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

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General introduction and thesis overview

This chapter has been modified after: I. Mateo Leach, B.A. Pannebakker, M.V. Schneider, G. Driessen, L. van de Zande and L.W. Beukeboom. Thelytoky in Hymenoptera with *Venturia canescens* and *Leptopilina clavipes* as case studies. In: *Lost Sex*. I. Schön, P. van Dijk and K. Martens (eds). Springer (*in press*).

Sexual reproduction (fusion of male and female gamete) is the most common mode of reproduction among eukaryotic organisms. Asexual reproduction (parthenogenesis: females reproducing without males) is less common, but it is not rare and has evolved many times in many eukaryotic groups. If sexuality is so much more wide spread than asexual reproduction it must be advantageous to reproduce sexually. Many theories have been proposed in the past decades to explain the advantages of sexual reproduction. They can be divided into two groups: (a) sexual reproduction brings together good combinations of genes that allow a better adaptation or (b) sex purges deleterious mutations more efficiently. Recombination between chromosomes of two individuals is exclusive of sexual reproduction and brings together advantageous combinations of new mutations allowing a faster adaptation to changing environments (Fisher-Muller accelerated evolution theory, Fisher, 1930; Muller 1932; Red Queen hypothesis, van Valen, 1973; Tangled bank hypothesis, Ghiselin, 1974). In contrast asexual populations have the long term disadvantage of accumulating deleterious mutations (Muller's ratchet, Muller, 1964; Deterministic mutation hypothesis, Kondrashov, 1982) although certain forms of asexual reproduction fix heterotic combinations of genes and reduce gene loss (Slobodchikoff & Daly, 1971).

Not everything is advantageous in sexual reproduction. Sex is costly. Sexually reproducing individuals need time to find mates (Bell, 1982) and have to invest time in mating (Daly *et al.*, 1978). This cost is reduced in hermafroditic that self-fertilise. Sex is associated with transmission of sexual diseases, breakdown of favourable gene combinations by recombination and possible negative interactions between both genomes (outbreeding effect). But, the most obvious cost of sex is the one of producing males. Sexual females produce sons and daughters, whereas asexual females produce only females, thus all her offspring will contribute to the next generation (cost of sex, Maynard Smith, 1971, 1978). Genes that confer parthenogenetic reproduction (asexuality) are expected to rapidly spread in a sexual population, because they are transmitted twice as efficiently as sexual genes. In the same way, asexual females transmit 100% of their genome to the next generation while under sexual reproduction the genome is diluted by half every generation (cost of meiosis, Williams, 1975).

Many modes of reproduction have evolved, such as diploidy and haplodiploidy. Diploidy (or amphimixis) is the most well-known mode of sexual reproduction, where males and females are both diploid and produced from fertilized eggs. Haplodiploidy occurs within the order Hymenoptera. Most species reproduce by arrhenotoky (sexual reproduction, see below) and more rarely by thelytoky (parthenogenesis or asexual reproduction, see below). In some species both reproductive modes can coexist and this raises many evolutionary questions since asexual reproduction has the (theoretical) short term competitive advantage over sexual reproduction. This makes some Hymenopteran species very suitable to test the maintenance of sex theories and to study the paradox of sex.

Reproductive modes in Hymenoptera

All species within the Hymenopteran order have a haplodiploid mode of reproduction. Males always develop from unfertilized eggs and are haploid. Females are always diploid and can develop from both fertilized and unfertilized eggs. Within haplodiploidy, arrhenotoky is the most common mode of reproduction: unfertilized eggs develop into males that are haploid and 100% related to their mother, whereas fertilized eggs yield diploid females with a haploid complement of both parents. Hence, virgin arrhenotokous females can reproduce, but produce all-male progenies. Mated females typically store sperm in the spermatheca and can control the sex of their offspring by allowing a sperm to fertilize the egg upon oviposition.

Thelytoky is a less common mode of reproduction. Thelytokous females develop parthenogenetically from unfertilized eggs after restoration of diploidy and are 100% related to their mother. Males do not occur. Arrhenotoky is thought to be the ancestral mode of reproduction and thelytoky has evolved from it in several groups independently (Cook, 1993; Godfray, 1994). Note that the terms arrhenotoky and thelytoky refer to sexual and parthenogenetic female production under haplodiploidy, respectively. Males are always produced parthenogenetically in arrhenotokous species only. Hence, arrhenotoky can be considered as a mixed mode of sexual and parthenogenetic reproduction, whereas thelytoky is strictly parthenogenetic.

Types and incidence of thelytoky in Hymenoptera

Thelytoky occurs in all major groups of Hymenoptera, but is especially present among sawflies (Symphyta) and some parasitoid families such as the Chalcidoidea and Cynipoidea (Cook, 1993; van Wilgenburg *et al.*, 2006). Two distinctive classes of thelytoky can be distinguished based upon the causal mechanism: thelytoky can be induced by nuclear genes or be based on cytoplasmic genes including microorganisms. Most cases of thelytoky concern species that are infected with parthenogenesis-inducing microorganisms (Stouthamer, 1997; Braig *et al.*, 2002; van Wilgenburg *et al.*, 2006). A genetic basis for thelytoky has been shown for few studied species only (see below).

