Bio-systematics of predatory mites used for control of the coconut mite
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Citation for published version (APA):
Famah Sourassou, N. (2012). Bio-systematics of predatory mites used for control of the coconut mite
General introduction

Over the past 50 years there has been considerable progress in the use of phytoseiid predators for control of mite pests in agricultural crops (Helle and Sabelis 1985; Lindquist et al. 1996; Gerson et al. 2003). Essential to the development of these biocontrol methods is to provide a reliable description of the control agent (Moraes 1987; McMurtry 2010). A description can be obtained from taxonomic studies that are based on morphological criteria for species identification. However, taxonomic identification of species alone does not always suffice because populations of the same predator species may largely differ in their capacity to control the pest. A recent example is the finding that the predatory mite *Phytoseiulus longipes* from Brazil and Argentina can control *Tetranychus evansi* on tomato, but *P. longipes* from Chili and South-Africa cannot (Tixier et al. 2010). These between-population differences in control capacity appear to have a genetic basis. Another example, is the presence of five cryptic species from different hosts and geographic localities (*Euseius tularensis* from citrus, *E. hibisci* from avocado, *E. quetzali* from deciduous fruits and nuts, *E. obispersis* from avocado in San Luis Obispo county, *E. stipulatus* introduced from the Mediterranean region) in what was once assumed to be a single species (*E. stipulatus*) (McMurtry and Badii 1985; McMurtry 2010). However, there may also be within-population genetic variation with respect to genetic traits relevant to biological control. For example, it is shown that at a scale of 0.25 m² soil a population sample of the predatory mite *Hypoaspis aculeifer* exhibits a genetic polymorphism in preference for two species of astigmatic mites, *Tyrophagus putrescentiae* and *Rhizoglyphus robini*, that are pests in crops of lily bulbs (Lesna and Sabelis 1999, 2002). Thus, there is a need to identify and characterize the genetic entities (strain, population, biotype, ecotype, host race, geographic race, subspecies, cryptic species, species) that are relevant for assessing traits relevant to biological control (McMurtry 2010).

Before deciding on relevant genetic entities it is useful to briefly introduce and define some terms because there is still room for confusion in the general literature. First and foremost, is the biological species concept (Mayer 1940; Mallet 1995), which defines a species as a group of organisms capable of interbreeding and producing fertile offspring of both sexes and separated from other such groups with which interbreeding does not normally happen. With respect to the Phytoseiidae most species have been described solely based on morphological criteria (Chant and McMurtry 2007) and only some species have been studied for their reproductive isolation. However, not all isolating mechanisms are immediately apparent. For example, the presence of endosymbionts and their role in incompatibility between populations has long gone unnoticed, but now we know that this mechanism can isolate populations even when geographical-
ly very close to each other (see Hoy and Cave 1988 for an example in phytoseiid mites). Such isolating mechanisms cause reduced gene flow between populations and may give rise to genetic differences and incipient speciation (Telschow et al. 2005). This has led to the notion of cryptic species (complex of species), which are defined by Stebbins (1950) as ‘population systems which were believed to belong to the same species until genetic evidence showed the existence of isolating mechanisms separating them’. Grant (1981) defined cryptic species as ‘good biological species that are virtually indistinguishable morphologically. Cryptic species have been found in the Phytoseiidae based on morphological studies combined with crossbreeding studies (Muma and Denmark 1976; Mahr and McMurtry 1979; McMurtry et al. 1985; Congdon and McMurtry 1985), with behavioural studies (Beard 1999) and with molecular-genetic studies (Tixier et al. 2003, 2004, 2006, 2008). Whether these cryptic species are also sibling species, requires in depth phylogenetic analysis using a variety of morphological, behavioural and genetic traits. Such an analysis is still lacking with respect to the Phytoseiidae. Instead, classifications in the Phytoseiidae are purely based on morphological traits (Chant and McMurtry 2005).

Geographically isolated populations may or may not be reproductively isolated. They are often referred to as geographic races, subspecies or semispecies, but their status as a species is unclear until it is known whether they are reproductively isolated when the races become sympatric (Diehl and Bush 1984). The same applies to terms like host races, ecotypes, biotypes and strains. These terms are used to indicate that populations differ conspicuously in some genetically determined traits. Such differences are thought to result from reduced gene flow between populations and thus at least partial genetic isolation. However, the extent to which the partial isolation operates is not always known. Because gene flow is hard to assess and there is a practical need to distinguish populations with different traits (Clark and Walter 1995), the terms referred to above will persist, but it should be clear that the categories blend into one another (Diehl and Busch 1984).

