Genetics of arrhenotokous and thelytokous reproduction in Venturia canescens (Hymenoptera)

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CHAPTER 8

Summary and conclusions

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Egg development without fertilization occurs in all Hymenoptera. Most species reproduce by arrhenotoky (haploid males, diploid females), in which males develop parthenogenetically from unfertilized haploid eggs. However, females develop from unfertilized eggs under thelytokous reproduction which require cytological mechanisms that maintain or restore diploidy. Most thelytokous hymenopterans have a form of automictic parthenogenesis involving fusion of meiotic products (automixis) to restore the diploid number of chromosomes in the egg. This process can occur in three different ways (terminal fusion, central fusion and gamete duplication) which have severe consequences for heterozygosity in the following generation (Suomalainen *et al.*, 1987; Beukeboom & Zwaan 2005). Apomixis is less common in hymenopterans: thelytokous offspring are mitotically produced and the offspring are genetically identical to their mother.

Thelytoky can be classified in two categories according to the causal mechanism: microbe-induced and genetically determined. Several bacteria are now known to induce reproductive alterations in hymenoptera such as thelytoky, cytoplasmic incompatibility, male killing and feminization. *Wolbachia* has been the most widely studied of these microorganisms (e.g. O'Neill *et al.*, 1997; Stouthamer *et al.*, 1999), a growing number of studies show that *Cardinium* and bacteria of the order *Rickettsia* also induce the same reproductive alterations (e.g. Hagimori *et al.*, 2006; Perlman *et al.*, 2006). The cytological mechanism through which *Wolbachia* restores diploidy is gamete duplication (e.g. Stouthamer & Kazmer, 1994; Pannebakker *et al.*, 2004), but exceptions have also been reported (Weeks & Breeuwer, 2001). Although *Cardinium* and *Rickettsia* induce the same phenotypes as *Wolbachia*, the cytological mechanisms of diploidy restoration appear to be different (Adachi-Hagimori *et al.*, 2008). Genetically determined thelytoky occurs in some hymenopteran species but very little is known about the genetic regulation. An exception is the cape honey bee where the possible involvement of the thelytoky gene (*th*) as a transcription factor has recently been reported (Lattorff *et al.*, 2007). More information about the genetic basis of thelytoky is required for a better understanding of the evolutionary dynamics of parthenogenesis and the twofold cost of sex paradox.

Thelytokous reproduction in Hymenoptera can have a number of important implications for the genetic make-up of individuals and the amount and structure of genetic variation in populations. Although several different cytological mechanisms are involved, all automictic forms are believed to increase the level of homozygosity within individuals and populations. However, we are only at the beginning of understanding the interplay between the genetic consequences of the cytological mechanism of thelytoky and the population level processes that shape genetic variation in thelytokous populations. This requires more detailed population genetic studies with a larger array of thelytokous species.

The parasitoid wasp *Venturia canescens* is an example of a species with genetically determined thelytoky, whereas arrhenotokous individuals also occur. Previous studies on *V. canescens* have uncovered a number of basic differences in life-history

traits between arrhenotokous and thelytokous individuals, which may facilitate their co-occurrence (Schneider *et al.*, 2002). However, genetic aspects of thelytokous reproduction have hardly been considered which is however crucial for understanding the evolution and persistence of thelytokous reproduction.

Beukeboom & Pijnacker (2000) already showed that thelytoky in *V. canescens* is not caused by *Wolbachia*. In Chapter II I confirm that thelytoky in *V. canescens* is not caused by *Wolbachia* but also show that no other prokaryotic endosymbionts are involved. Antibiotic and temperature curing of thelytokous lines combined with PCR amplification with specific *Wolbachia* and universal prokaryotic primers gives clear evidence that no prokaryotic endosymbionts are present in the ovaries of *V. canescens*. Reineke & Asgari (2005) reported a small RNA containing virus (VcSRV) in the thelytokous strains of *V. canescens*. I checked for the presence of this virus in arrhenotokous and thelytokous wasps and found it to be present in wasps of both reproductive modes. This indicates that the VcSRV virus is not related to thelytoky in *V. canescens*. I further discuss the possible pitfalls of the experimental set up of trying to show the absence of endosymbionts. We conclude that (most likely) thelytoky in *V. canescens* is not caused by endosymbionts but instead has a genetic basis. These results formed the basis for the study reported in **Chapter VI** (see below).

At the beginning of this project I tried to cross arrhenotokous males with thelytokous females as indicated by Schneider *et al.* (2003). In this way arrhenotokous genes would be introgressed into a thelytokous background and the genetics of thelytoky could be studied in the way it has been done for the cape honey bee (Lattorff *et al.*, 2005). Unfortunately the crosses performed by Schneider *et al.* (2003) could not be repeated due to as yet unsolved reasons. Parallel to these crosses, I started developing microsatellite markers with the purpose of constructing a linkage map for location of genes of interest and for studying the genetic structure of arrhenotokous and thelytokous populations, as well as for the consequences of parthenogenesis on the genetic variation in this species. In **Chapter III** I report the development of 56 microsatellites for both reproductive modes. These markers were tested in arrhenotokous and thelytokous females, providing me with some initial information of the genetic make-up of individuals of both reproductive modes. Thelytokous females appeared to be more homozygous than arrhenotokous ones consistent with the mechanism of diploidy restoration that occurs in the thelytokous strains of *V. canescens* (central fusion automictic parthenogenesis, Beukeboom & Pijnacker, 2000).