A genetic basis for thelytokous parthenogenesis has been shown for *Trichogramma cacoeciae* (Stouthamer *et al.*, 1990b; Vavre *et al.*, 2004), several species of the genus *Lysiphlebus* (Belshaw *et al.*, 1999), the ant species *Plathythyrea punctata* (Schilder *et al.*, 1999), the ichneumonid *Venturia canescens* (Beukeboom & Pijnacker, 2000) and the cape honeybee *Apis mellifera capensis* (Tucker, 1958; Lattorff *et al.*, 2005). Virtually nothing is known about the underlying genetics of thelytokous parthenogenesis in these hymenopterans except for the egg laying workers of the cape honeybee. From crosses between arrhenotokous and thelytokous subspecies,

Ruttner (1988) concluded that thelytokous parthenogenesis is under the control of a single gene. Lattorff *et al.*, (2005) refined this study to show that thelytokous parthenogenesis is a qualitative character determined by a single major recessive gene, called thelytoky (*th*). Except for the cape honey bee, we know remarkably little about the genetic basis of thelytoky within Hymenoptera and there is a large need for genetic studies of thelytoky to be able to understand the evolutionary dynamics of asexual reproduction.

A different type of thelytoky is the one caused through infection by intracellular bacteria. So far, thelytoky-inducing bacteria have only been found among the Gram-negative bacteria, in the genera *Wolbachia*, *Cardinium* and *Rickettsia* (O'Neill *et al.*, 1997; Stouthamer *et al.*, 1999; Zchori-Fein & Perlman, 2004; Perlman *et al.*, 2006). They predominantly occur in the insect order Hymenoptera (Huigens & Stouthamer, 2003; Zchori-Fein *et al.*, 2001; 2004; Hagimori *et al.*, 2006), but sporadically in other groups as well, such as Coleoptera (Werren *et al.*, 1995), Thysanoptera (Arakaki *et al.*, 2001) and mites (Weeks & Breeuwer, 2001; Groot & Breeuwer, 2006). In general, bacterial induction of thelytoky is restricted to host species with haplodiploid sex determination, although Weeks *et al.*, (2001) found it in an entirely haploid mite species.

Besides inducing thelytoky, these bacteria are involved in a wider array of reproductive manipulations, including cytoplasmic incompatibility, feminization and male-killing (O'Neill *et al.*, 1997; Werren, 1997; Perlman *et al.*, 2006). *Wolbachia* is the most widespread and best known of these reproductive parasites, the manipulative phenotypes of *Cardinium* and *Rickettsia* have only recently started to become evident (Zchori-Fein & Perlman, 2004; Perlman *et al.*, 2006; Giorgini *et al.*, 2009; White *et al.*, 2009). All these bacteria live within the reproductive and other tissues of their host and are maternally inherited through the egg cytoplasm. Sperm cells do not contain enough cytoplasm and males are therefore considered an evolutionary dead end for these bacteria. The existence of these symbionts can therefore most easily be explained by their selective advantage of increasing the production of infected female offspring, and hence their transmission to the next generation. Their most extreme phenotype is the induction of all-female offspring by inducing thelytoky.

Cytology and genetic consequences of thelytoky in Hymenoptera

Several cytological mechanisms are responsible for thelytoky in Hymenoptera, each with different genetic consequences for the genetic make-up of the offspring (Fig. 1.1). Although any classification of thelytokous forms may be disputable (Suomalainen *et al.*, 1987), a first distinction can be made based upon whether the maternal ploidy level is maintained without fusion of nuclei (apomixis) or restored

after fusion of two division products from a single cell (automixis). In apomictic thelytoky, offspring are mitotically produced, whereas automictic forms of thelytoky involve meiosis and recombination. Apomictic reproduction (Fig. 1.1A) retains heterozygosity and results in offspring that are genetically identical to their mother because sister chromatids pair during meiosis (Suomalainen *et al.*, 1987; Beukeboom & Zwaan, 2005). Although apomixis is the most common form of parthenogenesis in insects (Suomalainen *et al.*, 1987), it occurs only sporadically in Hymenoptera. It is found in the sawfly *Strongylogaster maculata* (Peacock & Sanderson, 1939), the spring generation of the gall wasp *Neuroterus bacarum* (Doncaster, 1916; Dodds, 1939), the egg laying workers of queenless groups of the weaver ant *Oecophylla longinoda* (Ledoux, 1954), the parasitoid wasp *Trichogramma cacoeciae* (Vavre *et al.*, 2004) and the little fire ant *Wasmannia auropunctata* (Fournier *et al.*, 2005). Vavre *et al.*, (2004) found high levels of heterozygosity in field populations and no segregation of heterozygous genetic markers, which is consistent with apomictic reproduction.

Most thelytokous hymenopterans reproduce by some form of automixis. Typically, early stages of meiosis in automixis (meiotic oogenesis) are normal: pairing of chromosomes, crossing over, bivalent formation and chromosome reduction to form a haploid ovum. Diploidy is restored by fusion of two haploid nuclei from a single dividing oogonium. The restoration of diploidy may occur in different ways, each with different consequences for the genetic variation among offspring (Lamb & Wiley, 1987; Suomalainen *et al.*, 1987; Beukeboom & Zwaan, 2005). Terminal fusion (Fig. 1.1B) is the process in which two haploid daughter cells, the second polar body nucleus and the egg nucleus, fuse to form a diploid egg. This leads to an increase in homozygosity at loci proximal of crossovers (Suomalainen *et al.*, 1987). The saw flies *Pristiphora rufipes* (Comrie, 1938) and *Diprion polytonim* (Smith, 1941) and the parasitoid wasp *Aphytis mytilaspides* (Rössler & DeBach, 1973) produce their eggs through terminal fusion.