For the purpose of biological control it is surely interesting to know whether populations differ in a genetic trait relevant to this objective. However, releasing such a strain, ecotype, biotype or geographic race in another area with a morphologically similar population is problematic if reproductive isolation is not ensured. This is why I focus on reproductive incompatibility between geographic populations and assess their traits as far as I think they are relevant to biological control.

Hybridization studies have been conducted between populations or strains of phytoseiid species to test for reproductive compatibility. Such studies challenge taxonomists to examine specimens more critically for morphological characters. This is illustrated by a case study on *Typhlodromus exhilaratus* (Ragusa), which appeared to harbour two cryptic species, *T. exhilaratus* and *T. phialatus* (Athias-Henriot) (Tixier et al. 2006a). In other stud-
ies, synonymy was considered between phytoseiid mites previously identified as different species but showing full reproductive compatibility, for instance synonymy between *Amblyseius swirskii* (Athias-Henriot) and *A. rykei* (Pritchard & Baker) (Zannou et al. 2011), or between *Cytophromus idaeus* (Denmark & Muma) and *C. picanus* (Ragusa) (Tixier et al. 2011).

Reproductive barriers may arise from prezygotic isolation mechanisms (e.g., mate choice) or postzygotic mechanisms. Postzygotic incompatibility can result from genetic incompatibility (Navajas et al. 1999; Uesugi et al. 2003; Gotoh et al. 2005b; Pryke and Griffith 2008; Koevoets and Beukeboom 2009) or more specifically from endosymbionts, such as *Wolbachia* and *Cardinium* (Laven 1967; O’Neill and Karr 1990; Perrot-Minnot et al. 1996; Breeuwer 1997; Gotoh et al. 2007) as well as their interactions with nuclear genes (Gotoh et al. 1995). Endosymbiont-mediated reproductive incompatibility, so-called ‘cytoplasmic incompatibility’ is a sterility phenomenon that has been first reported between strains of insects (Laven 1959, 1967), and is now known to occur in a wide range of taxa. Two basic forms of cytoplasmic incompatibility are known: unidirectional (nonreciprocal) cytoplasmic incompatibility and bidirectional cytoplasmic incompatibility. Unidirectional (nonreciprocal) cytoplasmic incompatibility occurs in crosses involving infected and uninfected populations and results in normal progeny in one direction but in few viable hybrids or no progeny in the other. Bidirectional cytoplasmic incompatibility is observed in crosses between populations infected with different strains of endosymbionts (Perrot-Minnot et al. 1996). In the Phytoseiidae, unidirectional incompatibilities are the most common, and are associated with shrivelled eggs, a low number of eggs, low survival of immature stages and reduced fecundity in F1 individuals (if present) (Johanowicz and Hoy 1998; Norhona and Moraes 2004). Although compared to insects and isopods little is done to assess the cause of the reproductive incompatibilities in the Phytoseiidae, there is one case study on *Metaseiulus occidentalis* Nesbitt, demonstrating that *Wolbachia* mediates unidirectional incompatibility in phytoseiid species (Hess and Hoy 1982; Hoy and Cave 1988; Johanowicz and Hoy 1998). Yet, in the Phytoseiidae, no study has reported *Cardinium*-mediated cytoplasmic incompatibility, an endosymbiont well known from insects or other mite taxa (spider mites and false spider mites).

To discriminate between genetic entities a comprehensive biosystematic approach is required (McMurtry et al. 1976; McMurtry 1980). Over the past decade, molecular methods are being emphasized in systematics as tools to discriminate between populations and/or species. Such techniques are complementary to morphological analysis and crossbreeding studies, and vice versa. DNA-based tools are currently applied to detect genetic variation at any taxonomic level and to measure relatedness between taxa in mites. Mitochondrial DNA (mtDNA) provides useful markers for the genetic characterization and studies of phylogenetic relationships of...
organisms at different taxonomic levels (Hebert et al. 2003a,b; Tixier al. 2003, 2004, 2006b, 2008a,b; Okassa et al. 2010). DNA barcoding is a method proposed to classify animals to the species level using a fragment of mtDNA, usually the cytochrome oxidase subunit I (COI), well suited for population and species identification (Hebert et al. 2003a,b). The combination of morphological and molecular data has led to separation of species that were hard to distinguish based on morphological criteria only (e.g., Tixier et al. 2006b).

