when the host plant has a high economic threshold and the infestation is at an initial phase. In greenhouse experiments the Early-dispersal line, which behaves as a Milker line, controlled spider-mite infestations equally well as the Koppert line, which is a more Killer-like line (Chapter 2), but produced more dispersers (Revynthi et al. unpubl. data). Hence, a Milker line has the ability to control a mite infestation well at a local scale but provides protection of plants that are located further away from the point of release.

Other important parameters are prey dispersal and the cultivation system that the grower is using, which affects the plant distribution over the greenhouse. As was already discussed in a previous section of this thesis, the prey also have exploitation and dispersal strategies, and the ability of predators to control spider-mite infestations is affected by the behavior of the prey. The planting system that is used by the grower can also affect the efficiency of biocontrol. Highly dense plant systems provide good connection of the local prey population and accommodate predator dispersal (Takafuji 1977); hence Killer-like host exploitation strategies are favored. Plant systems that allow sufficient space among plants or rows can result in more Milker-like host exploitation strategies and reduced predator dispersal (Takafuji 1977).

### 6. Cannibalism in biocontrol: is it really a problem?

Overexploitation leads to food scarcity and an alternative way to obtain food and nutrients under these conditions is cannibalism (review in Fox 1975; Polis 1981; Schausberger 2003). It is known that *P. persimilis* starts feeding on conspecifics after exterminating its prey. Several studies have investigated the cannibalistic behavior of this mite species (Yao and Chant 1989; Walzer and Schausberger 1999; Schausberger and Croft 2001; Schausberger 2007; Schausberger and Hoffmann 2008), but none of them discussed the potential interference with biological control. The question that arises is whether and in which way cannibalism can affect biological control.
Cannibalism can affect biological control either negatively or positively. If the predators have high cannibalistic tendency and initiate cannibalism before prey depletion, then biocontrol is negatively affected. When predators cannibalize before prey are exterminated, they remove potential biological control agents that can contribute to the control of spider-mite infestation. When prey are exterminated, however, cannibalism may have a positive effect on biocontrol. This predatory species can persist for more than 2 weeks in the presence of water (15.7 days on average at 26 °C; Sabelis 1981) hence, when it feeds on its conspecifics it can persist even longer on the crop and be present if another spider-mite outbreak appears. The results of CHAPTERS 4 and 5 show that predators that are deprived of food for at least 48 h engage in cannibalism, even when they have the option to disperse. Moreover, in CHAPTER 5 I showed that predators will engage in cannibalism despite their dispersal behavior.

Phytoseiulus persimilis is massively cultured and sold by several biocontrol companies. The predators can be cultured in open or closed systems. Open systems allow the prey and predators to freely disperse from one plant to another and this may even result in selection for high dispersal tendency, since the prey are allowed to disperse and colonize new hosts. As a result, selection for cannibalism is not expected because when prey are locally exterminated, the predators can disperse and find a new patch. In closed mass cultures, barriers prevent the predators from dispersal. This method concomitantly selects artificially for predators with a low dispersal tendency that can deplete prey population fast, i.e., Killer predators (Elliot et al. 2002). As a consequence of the rearing method, these predators could also have increased their cannibalistic tendency. Because predator densities in this type of cultures eventually become high, prey becomes limited, thus there is high competition for food but also for mates. Therefore the predators are also selected for higher cannibalistic tendency, because via cannibalism they can obtain food and remove competitors. The results of CHAPTER 4 support this hypothesis and show that prolonged culture in closed systems increase the cannibalistic tendency of the predators. Nowadays, biological control companies are interested in lines of Phytoseiulus persimilis that are able to quickly control local spider mite infestations, have high dispersal tendency and low cannibalistic tendency. The findings of CHAPTER 3 can provide a basis for breeding programs to create Milker- or Killer-like strains that can be used depending on the specific situation, i.e., infestations in ornamental or vegetable crop.