In central fusion (Fig. 1.1C) the second polar body fuses with a descendant of the first polar body to form a diploid egg. These two nuclei are non-sister ootids (originate from different secondary oocytes) and although a certain level of heterozygosity will be maintained, homozygosity increases each generation. Loci close to the centromere have a higher chance to remain heterozygous because less recombination occurs in this region of the chromosome. This mechanism is known from the cape honeybee *Apis mellifera capensis* (Tucker, 1958; Verma & Ruttner, 1983), the parasitoid wasp *Venturia canescens* (Speicher, 1937; Beukeboom & Pijnacker, 2000), and the ant *Cataglyphis cursor* (Pearcy *et al.*, 2004).

Gamete duplication (Fig. 1.1D) is a post-meiotic process in which chromosome number doubles during the first cleavage division of the egg (fusion of cleavage nuclei) through endomitosis. To date all studied cases of thelytoky induced by *Wolbachia* involve gamete duplication, resulting in complete homozygosity of the offspring. This has been shown for the gall wasps *Diplolepis rosae* (Stille & Dävring,

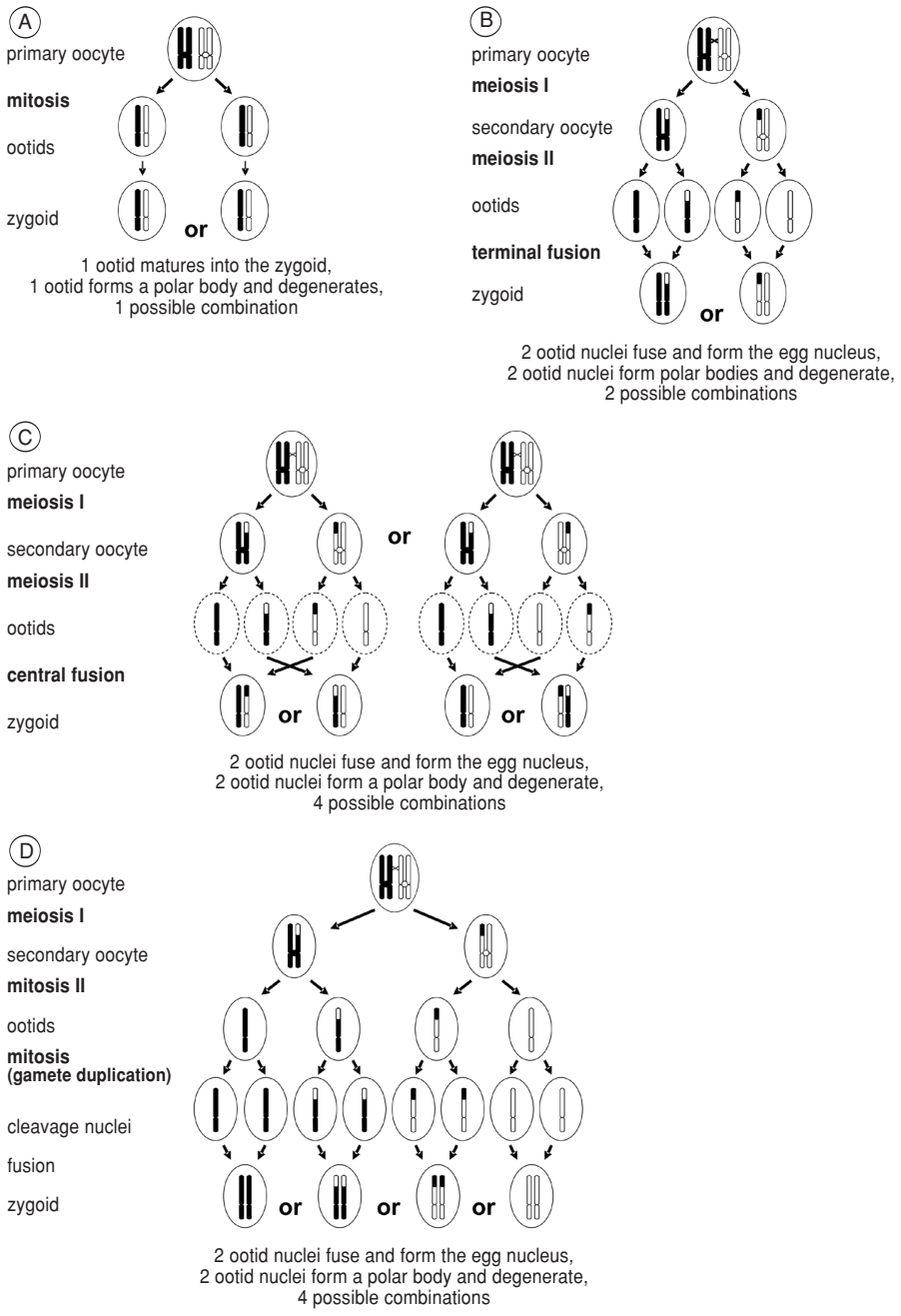


Figure 1.1. Cytological mechanisms of thelytoky in Hymenoptera. (A) apomixis, (B-D) automixis, (B) terminal fusion, (C) central fusion and (D) gamete duplication. Diagrams show all possible combinations of nuclei, but each primary oocyte always yields only one zygoid. Alternative segregations occur depending on orientation on the metaphase plate in secondary oocytes. Adapted from Suomalainen *et al.* (1987).