The set of markers allowed for the construction of the first linkage map for *V. canescens* containing 29 microsatellites and 19 AFLP markers (**Chapter IV**). The linkage map has a resolution of 11 linkage groups, which is the haploid complement of *V. canescens* (N = 11), but the map is unsaturated and more markers are needed to improve its resolution. I have mapped two genes of interest in *V. canescens*: the Virus Like Particle (*vlp*) gene at the tip of linkage group II and the

complementary sex determining locus (csd) on linkage group IV. Virus Like Particles have been very well characterised in *V. canescens* and the polymorphism present in one of the genes has been used as a molecular marker in several studies. I have confirmed the prediction of Malmberg *et al.* (2000) about the *vlp* map location: *vlp* is more homozygous in thelytokous populations than in arrhenotokous as distal loci in a linkage group tip are expected to become homozygous over time under central fusion automixis. The *csd* locus shows linkage to marker *Vcan*071, but more fine scale mapping is needed to further locate and isolate this gene. I intended to study the homozygosation process in the thelytokous genome in more detail, but a more saturated map is required for this type of analysis. The current map provides a starting point for more detailed genetic studies in *V. canescens*, such as for future QTL mapping of behavioural and life history traits using divergent arrhenotokous populations or selection lines.

The unsuccessful outcome of the introgression experiments in which crosses between arrhenotokous males and thelytokous females did not yield hybrid offspring, made me reconsider the results of Schneider *et al.* (2002; 2003). Are the events of occasional sex that they describe common? How related are arrhenotokous and thelytokous populations? Does thelytoky arise frequently from arrhenotoky? Fortunately, I had access to individual samples from the same populations used by Schneider *et al.* (2002) and I expanded this study with some newly collected samples. I carried out a similar genetic diversity study, but this time I used different markers than AFLPs and RFLPs (microsatellites and COI mitochondrial sequences). In **Chapter V**, I show that genetic variation of thelytokous individuals is lower compared to that of arrhenotokous ones. As mentioned in **Chapter III**, this is due to the diploidy restoration process that takes place in thelytokous eggs; a process known as "genome homozygosation". I found a clear segregation of the two reproductive modes for both nuclear and mitochondrial markers which indicate that the gene flow suggested by Schneider *et al.* (2002) does not occur frequently in the field. This is consistent with my result that crosses between arrhenotokous males and thelytokous females under laboratory conditions do not easily produce hybrid offspring. The incongruence of the results of both studies may be due to the markers used: AFLPs used by Schneider *et al.* (2002; 2003) are dominant markers that may overestimate similarities between both reproductive modes in comparison with microsatellites used in this study due to non-homologous AFLP fragments of similar size. Furthermore, it is known that AFLP genotyping generates reproducible artefacts that can be mistaken for real bands. Mitochondrial sequences give more precise information on the haplotypes than RFLPs used in Schneider's study. My results suggest that one needs to be cautious with using AFLP markers for population genetics studies. The results of **Chapter V** indicate that there is one widespread thelytokous clone with little genetic similarity to arrhenotokous wasps. It is now apparent that genetic exchange between individuals of different reproductive mode is very rare.

In Chapter **VII** I present a study that compares fitness between females of the two reproductive modes. I found that thelytokous wasps invest more in egg production and superparasitism whereas arrhenotokous wasps spread their egg production over a longer time (having higher longevity). These results are consistent with available information about differences in life history between both reproductive modes (Chapter VI in Schneider, 2003). Thelytokous females are generally found in rich environments (e.g. bakeries) with high host density (references in Schneider *et al.*, 2002), while arrhenotokous wasps appear more adapted to field situations with fewer hosts at larger travel distances (Driessen *et al.*, 1995).

All results taken together and considered in the context of the evolution of sex theories (**Chapter I**) indicate that arrhenotokous and thelytokous populations are genetically differentiated. Thelytoky probably arises from arrhenotoky as a rare event and genetic exchange between the two modes is very rare. Arrhenotokous and thelytokous individuals inhabit different habitats and exploit different resources, and are therefore not direct competitors in the field. This explains the apparent coexistence of both reproductive modes in nature. Are these two reproductive modes so different that we should be considering them as two different species? I do not think so. If arrhenotokous and thelytokous individuals would belong to different species, it would be difficult to explain why two arrhenotokous individuals share the same mitochondrial haplotype as thelytokous ones (**Chapter V**) or the possible rare events of gene flow.

A different meiotic segregation pattern of chromosomes between unfertilized arrhenotokous and thelytokous eggs was observed by Beukeboom & Pijnacker (2000). RNA and protein comparison of unfertilised arrhenotokous and thelytokous eggs was used to gain insight into the genetic control of diploidy restoration in thelytokous eggs. In **Chapter VI** I present a description of putative transcripts and proteins involved in arrhenotokous and thelytokous egg development. More expression products are present in arrhenotokous ovaries indicating that this mode requires more complex processes and more gene products than under thelytokous development, such as processing the sperm nucleus after fertilization. Thelytokous females do not fertilise their eggs, or might do only very rarely upon mating with an arrhenotokous male. Apparently in most cases, they have lost this function due to mutation accumulation (e.g. Carson *et al.*, 1982; Pannebakker *et al.*, 2005). I found several genes and proteins that show an absence-presence differential expression between both reproductive modes. The explicit role of these candidate genes and proteins is difficult to assess in the context of diploidy restoration, fertilization or any other processes that differ between the reproductive modes. VLP2, tubulin and actin are the most likely candidates involved in diploidy restoration. These three proteins are associated with microtubule structure or microtubule mediated processes, as well as spindle formation and orientation which is consistent with the observations by Beukeboom and Pijnacker (2000). We are only at the beginning of elucidating the genetic regulation of parthenogenetic

reproduction and more detailed functional studies are needed to further characterise the role of putative genes and proteins in the context of diploidy restoration and (absence of) fertilization. Next generation genomic techniques including availability of whole genome sequences of an increasing number of organisms will help to reach this goal.

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