The Milker-Killer dilemma in a broader context
The Milker-Killer dilemma is broadly applicable to predator-prey systems that are characterized by local overexploitation and metapopulation dynamics, typical for many arthropod predator-prey systems (Hassell 1978). Analogies may also be found in host-parasite interactions, extending to systems such as parasitoid-host and pathogens-host systems. In the same way as a predator can drive local prey to extinction rapidly or slowly, a parasite can reproduce fast in its host, thereby decreasing the host’s longevity, or it can reproduce more slowly without affecting the longevity of its host much. This results in a continuum ranging from extreme virulence to extreme avirulence (van Baalen and Sabelis 1995a; Sabelis et al. 2002).

The trade-off hypothesis states that the parasites have to pay a cost when they increase the duration of infection by being avirulent (Anderson and May 1982). This hypothesis
describes the trade-off between parasite transmission and virulence – higher transmission increases parasite fitness, whereas higher virulence decreases parasite fitness, as the parasite overexploits its host quickly (Anderson and May 1982; Frank 1996; Alizon et al. 2009). When a parasite exploits its host alone, selection will favor the prudent exploitation strategy. However, in the case of multiple infections, the prudent exploitation strategy is not evolutionarily stable (Hamilton 1972). Less virulent parasites are outcompeted by more virulent parasites, hence in cases of coinfection, natural selection will favor increased virulence (Frank 1996; van Baalen and Sabelis 1995b). Regardless the exploitation strategy, the question is still the same: how can a parasite kill its host and yet not go extinct? Before the host dies or becomes resistant, the parasite should infect another host (primary or secondary) or a vector, otherwise it will go extinct.

The analogies among parasite-host interactions and local predator-prey interactions are quite obvious; however, they have different spatial structures. In predator-prey systems, the prey and predators often show a similar spatial structure, i.e., a prey patch can become a predator patch, and their metapopulations are of the same spatial scale. In contrast, in host-parasite and especially in host-pathogen systems, one host often contains a local population of the parasite or pathogen, so the spatial scales of their populations differ. For the host, a metapopulation consists of several host populations, but for the pathogen a metapopulation can consist of several individual hosts belonging to the same population. These differences may have consequences for the evolution of exploitation strategies, for example, because similar exploitation strategies as in the predators may occur within a prey population, which implies that the prey, rather than the predator, may determine the duration of the local interaction, offering less opportunities for the evolution of different predator exploitation strategies. Although there is much literature on the evolution of virulence in viruses and pathogens, we lack studies on the evolution of alternative exploitation strategies in arthropod systems.

**Conclusions – Future perspectives**

In this thesis I studied how exploitation strategies of predators can be affected by both timing of dispersal and their cannibalistic tendency. I showed that there are differences in dispersal and cannibalistic behavior between the two genders in haplodiploid systems, which should be taken into account. In this chapter I mentioned possible applications of the Milker-Killer dilemma to biological control and a generalization of the theory to other systems that are characterized by overexploitation. Nevertheless, many questions remain. An interesting question that asks for an answer is what happens when a Killer predator invades a patch of Milker predators. Theory predicts Milker predators will then be outcompeted by Killer predators (van Baalen and Sabelis 1995a); however, we lack experimental evidence. Furthermore, Pels et al. (2002) show that the Milker strategy can be evolutionarily stable in spatially structured environments and at a metapopulation level. Up to today, we lack genetic markers that we could use to discriminate between strains with different dispersal strategies. To the best of my knowledge, there are no genetic markers for dispersal behavior in *P. persimilis*. If these markers would exist, it would greatly help in designing experiments where Milker and Killer strains will be brought into competition to test the predictions of Milker-Killer theory.
The experiments presented in Chapter 4, give a different perspective to cannibalism in haplodiploid species. Male cannibalism was studied in *P. persimilis* for the first time. As was briefly mentioned in the section *Can cannibalism be assumed as a form of prudent predation?*, all the experiments so far, including Chapters 4 and 5, show the propensity to cannibalize. We need more experiments that will offer the predators or their offspring the choice to escape cannibalism and then study whether they will engage in it. In Chapter 5, I quantified cannibalism in relation to dispersal, but in an artificial system without heterospecific prey. A good extension of this experiment would be the addition of *T. urticae*, which will give us the opportunity to study how the exploitation behavior can be affected by cannibalism. Moreover, the consequences of the differences in the behavior of males and females in addition to the asymmetries regarding inclusive fitness loss in haplodiploid species for population dynamics and evolution deserve further study.