1980) and *Diplolepis spinosissima* (Plantard *et al.*, 1998), and the parasitic wasps *Muscidifurax uniraptor* (Legner, 1985; Gottlieb *et al.*, 2001), *Leptopilina clavipes* (Pannebakker *et al.*, 2004) and some species of *Trichogramma* (Stouthamer & Kazmer, 1994). Although no cytological study was done, genetic analysis of thelytoky in the *Wolbachia*-infected mite *Bryobia praetiosa* showed the production of heterozygous progeny, suggesting a different mechanism of thelytoky than gamete duplication (Weeks & Breeuwer, 2001). The cytological mechanisms involved in *Cardinium* and *Rickettsia*-induced thelytoky have still been little studied. Adachi-Hagimori *et al.* (2008) have shown that the parasitoid *Neochrysocharis formosa* infected with *Rickettsia* maintains diploidy by a newly described functionally apomictic cloning mechanism that differs entirely from the mechanism induced by *Wolbachia*. This mechanism appears to maintain heterozygosity over generations.

Thelytoky and sex determination

Different sex determination mechanisms exist in Hymenoptera, but little is known about the underlying genetics (Beukeboom, 1995; Heimpel & de Boer, 2008). Under haplodiploidy, sex determination does not depend on heteromorphic sex chromosomes, but on the number of chromosome sets (haploid males and diploid females). Under single locus complementary sex determination (sl-CSD), first described by Whiting (1943), sex depends on the allelic composition at a single *csd* locus: hemizygous haploids develop into males, heterozygous diploids into females and *csd*-homozygous diploids into diploid males. Diploid males typically have low viability or fertility, or produce diploid sperm that will yield sterile triploid offspring (Agoze *et al.*, 1994). Hence, CSD is considered disadvantageous under inbreeding conditions because more homozygous diploid males are produced. They pose a genetic load to the population and selection will therefore favour rare *csd* alleles in the population (Cook & Crozier, 1995). There is some recent evidence for multiple *csd* loci (multi-locus CSD) in the parasitoid wasp *Cotesia vestalis* (De Boer *et al.*, 2006), which may be an alternative way to reduce diploid male frequencies and lessen the effects of inbreeding. Van Wilgenburg *et al.* (2006) have recently reviewed the incidence of CSD and found it to be present in over 60 species of Hymenoptera occurring in each major subgroup, including the social Hymenoptera. This suggests that it is the ancestral mode of sex determination although the basal groups have been poorly studied (Cook & Crozier, 1995). The *csd* gene has recently been cloned and sequenced in the honey bee but the molecular regulation of CSD remains unknown (Beye *et al.*, 2003).

The fact that thelytokous species can have a form of complementary sex determination may look contradictory because most forms of thelytoky lead to increased homozygosity, which would result in a high proportion of diploid males. The most extreme case is gamete duplication mediated by microorganisms which leads

to complete homozygosity within a single generation. Therefore, it is believed that CSD may prevent the evolution of thelytoky induced by *Wolbachia*. There is some phylogenetic information that supports this idea: *Wolbachia* induced thelytoky appears mostly absent in hymenopteran groups for which CSD has been described, such as the sawflies (Tenthredinoidea) and bees and wasps (Wenseleers & Billen, 2000), and is particularly abundant in the non-CSD parasitoid groups Chalcidoidea and Cynipidae (van Wilgenburg *et al.*, 2006). However, not all cases of thelytoky are incompatible with complementary sex determination. Heterozygosity may be completely (apomixis) or partially preserved (central and terminal fusion). An example is the parasitoid wasp *Venturia canescens* which combines sl-CSD with thelytokous reproduction.

Evolutionary consequences of thelytoky

At the beginning of this chapter I mentioned the advantage of sex. Haplodiploidy gives an extra advantage to sexual reproduction allowing for a reduction of genetic load because recessive deleterious alleles are exposed to selection in haploid males (Crozier, 1985) while asexual populations have the long term disadvantage of accumulating deleterious mutations (Muller, 1964; Kondrashov, 1982). Haccou and Schneider (2004) showed theoretically that segregation and recombination do reduce the accumulation of deleterious alleles under several forms of automictic thelytoky, although not to the same degree as under arrhenotoky.

In strictly thelytokous populations, genes involved in sexual reproduction are no longer maintained by selection and the traits determined by these genes are expected to become reduced or disappear completely (Fong *et al.*, 1995). This process occurs in thelytokous populations regardless of the underlying mechanism, but it is easiest studied for cases of microbe-induced thelytoky because these can be reverted to the sexual mode of reproduction by curing infected females from their infection using high temperature or antibiotic treatments (Stouthamer *et al.*, 1990a). Cured females produce males from unfertilized eggs and this allows for testing deterioration of traits involved in sexual reproduction by determining the functionality of male and female sexual traits, such as sperm production and egg fertilization ability (Stouthamer, 1997; Huigens & Stouthamer, 2003; Zchori-Fein *et al.*, 2001, 2004; Hagimori *et al.*, 2006).

