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Vojvodic, Svjetlana

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Svjetlana Vojvodic

University of Copenhagen
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Supervisor Jørgen Eilenberg
Co-supervisor Annette Bruun Jensen
PREFACE

This thesis has been submitted to The Faculty of Life Sciences, University of Copenhagen, in partial fulfillment of the requirements for the degree Doctor of Philosophy in the Department of Agriculture and Ecology, University of Copenhagen.

This thesis consists of an introduction, which describes the background and objectives of this thesis and the following three chapters are manuscripts written to report the findings of this PhD project. During the project I spent one month in USDA-ARS Logan, Utah, USA, hosted by Rosalind R. James.

Svjetlana Vojvodic
Copenhagen Denmark
August 2010
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ABSTRACT

Among honey bees (*Apis mellifera*) an exceptionally diverse set of parasites has been described that can lead to reduced productivity and colony failure. In recent years, due to the dramatic increase in worldwide losses of managed honey bee colonies, great emphasis has been placed on understanding honey bee parasites and host–parasite interaction. Certain diseases, such as the fungal brood–disease known as chalkbrood, persist in many honey bee colonies. Chalkbrood is caused by the fungus *Ascosphaera apis*, a specialist parasite that reproduces only after invading honey bee larvae. Other *Ascosphaera* species such as *Asc. proliperda* have been found to cause diseases only in solitary bee brood, while the avirulent pollen fungus *Asc. atra* has been commonly described in association with solitary bees. While obligate parasites such as chalkbrood can be expected to have evolved adaptations to exploit specific situations of reduced innate or behavioral host immunity, this is less obvious for facultative parasites. Several species of the fungal genus *Aspergillus*, such as *Asp. flavus*, are facultative parasites that kill honey bees in all stages of development and cause a honey bee brood disease known as stonebrood. In this thesis, I investigated the etiology and the evolution of chalkbrood disease; in particular I focused on how temperature and the presence of other types of fungal parasites shape the outcome of host-parasite interactions and parasite-parasite dynamics. In order to have controlled experimental conditions, I developed protocol for *in vitro* rearing of honey bee larvae that was used throughout this thesis.

In general, entomopathogenic fungi virulence, expressed as the number of infected and killed hosts, has been observed to depend on parasite concentration (dosage). In my first chapter I have shown that an increase in parasite concentration at infection had a more dramatic effect on host mortality induced by the facultative parasite causing stonebrood compared to the obligate parasite causing chalkbrood. In addition, previous colony infection trials with chalkbrood disease have indicated that accidental drops in brood temperature increase the prevalence of chalkbrood, but it has remained unclear whether parasite virulence is directly temperature-dependent. I have shown that chalkbrood and stonebrood inducing parasites use different strategies to invade the honey bee host. I found that a 24 hour cooling period after exposure to chalkbrood decreased larval survival, whereas such a cooling period improved survival of stonebrood exposed larvae. These results raise interesting questions about honey bee defenses against obligate and facultative parasites and about the extent to which stress factors in the host (dis)favor parasites with lesser degrees of specialization.

Infection with several parasites (mixed infections) can often result in increased virulence relative to single infections, possibly due to increased parasite densities or due to the inability of a host to defend itself against multiple parasites at once. In my second chapter, using the honey bee larva as a model organism, I investigated whether the virulence of *Asc. apis* can be altered by the presence of other *Ascosphaera* species that usually do not induce infections. Mixed infections of *Asc. apis* with *Asc. atra* - a pollen saprophyte of solitary bees, led to increased virulence compared to *Asc. apis* alone. In addition, mixed infections of *Asc. apis* and *Asc. proliperda* - the causative agent of chalkbrood in solitary bees, did not differ in virulence that was induced by *Asc. apis* alone.
This study demonstrated that some parasites that are thought to be avirulent may in fact interact with the virulent parasites, affecting the outcome of host-parasite interactions, and in some cases resulting in increased host mortality.

In my final chapter I found that both parasite strain and colony of larval origin affected mortality rates. Genetically different strains of chalkbrood caused different levels of larval mortality, suggesting that one level of virulence is not optimal for all host genotypes. Significant variation in host susceptibility to chalkbrood has also been observed across honey bee colonies. Together these results showed that chalkbrood and honey bee genotype interact to determine virulence, as expected under parasite-host co-evolution. From an applied perspective, understanding the timing and factors that trigger and mediate host-parasite interactions can offer important insight for the control and prevention of honey bee fungal infections. Finally, the results of my thesis indicate that the interaction between chalkbrood and honey bees, and chalkbrood and other fungal parasites provide an attractive model system to study host-parasite co-evolution in social insects.
RESUMÉ (ABSTRACT IN DANISH)

