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**The sympatric coexistence of two
reproductively independent lines of the
endoparasitic wasp *Venturia*
*canescens***

by

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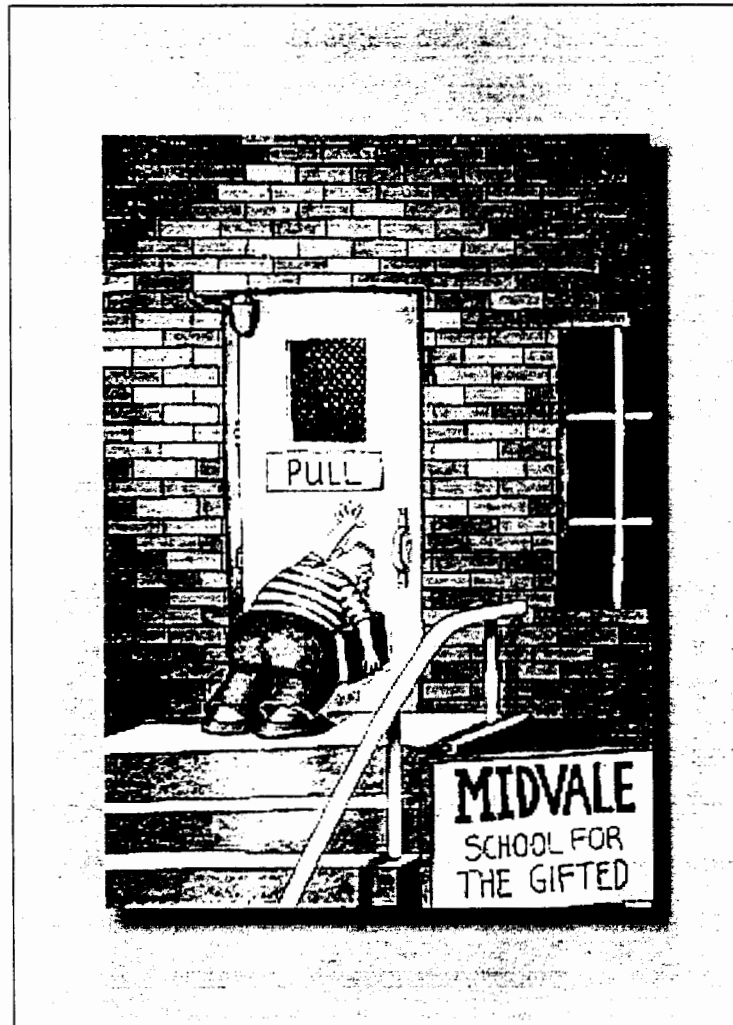
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Chapter One

Overview of the Thesis.



Gary Larson and Steve Martin, (2003) *The Complete Far Side*. Andrews McMeel Publishing, New York

The principle of competitive exclusion (Volterra, 1926; Gause, 1934) states that it is impossible for two species that are limited by the same resource to coexist indefinitely. While mathematical models incorporating non-linear phenomena suggest that sympatric coexistence is possible under certain conditions (e.g. Levins, 1979; Armstrong and McGehee, 1980; Durrett and Levin, 1998), the basic theory remains popular (Vandermeer *et al.*, 2002). In part, this is due to the numerous examples in the literature of competitive displacement of one species by another, but also because of the difficulty of demonstrating that there is no niche differentiation involved in those cases where species have been shown to be coexisting (see DeBach, 1966).

However, recent research has demonstrated that a laboratory culture of the asexual (=thelytokous) parasitoid wasp *Venturia canescens* contains two genetically distinct lines, coexisting on their host the flour moth *Ephesia kuehniella* (Hellers *et al.*, 1996; Beck *et al.*, 1999, 2000, 2001). The two lines are addressed as RP (repeat plus) and RM (repeat minus) for the presence or absence of a 54 base-pair tandem repeat sequence in the gene coding for a virus-like particle (VLP1) protein (Hellers *et al.*, 1996). The lines are genetically stable, and differ in a range of phenotypic characters, including ovarian morphology, calyx gland secretions and reproductive success (Beck *et al.*, 1999, 2000, 2001). Since the laboratory culture had been maintained without the addition of new stock for over 400 generations, the two lines must have been coexisting sympatrically while competing for the same resource.

The basis to the coexistence of the two lines appears to relate to differences in their reproductive success under single parasitism and superparasitism. In a simulation of laboratory conditions, Beck *et al.*, (1999) observed that when RM- and RP-wasps were allowed to compete for hosts for a 24-hour interval the RM-wasps produced significantly more offspring than the RP-wasps. However, under intra-line competition the RM-wasps produced significantly fewer offspring than the RP-wasps.