Venturia canescens as a case study

Venturia canescens (Gravenhorst) (Fig. 1.2) is a solitary endoparasitoid wasp of lepidopteran larvae (Beling, 1932; Salt, 1976) that has been widely used as a biological model in behavioural, population dynamical, genetic and physiological studies

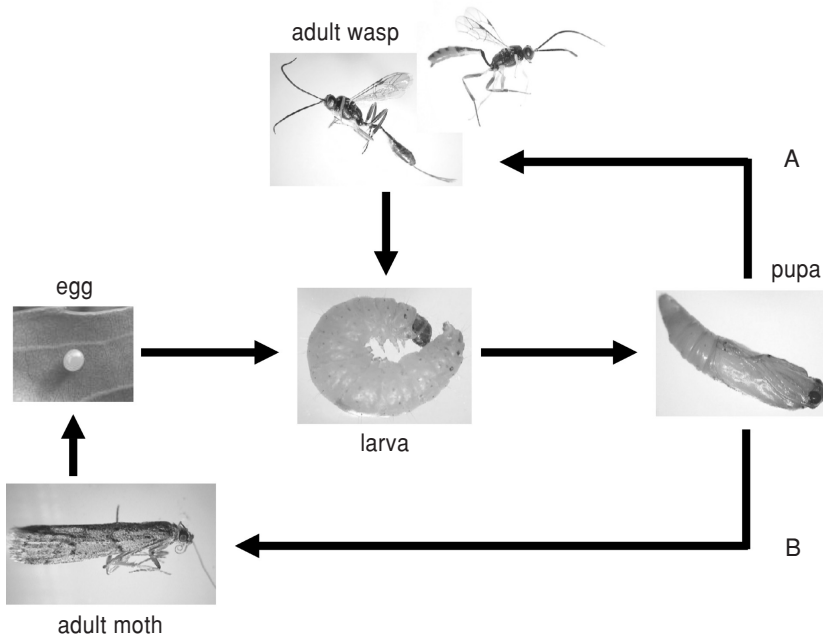


Figure 1.2. Life cycle of the parasitoid *Venturia canescens* (A) and its host *Ephestia kuehniella* (B). The *V. canescens* adult and pupa and the adult *Ephestia* pictures were taken at the Evolutionary Genetics laboratory in Groningen. The *Ephestia* egg and *V. canescens* larva pictures were taken from the internet without a reference source.

(e.g., Ahmad, 1936; Simmonds, 1943; Corbet & Rotheram, 1965; Arthur, 1971; Rogers, 1972; Hubbard & Cook, 1978; Waage, 1979; Harvey *et al.*, 1993; Begon *et al.*, 1995; Driessen *et al.*, 1995; Hellers *et al.*, 1996; Marris *et al.*, 1996; Corley, 1999; Driessen & Bernstein, 1999; Beukeboom & Pijnacker, 2000; Beukeboom, 2001; Schneider *et al.*, 2002, 2003; Amat, 2004; Desouhant *et al.*, 2005; Reineke *et al.*, 2006; Thiel *et al.*, 2006; Metzger *et al.*, 2008; Amat *et al.*, 2009). It has both sexual (arrhenotokous) and parthenogenetic (thelytokous) reproduction and females of either mode occur sympatrically in Southern Europe (Schneider *et al.*, 2002). Thelytokous females have an extended distribution range because they also inhabit man-made environments, such as bakeries and granaries. These environments are considered relatively constant, which would allow thelytokous strains to outcompete arrhenotokous ones due to the reproductive advantage of parthenogenesis over sexuality (Schneider *et al.*, 2002).

Cytology and genetics of thelytoky

Thelytokous females produce haploid eggs meiotically that subsequently undergo diploidy restoration and develop into females (Speicher, 1937; Beukeboom & Pijnacker, 2000). Beukeboom and Pijnacker (2000) showed that thelytoky in

Venturia canescens is not due to infection with *Wolbachia* and supposed a genetic basis for thelytoky.

Diploidy restoration in the thelytokous strains of *V. canescens* is described as a form of central fusion automictic parthenogenesis (Beukeboom & Pijnacker, 2000) (Fig. 1.3). Speicher (1937) already described the cytological mechanism to consist of an aberrant first meiotic division. Later genetic studies (Speicher *et al.*, 1965) were ambivalent about the nature of the second division, it either being reductional or equational. Beukeboom and Pijnacker (2000) showed that the first meiotic division is followed by an equational division of the restituted number of diploid chromosomes. The chromatid of one sister chromosome can segregate with either one of the other sister chromosome, resulting in two possible segregation combinations (Fig. 1.3). Subsequently, one of the two nuclei becomes a polar body and degenerates; whereas the other develops into the diploid embryo. This cytological mechanism of diploidy restoration does not instantly lead to complete homozygosity. It enables heterozygosity to be maintained for loci close to the centromere, but distal loci will become homozygous over generations in half of the segregation combinations. The fact that thelytokous females can be genetically heterozygous (Chapter V of this thesis) is consistent with this.