Der kendes et betydeligt antal forskellige parasitter fra honningbier (Apis mellifera), og flere af disse kan føre til nedsat produktivitet og tab af bifamilier. I de seneste år har der, grundet en dramatisk stigning i det globale tab af bifamilier, været fokus på at opnå bedre forståelse af honningbiens parasitter, samt vært-parasit interaktioner. Visse sygdomme, så som yngelsygdommen kalkyngel, optræder i mange bifamilier. Kalkyngel er forårsaget af svampen Ascosphaera apis, en specialiseret parasit, der kun reproduceres efter at have inficeret honningbilarver. Andre Ascosphaera arter, så som Asc. proliperda har vist sig kun at forårsage sygdomme i yngel fra enlige bier, mens den avirulente pollensvamp Asc. atra findes almindeligt i redesteder før enlige bier. Obligate parasitter, så som kalkyngel, må forventeligt have udviklet specielle tilpasninger for at udnytte de specifikke situationer, hvor værtens innate (medfødte) eller adfærdsmæssige immunitet er nedsat. Derimod er det mindre sandsynligt at fakultative parasitter udvikler specielle tilpasninger. Flere arter inden for svampeslægten Aspergillus, for eksempel Asp. flavus, er fakultative parasitter, der kan inficere honningbier i alle udviklingsstadier og kan forårsage yngelsygdommen stenyngel.


Generelt måles virulens af insektpatogene svampe som antal svampedræbte værter (af eksponerede), og det er kendt, at virulensen er afhængig af parasitkoncentrationen (dosis). I første kapitel viser jeg, at en øget parasitkoncentration af en fakultativ parasit, der giver stenyngel, har en mere dramatisk effekt på værtsdødeligheden i forhold til det obligate parasitter, der giver kalkyngel. Desuden har tidligere infektionsforsøg i hele kolonier med sygdommen kalkyngel antydet, at nedkøling af yngellejet øger prævalensen af kalkyngel, men det er stadig uvist, om virulensen er direkte temperaturafhængig. Forsøgene viste at kalkyngel- og stenyngelinducerende svampe bruger forskellige strategier til at invadere honningbiværten. Jeg fandt, at en 24-timers nedkølingsperiode efter eksponering af kalkyngel nedsatte larvernes overlevelse, hvorimod at samme nedkølingsperiode øgede larvernes overlevelse, når larverne blev eksponeret for stenyngel. Disse resultater rejser interessante spørgsmål dels om honningbiens forsvar mod obligate og fakultative parasitter, og dels om i hvilket omfang stressfaktorer i værten favoriserer parasitter med en mindre grad af specialisering.

Infektioner med flere parasitter på en gang (blandede infektioner) vil ofte resultere i øget virulens i forhold til enkelte infektioner. Muligvis på grund af øget parasittæthed eller på grund af værtens manglende evne til at forsvare sig mod flere parasitte på samme tid. I mit andet kapitel, hvor jeg atter brugte honningbilarver som modelorganisme, har jeg undersøgt om virulensen af Asc. apis ændres ved tilstedeværelsen af andre Ascosphaera arter, der normalt ikke inducerer infektion. Blandede infektioner med Asc. apis og pollensaprofytten Asc. atra resulterede i øget virulens i forhold til infektioner med Asc. apis alene. Derimod gav forsøg med Asc. apis og Asc. proliperda, der inficerer og forårsager
kalkyngel i enlige bier, ingen forskel i blandede eller enkelte infektioner. Dette forsøg
demonstrer, at organismer, der menes at være avirulente, faktisk kan interagere med
virulente parasitter og påvirke udfaldet af vært-parasitinteraktionen, og i nogle tilfælde
resultere i øget værtsdødelighed.

I mit sidste kapitel fandt jeg, at både parasitstamme og bifamilie, hvorfra larverne kom,
påvirkede dødeligheden. Genetisk forskellige kalkyngelstammer gav forskellig
larvedødelighed, hvilket tyder på, at et virulensniveau ikke er optimalt for alle
værtsgenotyper. Der blev også fundet signifikant variation af modtagelighed over for
kalkyngel på tværs af bifamilier. Disse resultater påpeger, at kalkyngel og
hønningbigenotyper interagerer og at samspillet er en vigtig faktor for udfaldet af
virulensen, som forventet i forhold til vært-parasit co-evolutionsmodeller. Fra et anvendt
perspektiv er forståelsen af timing og faktorer, der er afgørende for vært-
parasitinteraktioner, vigtig og kan give informationer af afgørende betydning for
bekæmpelse og forebyggelse af hønningbiens svampeinfektioner. Endeligt har
resultaterne af mine studier også vist, at samspillet mellem kalkyngel og hønningbier
samt kalkyngel og andre svampe parasitter er et attraktivt modelsystem, der kan bruges til
at studere vært-parasit co-evolution i sociale insekter.