Finally, greenhouse experiments can answer questions about the efficiency of Milkers and Killers in biocontrol. Variation of the initial predator-prey ratio, but also distribution and connectivity of the local patches can provide information on how to optimize the use of *P. persimilis* under greenhouse conditions but also to experimentally test the predictions of the Milker-Killer dilemma. Furthermore, we can use this knowledge to define a stable mixture of Milkers and Killers that can provide a better biological control. Application of the Milker-Killer dilemma to different biocontrol situations might allow for more efficient control in more crops. This action, however, would probably also require more information from the grower regarding the state of the infestation, the use of pesticides and other biological control agents before utilizing a Milker or a Killer strain. Direct application of the Milker-Killer dilemma as well as greenhouse experiments would give perspective for better biological control, but also would give ample opportunities to test theory.

**References**


Summary

Overexploitation is the harvesting of a resource at a rate which is higher than the resource renewal rate and is a phenomenon that occurs at various trophic levels. For example, a local plant population can be driven to extinction by a herbivore population and a local herbivore population can be overexploited by a predator population. In nature, however, species can persist despite local extinctions, because of dispersal among local patches, which together form a metapopulation. As long as the dynamics in the various local patches is asynchronous, there will always be patches with individuals. In predator-prey interactions, early dispersal of some predators after some reproduction decreases the predation rate and as a consequence the offspring of the dispersed predator will have more food available, resulting in a longer interaction period between the predators and their prey in the local patch. Conversely, late predator dispersal drives the local prey population to extinction faster, resulting in a shorter interaction period between the predator and its prey. The early dispersal strategy is called ‘Milker’, and the late dispersal strategy is called ‘Killer’, which are the extremes of a continuum of exploitation strategies. The Milker-Killer dilemma theory describes the effects of dispersal on local population dynamics. In this theory, the Milker strategy is presented as a form of prudent predation that can only be evolutionary stable when the probability of subsequent invasion of a patch by a Killer is low. This is because predators that employ the Killer strategy outcompete Milkers in local patches by exploiting the prey population faster. Nevertheless, predators of both strategies eventually will drive the local prey population to extinction.

When the local prey population is depleted, an alternative way for the predators to temporarily obtain food is through cannibalism. Such intraspecific predation is common in a wide range of animal taxa and may significantly affect population dynamics. Even though cannibalism and dispersal are important phenomena for the persistence of populations and can be driven by overexploitation, they hardly ever have been studied together, while they are likely to mutually affect each other. In this thesis, I used an acarine predator-prey system and I experimentally tested the Milker-Killer dilemma. In addition, I investigated predator behavior under conditions where the predators could cannibalize, disperse, or both and to study how dispersal and cannibalism interact.

In Chapter 2, I present a comprehensive survey of predator dispersal and exploitation strategies using strains of the predatory mite *Phytoseiulus persimilis* that I collected in Turkey and Sicily. My aim was to investigate whether there were differences among the collected strains in dispersal behavior and whether these differences would result in the local population dynamics predicted by the Milker-Killer theory. I found significant variation in the exploitation and dispersal strategies among predator populations. However, none of the collected strains showed the extreme Killer or Milker strategy. The results suggest that there is genetic variation for prey exploitation and dispersal strategies. Thus, different dispersal strategies in the Killer-Milker continuum may be selected for under natural conditions, which affects the predator-prey dynamics in local patches and is likely to determine persistence of the system at the metapopulation level.
In CHAPTER 3, I asked whether it is possible to select for Milker-like and Killer-like predatory mite lines. I aimed to explore whether artificial selection for early and late timing of dispersal results in differences in the dispersal rate between the two selected lines, and whether this affected the interaction period and the number of dispersers that are produced. I showed that 6 rounds of selection for early or late timing of dispersal resulted in predator lines displaying earlier or later dispersal. In a population dynamics experiment, I furthermore showed that selection for timing of dispersal also resulted in the predicted differences in the local interaction time with the prey and in the cumulative number of dispersers. I conclude that timing of dispersal is a heritable trait that can be selected for and results in lines with quantitative differences in local predator-prey dynamics.