The sex determining mechanism in *V. canescens* is a single locus complementary sex determination (sl-CSD), which was originally discovered by comparing sex ratios between crosses of related and unrelated individuals using an arrhenotokous strain (Beukeboom, 2001). Typically, sl-CSD is tested by brother-sister matings in which, due to haplodiploidy, 50% of males are expected to share a *csd* allele with their sisters. Such matched crosses will yield 50% homozygous and 50% heterozygous *csd* offspring which develop into diploid males and females respectively. Crosses between unrelated males and females are used to compare progeny sizes, because diploid males in some species are unviable (Agoze *et al.*, 1994). These inbreeding experiments resulted in higher sex ratios at frequencies that were consistent with the presence of viable diploid males as predicted under sl-CSD (for details see Beukeboom, 2001). Recently, the generation of diploid males under inbreeding was confirmed by flow cytometry (Mateo Leach, unpublished data). How can the mechanism of diploidy restoration in *V. canescens* be compatible with sl-CSD? Both can operate as long as the *csd* locus is located in a chromosomal region where heterozygosity is maintained, e.g. close to a centromere on one of the eleven autosomes or in a region in which recombination is prevented by inversions. Interestingly, occasionally males are found in thelytokous laboratory populations (Chapter II of this thesis).

Very little is known about the genetic basis of parthenogenesis in animals and in hymenopterans in particular. In the cape honeybee, parthenogenesis in the egg-laying worker is a heritable trait probably determined by a single recessive locus (Lattorff *et al.*, 2005). In *V. canescens*, we do not know whether a single or multiple genes are responsible for thelytoky. We also cannot exclude the possibility that

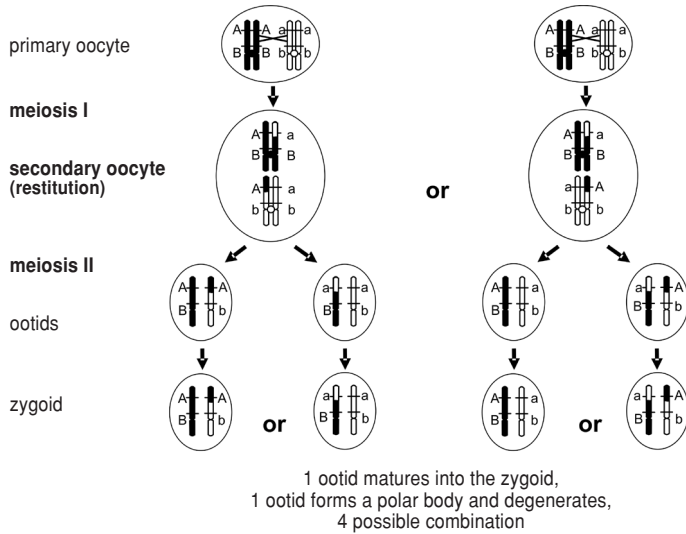


Figure 1.3. The cytological mechanism of thelytoky in *Venturia canescens*. The first meiotic division results in a diploid restitution metaphase, which divides and of which one nucleus becomes a polar body and one nucleus develops into the zygoid. Alternative segregations occur depending on orientation on the metaphase plate in secondary oocytes. Loci distal of crossing-over (locus A) have a 50% chance of becoming homozygous (left diagram) or remain heterozygous (right diagram) depending on the segregation combination), loci proximal of crossing-over (locus B) always remain heterozygous. For details, see Beukeboom & Pijnacker (2000).

thelytoky is encoded by mitochondrial genes that are maternally inherited. Schneider *et al.*, (2003) performed crosses between arrhenotokous males and thelytokous females and discovered paternal genetic markers in the hybrid female offspring. Such introgression of arrhenotokous genes would allow for studying the genetic basis of thelytoky in *V. canescens*. Assuming a nuclear basis of thelytoky and recombination, segregation of arrhenotokous and thelytokous reproduction among offspring may be expected when a sufficient proportion of arrhenotokous genes were introduced into a thelytokous background. We set out to do just this, but, unfortunately, were not able to repeat the results of Schneider *et al.* (2003) even though several laboratory and field strains were used under different conditions. Although thelytokous females mated with arrhenotokous males and males transferred sperm, as evidenced from dissection of spermatheca of mated females, females did not lay eggs that contained the paternal genotype (Mateo Leach, unpublished data). Unfortunately, the particular laboratory strain (collected from Golfe, Southern France) used by Schneider *et al.* (2003) was lost. Hence, the most likely explanation is that gene exchange between arrhenotokous males and thelytokous females is very rare.