In this thesis I will focus on geographic populations as genetic entities and will study whether they are reproductively isolated from other populations, whether they are morphologically and/or genetically different and finally in which biocontrol-relevant traits they differ. Hence, I will perform morphological and molecular analysis, cross-breeding experiments and – in case populations are not compatible – also experiments to assess the causes of incompatibility (especially the role endosymbionts) (CHAPTERS 2-4). This approach has the practical advantage that genetic integrity of a promising population of biocontrol agents is maintained even after it is introduced in the habitat of another conspecific population. Finally, I will determine differences between populations and species in competitive abilities (CHAPTER 5) and in the traits thought to be relevant for biological control (CHAPTER 6).

Thesis outline
In this thesis, I focus on mainly two species of predatory mites (Acari: Phytoseiidae) that are found under the perianth of coconuts: *Neoseiulus paspalivorus* DeLeon and *N. baraki* Athias-Henriot. There, they feed on coconut mites (*Aceria guerreronis* Keifer) which in turn are plant parasites in that they consume the contents of meristematic cells in the tissue protected by the coconut perianth. Each of the two predator species has been found in the continents and countries explored for their fauna of predatory mites inhabiting coconuts (Fernando et al. 2003; Lawson-Balagbo et al. 2008; Reis et al. 2008; Fernando and Aratchige 2009; Banerjee and Gupta 2011; Negloh et al. 2011; Zannou et al. unpublished; Fernando et al. unpublished). Thus, they occupy the same niche in that they share coconut mites as prey and they are likely to be each other’s competitor. Based on morphological criteria these species belong to the same genus and they are closely related systematically (Zannou et al. 2006; De Moraes et al. 2004). These two predator species are considered to be candidates for the biological control of coconut mites. To provide a sound basis for identification and characterization of these natural enemies for utilization in biological control programs (Moraes et al. 1987; McMurtry 2010), I investigated several geographic populations of each of the two species. I assessed the degree of reproductive compatibility and examined the extent of morphological and genetic variation between these populations of predatory mites. For each of these populations, I studied some traits thought to be relevant for biological control.
In CHAPTER 2 three populations of predatory mites identified as *N. paspalivorus* – from Benin, Ghana and Brazil – were subject to a test on reproductive compatibility and multivariate analysis on 32 morphological characters. Multivariate morphometry is the most suitable approach for analyzing morphological variation on a wide set of morphometric and meristic traits. These methods are currently used to provide information on character variability within and between phytoseiid mite populations and to contribute to the taxonomy knowledge of the studied group (i.e., Tixier al. 2003, 2004, 2006b, 2008a,b; Okassa et al. 2010).

In CHAPTER 3 I investigate the genetic variation in the three populations identified as *N. paspalivorus* as well as the cause of their reproductive isolation. First, I used the mitochondrial cytochrome oxidase subunit I (COI) primers to assess the genetic variation between populations. Then, I used 16S rDNA primers to check for the presence of endosymbiotic bacteria *Wolbachia* and *Cardinium* in those populations, and to analyze the phylogenetic relationships of each endosymbiont from each host population and from other mite and insect populations of which the endosymbiont sequences are known from GenBank. In addition, an antibiotic test was also conducted to determine whether each symbiont can cause cytoplasmic incompatibility in its host.

In CHAPTER 4 I applied an integrative approach combining multivariate morphometry, crossbreeding and molecular analysis to four populations previously identified as *N. baraki*, one from Benin, two from Tanzania and one from Brazil.

In CHAPTER 5 I report on intraguild interactions observed in cross-pairing experiments conducted with the aim to assess reproductive barriers between *N. baraki*, *N. paspalivorus* and *N. neobaraki*. These intraguild interactions may play a role in niche competition between different species, but they are possibly also relevant in creating prezygotic barriers between populations of the same species.

In CHAPTER 6, I review the literature on predatory mites found under the perianth of coconuts and focus on the traits that are thought to be vital for their impact on coconut mite control. First, I assess the features of numerical abundance of the predator species. Then, I focus on interspecific and inter-population differences in size, life history on a diet of coconut mites or alternative food (pollen), as well as predation. Finally, I present a phylogenetic analysis for all populations of the three predator species based on COI sequences to test the hypothesis that they represent a complex species.

The general discussion (CHAPTER 7) of this Thesis is devoted to the question which of the geographic populations may have the best combination of the traits for biological control of coconut mites. Moreover, I try to provide different alternative scenarios for the worldwide invasion of the three species of predatory mites studied in this Thesis. Finally, I scrutinize the critical assumption underlying this Thesis that reproductive isolation is a fixed trait, rather than a context-dependent plastic phenomenon.
Acknowledgements
I am very grateful to Maurice W. Sabelis for his valuable comments on earlier versions of this chapter.

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