In CHAPTERS 4 and 5, I focused on the cannibalistic behavior of *P. persimilis* and how this behavior could affect its dispersal tendency. Cannibalism can delay dispersal of the cannibals but accelerate dispersal of individuals that stand to be cannibalized. In CHAPTER 4 I asked to what extent prolonged culturing of predators affects the cannibalistic behavior of adult females and males. I tested two contradictory hypotheses about adult cannibalism on juveniles. The first hypothesis predicts that strains that have been in culture for a long time have higher tendency to cannibalize than strains in the field, because laboratory strains are unintentionally selected for higher tendency to cannibalize due to food limitation and lack of opportunities to disperse. This prediction will hold especially for females because they are the first to disperse from a patch when food is scarce. The second hypothesis is based on the increase in kin-relatedness among individuals of populations that were cultured for a long time. Owing to this increase in relatedness, I expected lower rates of cannibalism in strains that have been in culture for a long time than strains that were recently collected from the field. Using a laboratory and a field strain in a closed system, I observed the behavior of adult predators that were offered conspecific larvae as prey. The results show that males engaged more often in cannibalism than females, and females of the strain with a long culture history engaged more in cannibalism than those of the recently collected strain, both in agreement with the first hypothesis. To test the second hypothesis, I created an isofemale line from each strain and compared the cannibalistic behavior of adult male and female predators. The cannibalistic tendency of the strains and their isofemale lines was similar, suggesting that kin-relatedness did not affect their cannibalistic behavior. These results did not support the second hypothesis.

In CHAPTER 5 I investigated cannibalism in lines that were selected to employ different dispersal and exploitation strategies. In this chapter I asked (1) when food is limited and given the option to disperse, will predators choose to cannibalize or not and to disperse or not? And (2) if the predators cannibalize when they have the option to disperse, which line and gender of *P. persimilis* is more voracious towards its conspecifics? Using wind tunnels, I observed the behavior of adult predators with different dispersal strategies, when offered conspecific larvae as prey. The results show that both selection lines had similar dispersal and cannibalistic behavior, but differences occurred between males and females. Male predators stayed significantly longer in the patch and engaged more often in cannibalism, whereas females departed earlier and often did not cannibalize. I hypothesized that this result may be caused by differences in the biology of males and females. *Phytoseiulus persimilis* is a
pseudo-arhenotokous species and therefore females require insemination and relatively large amounts of food to be able to oviposit. Adult males, in contrast, do not need much food, thus do not search for food but for mates. Hence, males search for females, which they can do on the local patch, and mated females search for food, which they could only find on other patches. While searching for mates, males also encounter more potential victims to cannibalize, whereas females disperse and thus encounter fewer potential victims. An alternative explanation for the observed differences in cannibalistic tendencies between males and females is based on the existing asymmetries in the relatedness of parents with their offspring. In haplodiploid species like *P. persimilis*, the cost of cannibalism regarding inclusive fitness loss is higher in females than in males. The diploid females have more offspring than the haploid males, because the latter only contribute genes to daughters. Thus, it is expected that females will suffer from greater inclusive fitness loss when engaging in cannibalism. Hence I hypothesize that under food limitation, adult females are less prone to cannibalize than adult males and will more often choose to disperse in order to refrain from cannibalism.