Thelytoky and genetic diversity

Central fusion automixis has important consequences for genetic variation in thelytokous populations. Due to cross-overs in the primary oocytes and the subsequent fusion of nuclei, recombination leads to an increase in homozygosity rather than a decrease as may intuitively be expected. Each generation, loci distal of a chiasma have a 50% chance of becoming permanently homozygous in an individual (Fig. 1.3). Hence, depending on the number and location of chiasmata in the primary oocytes of the parental generation, offspring will become more and more homozygous. Moreover, at the level of individual genomes, an increasing homozygosity gradient is expected to become established from the centromeres to the telomeres. In theory, this process of “genome homozygosity” can be followed using polymorphic genetic markers and the degree of homozygosity may be used as a “genomic clock” to determine the age of thelytokous lineages. At this moment genetic linkage maps of *Venturia canescens* have insufficient detail to perform such an analysis. However, in the cape honeybee, *Apis mellifera capensis*, such a study has been done (Baudry *et al.*, 2004). The authors indeed observed gradients of homozygosity and were able to map centromere positions for most of the linkage groups. However, they also found that the recombination rate was reduced by more than tenfold during meiosis in thelytokous workers. This points towards the existence of genetic mechanisms for reducing homozygosity in thelytokous Hymenoptera which will have important evolutionary consequences. More studies are needed on the effects of particular cytological mechanisms of thelytoky on genetic variation in populations. Such studies should investigate how recombination events change individual genomes over successive generations and how this affects the structure of genetic variation in natural populations.

The simultaneous occurrence of arrhenotokous and thelytokous reproduction poses an evolutionary problem because thelytokous populations are expected to rapidly outcompete arrhenotokous ones due to the twofold cost of sex (Maynard Smith, 1978). Schneider *et al.* (2002) studied the geographical distribution and genetic diversity of arrhenotokous and thelytokous populations of *V. canescens* in Southern Europe. Arrhenotokous wasps were more abundant than thelytokous ones, but simultaneous occurrence of both reproductive modes in the same localities was found frequently. Analysis of the genetic structure of the populations at the Côte d’Azur revealed that there was one widespread thelytokous lineage and a few rare ones. They propose two explanations for the occurrence of few thelytokous individuals with high genetic similarity to arrhenotokous ones: apart from the widespread lineage, new lineages might recently have arisen from local arrhenotokous populations by a loss of sex, and/or introgression of arrhenotokous genes into thelytokous lineages had taken place through occasional sex between males and thelytokous females. Schneider *et al.* (2003) claimed that thelytokous females can mate with arrhenotokous males and produce biparental offspring.

An independent mitochondrial DNA analysis was subsequently used to compare maternal haplotypes between both reproductive modes. All arrhenotokous wasps (95 individuals from 22 sites) carried haplotype II, and except for one thelytokous wasp with haplotype II, all thelytokous wasps (31 from 7 sites) had haplotype I. Schneider (2003) confirmed these results in a larger scale study and concluded that mitochondrial haplotypes are largely divergent between both reproductive modes. What do the nuclear and mitochondrial marker data reveal about the genetic structure of *V. canescens* populations? Both studies show the occurrence of one widespread thelytokous lineage. When new thelytokous lineages arise regularly from local arrhenotokous populations, thelytokous wasps with the arrhenotokous haplotype II should have been found. Since only one such case was found, it can be concluded that recurrent arisal of new thelytokous lineages may occur but probably plays a minor role, if any, at the level of the local dynamics of both reproductive modes. If this particular case was the result of a recent mutational loss of sex, a high nuclear similarity of this wasp would be expected with its sympatric arrhenotokous conspecifics. This was not the case and therefore this wasp might be a member of another thelytokous lineage. In addition to thelytokous females resembling arrhenotokous ones, Schneider *et al.* (2002) also found some arrhenotokous females that shared many nuclear markers with females of the widespread thelytokous lineage. This indicates occasional introgression of thelytokous genes into the arrhenotokous cytotypes. One possible explanation is that thelytokous females occasionally produce males that mate with arrhenotokous females. Rare males were observed in thelytokous laboratory populations (Schneider *et al.*, 2003) and were haploid (Mateo Leach, unpublished). However, when tested (N = 4) such males were never reproductively active under laboratory conditions.

Many questions about the dynamics of thelytokous and arrhenotokous reproduction in *V. canescens* remain unanswered: What are the genetic mechanisms involved, i.e. how can sex be lost and is a return to sex of thelytokous wasps possible? More detailed population genetic analyses of co-occurring thelytokous and arrhenotokous populations as well as laboratory crosses are needed to reveal which mechanisms can cause gene flow between both reproductive modes in nature. The persistence at the same time and place of both reproductive modes within this species remains an intriguing paradox which might be enlarged even more by the presence of gene flow between the modes (Schneider, 2003). Studies on the ecological mechanisms behind their coexistence are highly relevant for gaining a better understanding of the problem of the 'maintenance of sex'.

Coexistence of arrhenotokous and thelytokous wasps

The vast majority of publications on *Venturia canescens* during the passed 80 years dealt with wasps from thelytokous populations that were collected from granaries, mills and other food storages infested with phycitid moths. It was only recently discovered that arrhenotokous populations are widespread under outdoor condi-

tions (Beukeboom *et al.*, 1999; Schneider *et al.*, 2002). Occasionally, wasps of both kinds have been found at the same site or even caught in the same tree. Arrhenotokous wasps have never been reported from indoor samplings: a year round monitoring of sticky traps in a granary in South of France yielded thousands of female wasps but not one male (L. Lapchin, pers. comm.). From a theoretical point of view, all else being equal, we would expect that the sexual form would be outcompeted by the asexual one due to the demographic costs of sex. However, the above observations suggest that the reproductive modes occupy different niches and that the coexistence could be facilitated by ecological differences.