V. canescens lays eggs directly into the body of the larva of its host (=endoparasitic), where the developing parasitoid feeds on the haemolymph. When more than one egg is deposited into a host, it is described as superparasitised. However, irrespective of the number of eggs laid, at most a single wasp emerges from a host (= solitary parasitoid). When the time

interval between ovipositions is around three days or less, parasitoids fight for possession of the host using strong sickle-shaped mandibles to attack competitors, and the outcome is uncertain. For greater time intervals the older larva prevails, probably by suppressing the development of its younger adversary by anoxia (Fisher, 1961, 1963).

While the findings of Beck *et al.* (1999, 2000, 2001) suggest the system represents an exception to Gauss' Competitive Exclusion Principle, the following broad questions remain unanswered:

- 1) What is the basis of the higher reproductive success of the RM-strain under competing superparasitism?
- 2) How is the development of *V. canescens* in *E. kuehniella* modified under superparasitism compared to single egg parasitism?
- 3) Under what conditions will the reciprocal differences in reproductive success for the two strains lead to coexistence? Is the coexistence of the two strains an artefact of the laboratory rearing conditions, or can it occur in field populations?

The primary aim of the research undertaken during my candidature was to provide answers to these questions. A secondary aim of the research, through work performed in collaboration with a number of others, was to explore a fourth question:

- 4) What is the function of the VLP1 protein, and what role (if any) does it play in the phenotypic differences observed between the strains?

To achieve these aims, a number of specific issues within these broad questions were addressed.

I. The basis of the higher reproductive success of the RM-strain under competing superparasitism.

A possible explanation of the RM line's advantage may be based on the maternal phenotype and on both wasps laying multiple eggs in a single host, a common occurrence under laboratory culture conditions. Oviposition rates of up to 50 eggs per hour, continuing until the oviducts are largely

depleted of eggs, have been reported (Harvey *et al.*, 2001), including under conditions of intra-line superparasitism (Beck *et al.*, 2001). Regardless of the respective line, the eggs of the wasp ovipositing first hatch first. Interlarval physical combat between the siblings commences shortly after, so by the time the eggs of the second wasp hatch a number of the first wasp's offspring have already eliminated each other. The larvae of the second wasp at this point outnumber the larvae of the first wasp and so it is more likely that one of them will be the ultimate victor.

Compared to RP-females, RM-females delay ovipositing after being provided with access to hosts, and the RM-offspring display longer embryonic development times (Beck *et al.*, 1999), leading to a greater overall lag time between an RM-wasp being given access to hosts and her larvae hatching compared to an RP-wasp. Thus, the relative reproductive success of the two lines when competing for hosts may show an overall bias in the RM line's favour.

The general alternative explanation is that there is some physiological difference between the RM and RP lines that increases the probability of an RM larva winning a one on one encounter with an RP larva. Most plausibly, this would involve an advantage in one of the two previously identified modes of competition, either physical combat or physiological suppression.

Chapter 3 describes experiments conducted to distinguish between these two explanations. The study compared the reproductive success of the two lines when one egg from each line was laid into a single host, for a range of time intervals between ovipositions. The results showed that the RM-line won a significantly higher fraction (around 60%) of the overall contests, and further, that the competitive abilities of the two lines were not symmetric, indicating that the advantage of the RM-line relates to one-on-one interlarval competition rather than differences in maternal behaviour. Further, dissection of parasitoid larvae from superparasitised hosts indicated that most contests between competing larvae had occurred within the first 24 h of the eggs hatching, suggesting the advantage of the RM-line relates primarily to physical combat rather than to physiological suppression.

Chapter 4 details research to determine the nature of the phenotypic differences between the RM and RP larvae underlying the differences in

reproductive success described in chapter 3. The study examined the outcome of inter-larval physical combat under *in vitro* conditions. The results showed that the outcome depended on both the relative and absolute ages of the contestants, and that the competitive abilities of larvae from the two lines were not symmetric. In contests involving two larvae, at least one of which was newly hatched, the RP larva tended to lose, while if both larvae were at least 8-10 hours post-hatching when the contest occurred then the larger of the two larvae tended to lose. Thus, the higher reproductive success of the RM line under competing superparasitism with the RP line is due to a physiological difference between the newly hatched larvae of the two lines which results in an advantage to the RM larva, which occurs independent of the order or time interval between ovipositions.

II. The development of *V. canescens* in *E. kuehniella* under single egg parasitism and superparasitism.

One of the major aims of the research was to create a mathematical model of the interaction between the two strains. Having investigated the reproductive success of the two lines, it was next necessary to quantify the life history parameters of *V. canescens* in *E. kuehniella* under single egg parasitism and superparasitism.

Chapter 5 describes research documenting the influence of host mass and the time interval between ovipositions on the survival and development of larvae from both the first and second laid eggs in superparasitised *Ephestia kuehniella*, in this case for two competitively similar strains. As the time interval between ovipositions increased both overall and superparasitism success decreased, however time between, and order of, ovipositions had little effect on other developmental parameters. Adult size increased with host mass under both parasitism and superparasitism, while host mortality decreased with host mass under superparasitism. In addition, wasps emerging from superparasitised hosts were larger than wasps from parasitised hosts.