In this thesis, I tried to answer how exploitation strategies of predators are shaped by both timing of dispersal and cannibalistic tendency. In addition, I showed that there are differences in dispersal and cannibalistic behavior between the two genders in haplodiploid systems, which require further study.

In the general discussion (Chapter 6) I tried to address questions about the evolution of prudent predation and cannibalism, as well as the applied aspect of the Milker-Killer dilemma to optimize biological control of spider mites. The Milker-Killer dilemma is broadly applicable to systems that are characterized by local overexploitation and metapopulation dynamics and analogies may also be found in host-parasites interactions. I briefly discuss the evolution of virulence, similarities and differences between predator-prey and host-parasite interactions. Future experimental and theoretical studies may help to answer questions about the evolution of prudent predation and the role of genders in this process. In addition, greenhouse experiments can answer questions about the efficiency of predators with different exploitation strategies in biocontrol.
Samenvatting

Overexploitatie is het oogsten van een voedselbron met zo’n intensiteit dat de bron zichzelf niet in stand kan houden, en dit is een fenomeen dat zich op verscheidene trofische niveaus voordoet. Ter illustratie, een locale populatie planten kan tot uitsterven worden gebracht door een populatie herbivoren en een locale populatie herbivoren kan overgeëxploiteerd worden door een populatie rovers. Soorten kunnen onder natuurlijke omstandigheden echter blijven voortbestaan ondanks dit proces van locale extincties omdat individuen dispergeren tussen locale leefgebieden die tezamen een metapopulatie vormen. Zo lang de dynamiek in de verschillende locale leefgebieden uit fase verloopt, zullen er altijd leefgebieden bevolkt zijn met individuen. In het geval van rover-prooi interacties verlaagt vroege dispersie van sommige rovers de predatiegedruck en als gevolg daarvan hebben de nakomelingen van de gedispergeerde rovers meer voedsel beschikbaar hetgeen resulteert in een langere interactieperiode tussen de rovers en hun prooi in het locale leefgebied. Late dispersie van rovers daarentegen leidt sneller tot locaal uitsterven van de prooipopulatie, resulterend in een kortere interactieperiode tussen de rovers en hun prooi. De vroege dispersie strategie wordt ‘Melker’ genoemd en de late dispersie strategie ‘Moordenaar’; dit zijn de extreem in een continuum aan exploitatie strategiën. De theorie van het Melker-Moordenaar dilemma beschrijft de effecten van dispersie op de locale populatie dynamiek. De Melker strategie staat in deze theorie voor een vorm van prudente predatie die alleen evolutionair stabiel kan zijn wanneer de kans dat een Moordenaar een locaal leefgebied met Melkers binnendringt klein is. De reden hiervoor is dat rovers met de Moordenaar strategie de Melkers wegeconcurren in locale leefgebieden door de prooipopulatie sneller te exploiteren. Desalniettemin zullen rovers van beide strategieën de locale prooipopulatie doen uitsterven.

Wanneer de locale prooipopulatie uitgeput raakt, hebben de rovers een alternatieve manier om voedsel te komen door kannibalisme. Dergelijke intraspecifieke predatie komt algemeen voor bij een waaier aan diergroepen kan een bepalende invloed hebben op de populatiedynamiek. Hoewel kannibalisme en dispersie belangrijke factoren zijn voor het in stand houden van populaties en aangedreven kunnen worden door overexploitatie, zijn deze factoren vrijwel nooit in samenhang bestudeerd, ondanks het feit dat ze elkaar waarschijnlijk wederzijds beïnvloeden. In dit proefschrift gebruik ik een rover-prooi systeem van mijten om experimenteel het Melker-Moordenaar dilemma te toetsen. Ik heb rover gedrag bestudeerd onder condities waar de rover kannibaliseren, dispergeren of beide, en de samenhang onderzocht tussen dispersie en kannibalisme.