A number of studies have now compared behaviour and life-history traits of arrhenotokous and thelytokous *V. canescens*. When foraging on single patches both kind of wasps have similar patch residence times and ovipositions (Lafortune & Driessen, unpublished; Amat *et al.*, 2006; Thiel *et al.*, 2006). However, when offered a sequence of patches, arrhenotokous wasps are less sensitive to patch encounter rate and keep to an oviposition strategy that results in a spreading of offspring across the habitat. This can be interpreted as an adaptation to CSD. Thelytokous wasps, on the other hand, adjust their oviposition much more in relation to patch encounter rate and follow a strategy that maximizes their foraging efficiency in a variable environment (Thiel *et al.*, 2006).

Arrhenotokous wasps are more sensitive to temperature changes compared to thelytokous wasps: they increase patch time and the number of ovipositions in response to a sudden drop in temperature (Amat *et al.*, 2006). This can be seen as an adaptive response to living outdoors, since sudden temperature drops may indicate unfavourable weather conditions in the immediate future and increase mortality risks. Luchetta *et al.*, (2007) investigated the response to host and food availability of wasps under different feeding regimes. Their results suggest that thelytokous wasps are more sensitive to the presence of food in the environment when they are exploiting a host patch. Food sources are scarce to absent in indoor conditions and hence they are much more valuable for thelytokous wasps.

Pelosse *et al.*, (2007) studied differences in energy allocation between arrhenotokous and thelytokous wasps. Arrhenotokous wasps emerge with more energy reserves, glycogen in particular, which can be seen as an adaptation to the outdoor life-style, where wasps have to search for host patches and food more actively. Thelytokous wasps, on the other hand, put more energy in eggs, which allows them to take advantage of occasional extreme host densities in granaries and mills (Ellers *et al.*, 2000). Higher egg loads at emergence in thelytokous wasps were also reported by Schneider (2003). Thelytokous wasps have a higher egg production throughout their lives resulting in higher life-time fecundity (Fig. 7.5C, Schneider, 2003). All in all, these studies suggest that each reproductive form has developed specific behavioural and life-history traits that make it the better competitor in its 'own' habitat and that niche differentiation is indeed an explanation for the coexistence of arrhenotokous and thelytokous forms of *V. canescens*.

Thesis overview

Much is known about the ecological and behavioural interactions between thelytokous and arrhenotokous *Venturia canescens*. Still, there are many open questions concerning the underlying mechanisms and genetics behind the reproductive modes: what triggers diploidisation of thelytokous eggs in absence of endosymbionts? How genetically variable are thelytokous individuals and populations? Is there gene flow between the two reproductive modes in the field?

The main goal of this thesis is to elucidate the genetic basis of thelytoky in *V. canescens* to better understand the evolution and persistence of asexual reproduction. We repeat a population genetics analysis to establish the robustness of previous studies and to determine to which degree arrhenotokous and thelytokous wasps are related to each other. In addition, we try to explain at the molecular level the cytological differences between both reproductive modes.

Before considering the genetics of thelytoky, we confirm in **Chapter 2** that thelytoky in *V. canescens* is not induced by endosymbionts. We use different approaches to show the absence of any prokaryotic infection in the ovaries of thelytokous females. Our data, in combination with those of Beukeboom and Pijnacker (2000) confirm a genetic basis of thelytoky in *V. canescens*.

In order to investigate the evolutionary dynamics of coexisting thelytokous and arrhenotokous wasps and origin of thelytokous populations we developed a new set of microsatellite markers that increase the limited number of existing microsatellites developed for thelytokous *V. canescens*. In **Chapter 3** we describe a set of 56 microsatellites for both reproductive modes. These markers are more suitable for intraspecific studies than the amplified fragment length polymorphism (AFLP) markers used in previous studies.

In **Chapter 4** we present the first genetic linkage map for the parasitic wasp *V. canescens* containing 29 microsatellites and 19 AFLP markers in addition to the *vlp* and *csd* loci. This map provides a basis for future genetic studies such as QTL mapping of behavioural traits or the genetics of thelytoky in *V. canescens*.

It is known that arrhenotokous and thelytokous *V. canescens* coexist in Southern Europe, although they exploit different habitats. **Chapter 5** presents a study on the genetic variation of individuals collected in the field of both reproductive modes to determine their degree of relatedness and possible origin of thelytokous lines. In this study we try to confirm the results of Schneider *et al.* (2002), by using 15 polymorphic microsatellites as nuclear markers and a partial sequence of the mitochondrial *COI* gene. Our results suggest that the two reproductive modes are isolated in the field.

Although we have shown that thelytoky is genetically determined in *V. canescens*, the underlying genetics of diploidy restoration remains unknown. In **Chapter 6** we aim to identify the genes and/or proteins that control thelytoky by comparing gene and protein expression in the ovaries of females of both reproduc-

tive modes. We detected tubulin and actin protein differences that are most likely functionally related to the two types of reproduction.

Theory predicts that asexual reproduction has a competitive advantage over sexual reproduction because of the cost of producing males. In **Chapter 7** we measured a number of fitness components of arrhenotokous and thelytokous *V. canescens* females. Thelytokous females invest more in egg production whereas arrhenotokous females had a higher longevity. These differences are considered in the context of different life histories of both reproductive modes.

The results of my study are summarized in **Chapter 8**.