Chapter 6 describes research documenting the previously unreported phenomenon of egg dumping by an endoparasitoid wasp when deprived of hosts. These data also provide another of the sets of life history parameters

required to model the interaction between the strains, the lifetime fecundity of female *V. canescens*. Female *V. canescens* maintained without hosts began to deposit eggs onto the sides of the culture vessel on the day of eclosion. The maturation of additional eggs was not inhibited once the maximum oviduct egg load was reached but rather continued for the duration of the experiment (up to 39 days), at a rate of around 5.8% of the remaining unmaturing eggs per day. When wasps were given access to hosts they matured additional eggs at an increased rate. Artificial damage to the ovipositor resulted in a reduced rate of egg maturation even though the oviducts were partly egg depleted, while damage to the auxiliary valvulae had no effect. These results suggest two conclusions. Under conditions of host deprivation the rate at which eggs are matured is determined by the rate of synthesis of precursors by the fat body that in turn is modified by feedback from the ovipositor, induced by physical stimulation. Further, the discarding of eggs is due to the involuntary unidirectional movement of eggs down the oviduct, facilitated by the ongoing maturation of additional eggs.

III. A mathematical model of the sympatric coexistence of two phenotypically distinct strains of *V. canescens*.

Having identified the basis of the differences in the reproductive success of the two lines under intra- and inter-line parasitism, and having quantified the relevant life history parameters, it was then possible to construct a mathematical model of the interaction between the two strains.

Chapter 7 presents the results of an iterative model that uses a range of experimental life history data to predict the stable composition of a mixed population of two lines displaying the laboratory phenotypes under different rates of superparasitism. Historically, the impossibility of showing that two species do not occupy separate niches has precluded any demonstration of sympatric coexistence in the field. The model predicts that sympatric coexistence of the two lines is possible when the overall rate of superparasitism is between 4 and 12% or greater. These values are within the rates reported for other solitary endoparasitoid wasp species in the field, and so demonstrate that the sympatric coexistence under natural conditions of two

species that display the phenotypes observed in the laboratory lines is, in principle, possible.

IV. The function of the VLP1 protein, and its role in the phenotypic differences observed between the strains.

The two *V. canescens* lines differ the presence or absence of a 54 base-pair tandem repeat sequence in the gene coding for a virus-like particle (VLP1) protein that is co-injected with the egg into the host (Hellers *et al.*, 1996). However, the role (if any) the VLP1 plays in the phenotypic differences observed between the strains is not clear.

Chapter 8 describes research on whether the *VLP1* gene is genetically associated with the phenotypes displayed by the RM and RP strains. The recent isolation of facultative sexual (arrhenotokous) and asexual *V. canescens* strains from the same location in Southern France enabled an investigation of the genetic basis for the observed phenotypic differences, by comparing the two asexual lines with the corresponding homozygous *VLP1* genotypes in arrhenotokous strains. This analysis showed similar patterns of morphological and functional differences exist in the ovaries of the two asexual *VLP1* lines and in the two homozygous *VLP1* genotypes from the field, suggesting that the *VLP1* gene alteration either causes the ovarian phenotype or is genetically closely linked to the putative gene. However, the *VLP1*-gene may not be the only gene contributing to the phenotypic effects observed in the asexual lines. Although the two *VLP1*-alleles segregate with the relative differences in the ovary distribution of eggs, the absolute egg numbers differ in the corresponding asexual and sexual genotypes. This suggests that an additional unlinked gene may be involved in the transfer of eggs from the ovarioles into the oviduct.

Chapter 9 details research that describes phenotypic changes in an RM strain following alteration in culturing conditions. Maintaining the RM line under conditions of low superparasitism resulted in rapid alterations in the phenotypic characters calyx eggload, egg maturation rate, and reproductive success under single egg and under competing superparasitism. The observed changes were not associated with changes in the RM-VLP1 allele, and in general are difficult to explain in terms of genetic change. The simplest

explanation for the observed phenotypic changes is that the RM phenotype is related to the action to a pathogen, specifically, an interaction between the RM genotype and a semi-permissive endosymbiont, whose transmission is vertical via maternal secretions from adult wasp to host and then horizontal from host to larval parasite. The data are also consistent with a mechanism based on maternal transmission of an inducible phenotype.

Chapter 10 discusses the findings of the research in the context of the two general explanations of the RM-phenotype, that the RM-phenotype is due to an endosymbiont or pathogen acting in some unknown fashion (Amat *et al.*, 2003), or due to pleiotropic effects of the allelic VLP1 gene locus (Beck *et al.*, 2000, 2001).

Other research conducted

During my candidature I was involved in several other projects, that were not directly related to the investigation of the sympatric coexistence of the two strains of *V. canescens*, but which led to a number of other publications. These are presented in *Appendix 1*, in order to give a full representation of the work I performed during my time as a PhD candidate.

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