In Hoofdstuk 1 presenteer ik een uitgebreide inventarisatie van dispersie en exploitatie strategiën van stammen van de roofmijt *Phytoseiulus persimilis* die ik verzameld heb uit natuurlijke populaties in Turkije en Sicilië. Mijn doel was om te onderzoeken of er verschillen waren in dispersie gedrag tussen de verzamelde stammen en of deze verschillen zouden resulteren in de locale populatiedynamica zoals voorspeld door de Melker-Moordenaar theorie. Ik vond significante variatie in de exploitatie en dispersie strategiën onder de rover stammen. Geen van de verzamelde stammen echter vertoonde de extreme Melker of Moordenaar strategie. De resultaten suggereren dat er genetische variatie is voor

In Hoofdstuk 2 vraag ik of het mogelijk is om te selecteren voor Melker-achtige en Moordenaar-achtige lijnen van roofmijten. Mijn doel was om na te gaan of artificiële selectie voor vroege of late timing van dispersie leidt tot verschillen in de dispersiesnelheid tussen de twee geselecteerde lijnen, en of dit effect heeft op de interactieperiode en het aantal geproduceerde dispergeerders. Ik laat zien dat 6 rondes van selectie voor vroege of late timing van dispersie resulteerde in lijnen van rovers die vroege of late dispersie vertonen. In een populatie-dynamisch experiment laat ik bovendien zien dat selectie voor timing van dispersie ook leidde tot de voorspelde verschillen in de lokale interactietijd met de prooi en in het cumulatieve aantal dispergeerders. Ik concludeer dat timing van dispersie een erfelijke eigenschap is waarvoor geselecteerd kan worden en dat resulteert in lijnen met kwantitatieve verschillen in locale rover-prooi dynamiek.

In Hoofdstukken 3 en 4 heb ik me geconcentreerd op het kannibalistisch gedrag van P. persimilis en de manier waarop dit gedrag haar neiging tot dispersie kan beïnvloeden. Kannibalisme kan leiden tot uitstel van dispersie van de kannibalen maar ook tot versnelde dispersie van individuen die kans lopen gekannibaliseerd te worden. In Hoofdstuk 3 vraag ik in welke mate het langdurig doorkweken van rovers het kannibalistische gedrag van volwassen mannetjes en wijfjes beïnvloedt. Ik testte twee tegenstrijdige hypotheses over kannibalisme door adults op juvenielen. De eerste hypothese voorspelt dat lijnen die lang doorgekweekt zijn in het lab een hogere neiging vertonen tot kannibalisme dan lijnen uit het veld, omdat laboratorium lijnen ongewild worden geselecteerd voor een hogere mate van kannibalisme vanwege voedselgebrek in combinatie met gebrek aan mogelijkheden te dispergeren. Deze voorspelling is met name van toepassing op wijfjes omdat zij de eerste zijn om uit een voedselplek te dispergeren wanneer voedsel schaars wordt. De tweede hypothese is gebaseerd op de toename van verwantschap tussen individuen van populaties die langer doorgekweekt zijn. Vanwege deze toenemende verwantschap verwacht ik een lagere mate van kannibalisme in lijnen die lang doorgekweekt zijn dan in lijnen die recent uit het veld verzameld zijn. Ik heb het gedrag bestudeerd van adulen rovers van een lab en een veld lijn in een gesloten systeem waar ze larven van dezelfde soort als prooi aangeboden kregen. De resultaten tonen aan dat mannetjes vaker kannibaliseerden dan wijfjes, en wijfjes van de lijn met een lange historie van doorkweken vertoonden meer kannibalisme dan die van de recent verzameld lijn. Beide bevindingen zijn in overeenstemming met de eerste hypothese. Om de tweede hypothese te toetsen, heb ik een isogene lijn gecreëerd van elke lijn en het kannibalistische gedrag vergeleken van adulte mannetjes en wijfjes van de rover. De neiging tot kannibalisme van de isogene lijnen was vergelijkbaar met de respectievelijke lijnen waaruit ze gecreëerd, hetgeen suggereert dat verwantschap geen effect had op het kannibalistische gedrag. Deze resultaten gaan in tegen de tweede hypothese.

In Hoofdstuk 4 heb ik kannibalisme onderzocht in lijnen die waren geselecteerd op verschillende dispersie en exploitatie strategieën. In dit hoofdstuk vroeg ik: (1) als voedsel gelimiteerd is, en de mogelijkheid tot dispersie gegeven wordt, kiezen rovers dan om te kannel
nibaliseren of niet, en om te dispergeren of niet? En (2) als de rovers kannibaliseren wanneer ze de mogelijkheid hebben om te dispergeren, welke lijn en welk geslacht van *P. persimilis* is meer roofzuchtig jegens zijn soortgenoten? Met behulp van wind tunnels heb ik het gedrag van adulte rovers met verschillende dispersie strategieën geobserveerd, terwijl ze larven van dezelfde soort als prooi kregen aangeboden. De resultaten laten zien dat beide selectielijnen vergelijkbaar dispersie en kannibalisme gedrag vertoonden, maar verschillen traden op tussen mannetjes en wijfjes. Mannelijke rovers bleven significant langer in de voedselplek en begingen vaker kannibalisme, terwijl wijfjes eerder vertrokken en zich vaak onthielden van kannibalisme. Ik hypothetiseer dat dit resultaat veroorzaakt kan worden door verschillen in de biologie van mannetjes en wijfjes. *Phytoseiulus persimilis* is een pseudo-arhenotoke soort hetgeen betekent dat wijfjes bevrucht dienen te worden voor reproductie, en relatief veel voedsel nodig hebben om eieren te kunnen leggen. Adulte mannetjes, daar- entegen, hebben niet veel voedsel nodig en zoeken dus niet zozeer naar voedsel als wel naar paringspartners. Dientengevolge zoeken mannetjes naar wijfjes, die ze kunnen vinden op de locale voedselplek, en gepaarde wijfjes zoeken naar voedsel, hetgeen ze alleen kunnen vinden in andere voedselplekken zodra de locale plek uitgeput raakt. Mannetjes komen dan meer potentiële slachtoffers tegen om te kannibaliseren tijdens hun zoektocht naar paringspartners, terwijl wijfjes dispergeren en dus minder potentiële slachtoffers tegenkomen. Een alternatieve verklaring voor de geobserveerde verschillen in kannibalistische neigingen tussen mannetjes en wijfjes is gebaseerd op de bestaande asymmetriëën in de verwantschap van ouders met hun nakomelingen. In haplodiploïde soorten als *P. persimilis* zijn de kosten voor kannibalisme, met betrekking tot verlies aan ‘inclusive fitness’, hoger voor de moeder dan voor de vader. De diploïde moeders hebben meer nakomelingen dan de haploïde vaders, omdat die laatste alleen genen bijdragen aan dochters. Daarom ligt het in de verwachting dat moeders meer *inclusive fitness* verliezen wanneer ze kannibaliseren. Ik stel dan ook de hypothese dat adulte wijfjes minder geneigd zijn tot kannibalisme bij gebrek aan voedsel dan adulte mannetjes en vaker zullen verkiezen te dispergeren om zich niet aan kannibalisme over te geven.

Dit proefschrift is gericht op het beantwoorden van de vraag hoe exploitatie strategieën van rovers worden gevormd door zowel timing van dispersie als kannibalistische neigingen. Daarnaast heb ik laten zien dat er verschillen bestaan in dispersie en kannibalistisch gedrag tussen de twee geslachten in haplodiploïde systemen, hetgeen nadere studie vereist.

In de *Algemene Discussie* behandel ik vragen over de evolutie van prudente predatie en kannibalisme, alsmede de toegepaste aspecten van het Melker-Moordenaar dilemma voor optimale biologische controle van spintmijten. Het Melker-Moordenaar dilemma is breed toepasbaar op systemen die gekenmerkt worden door locale overexploitatie en metapopulatie dynamiek, en analogieën kunnen ook gevonden worden in gastheer-parasiet interacties. Ik bediscussieer kort de evolutie van virulentie, overeenkomsten en verschillen met roverprooi en gastheer-parasiet interacties. Nadere studies, zowel experimenteel als theoretisch, kunnen helpen om vragen te beantwoorden over de evolutie van prudente predatie en de rol van geslachten in dit proces. Bovendien kunnen experimenten in kassen vragen beantwoorden over de efficiëntie van rovers met verschillende exploitatie strategieën voor biologische bestrijding.
Author contributions and project funding

1 – Prey exploitation and dispersal strategies vary among natural populations of a predatory mite
A.M. Revynthi, M. Egas, A. Janssen & M.W. Sabelis
MWS – originally formulated the idea; MWS, AMR – designed the experiments; AMR – sampled the mites used for this study and performed the experiments; AMR, ME, AJ analyzed the data and wrote the manuscript.

2 – Timing of aerial dispersal is a heritable trait in the predatory mite *Phytoseiulus persimilis*
A.M. Revynthi, D. Verkleij, A. Janssen & M. Egas
AMR, DV, ME – designed the experiments; AMR, DV – conducted the experiments; AMR, DV, AJ, ME – analyzed the data and wrote the manuscript

3 – Gender-specific differences in cannibalism between a laboratory strain and a field strain of a predator
A.M. Revynthi, A. Janssen & M. Egas
AMR – designed and conducted the experiments; AMR, AJ – analyzed the data; AMR, JA, ME wrote the manuscript

4 – To cannibalize or disperse? Males cannibalize and females disperse in the predatory mite *Phytoseiulus persimilis*
A.M. Revynthi, K. van Pol, A. Janssen & M. Egas
AMR, KvP – designed and conducted the experiments; AMR, KvP, AJ, ME – analyzed the data and wrote the manuscript

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Curriculum vitae

Alexandra was born and raised in Athens, Greece, embraced with family and friends, many linked to the beautiful island of Serifos, in the western Cyclades, her preferred place on earth. She attended high school at Chosteas-Geitonas School, where very early, at the age of 11, she became interested in Horticulture. Upon graduation, she began bachelor studies in Plant Sciences, and very rapidly developed a strong interest for mites. In 2006, she enrolled in the Department of Crop Science, Agricultural University of Athens, where she specialized in Plant Protection and Environment and obtained a degree in Agricultural Engineering and Agricultural Sciences in 2011 (equivalent to an MSc.). During her program, she was granted four years of consecutive scholarships from the Union of Greeks who come from Egypt.

Before graduating, she conducted several internships that provided her with basic skills on acarology research and exposure to multiple aspects of commercial agriculture. Upon graduation Alexandra begins correspondence with Prof. Maurice Sabelis who eventually became her mentor and major graduate advisor. She then moved to the Netherlands and became a guest researcher at the Institute for Biodiversity and Ecosystems Dynamics, Group of Population Biology, University of Amsterdam. In her first year in Amsterdam, she conducted research on chemical communication and antipredator behavior of Thrips (*Frankliniella occidentalis*). In view of her capacities as a researcher, Prof. Sabelis then offers her to join the Research Group of Population Biology and start a Ph.D. program to study dispersal strategies and the evolution of prudent predation using the predatory mite *Phytoseiulus persimilis*. During her time as a Ph.D. student she gained knowledge in statistics, food web dynamics, life history theory and molecular techniques. Her contributions to the group include providing supervision to two masters and one bachelor students as well as teaching assistance in the course of Ecophysiology and Evolutionary Biology. She has also demonstrated the clarity of thought needed to clearly communicate scientific findings in thirteen international scientific meetings.

Alexandra is ready for the next step in her professional career, equipped with 5 languages (Greek, English, German, Dutch and Spanish) and a strong interest in acarology, entomology, nematology, behavioural ecology, population dynamics, evolutionary ecology, chemical communication, biological control and integrated pest management.