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

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CHAPTER 5

Genetic diversity of thelytokous and arrhenotokous *Venturia canescens*

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Abstract

The parasitoid wasp *Venturia canescens* has both arrhenotokous and thelytokous modes of reproduction. Arrhenotokous and thelytokous individuals coexist in Southern Europe. Here we study the genetic variation of individuals of both reproductive modes collected in the field to determine their degree of relatedness and the possible origin of thelytokous lines. We use 15 polymorphic microsatellites as nuclear markers and a partial sequence of the mitochondrial *COI* gene. The two reproductive modes cluster in two unrelated groups using nuclear markers. All thelytokous wasps and two arrhenotokous individuals share the same mitochondrial haplotype whereas all the other arrhenotokous wasps cluster in four haplotypes. This indicates that there is one single widespread thelytokous clone and that gene flow between both reproductive modes is less frequent than previously suggested.

Introduction

In theory, asexual reproduction has the evolutionary advantage over sexual reproduction of not producing males (Maynard Smith, 1978), but still most known species reproduce sexually. Sympatric coexistence of sexual and asexual reproduction raises many evolutionary questions because sexual reproduction is costly. Sexually reproducing individuals have to invest time in finding mates (Bell, 1982) and mating (Daly *et al*., 1978). Sexual reproduction increases the risk of transmission of sexual diseases. Recombination breaks down favourable gene combinations and possible negative interactions between both genomes may appear (outbreeding effect). However, the most obvious cost of sex is the one of producing males (two-fold cost of sex, Maynard Smith, 1978). On the other hand, sexual reproduction has benefits such as more efficient elimination of deleterious mutations (Muller's ratchet, Muller, 1964) and faster combining of beneficial mutations. Sexual populations are believed to adapt faster to changing environments (the Red Queen hypothesis, Van Valen, 1973) and in theory they are better able to adapt to heterogeneous environments (the Tangled Bank hypothesis, Bell, 1985).

In the insect order of Hymenoptera, all species have a haplodiploid mode of reproduction. Arrhenotoky (sexual reproduction) is the most common mode of reproduction: unfertilized eggs develop into males that are haploid, whereas fertilized eggs develop into diploid females. Thelytoky (asexual reproduction) is less common: females develop parthenogenetically from unfertilized eggs after restoration or maintenance of diploidy and males do not occur (Crozier, 1977). Although arrhenotoky is the predominant reproductive mode, thelytoky occurs widespread and has evolved many times in all major groups of Hymenoptera (Cook, 1993). Thelytoky is often induced by microorganisms that manipulate the reproduction of their host. However, in a few cases rather than infectious, a genetic basis has been determined for thelytoky, i.e. for the chalcidoid wasp *Trichogramma cacoeciae* (Stouthamer *et al*., 1990; Vavre *et al*., 2004), several species of *Lysiphlebus* wasps (Belshaw *et al*., 1999), the ant *Plathythyrea puncata* (Schilder *et al*., 1999), the ichneumonid *Venturia canescens* (Beukeboom & Pijnacker, 2000) and the cape honeybee *Apis mellifera capensis* (Tucker, 1958; Lattorff *et al*., 2005).

Venturia canescens (Gravenhorst) 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 (references in Beukeboom & Pijnacker, 2000; Thiel *et al*., 2007; Chapter I). It has both arrhenotokous and thelytokous reproduction and females of either mode occur sympatrically in Southern Europe (Schneider *et al*., 2002). The presence of both reproductive modes in the same species makes this system very suitable for the study of genetic relatedness between arrhenotokous and thelytokous reproducing individuals and the origin and maintenance of thelytoky in the field.

According to the type of diploidy restoration that takes place in thelytokous eggs

(central fusion automictic parthenogenesis), recombination can occur in early stages of oogenesis leading to an increase of homozygosity. This process, known as genome homozygosation, is expected to increase over time: loci near the tip of the chromosome will become homozygous over generations while loci near a centromere position will remain heterozygous because recombination results in homozygosity and is reduced near the centromeres. Thus thelytokous individuals are expected to be more homozygous than arrhenotokous ones (Beukeboom & Pijnacker, 2000).

Schneider *et al.* (2002) studied the geographical distribution and genetic diversity of arrhenotokous and thelytokous populations of *V. canescens* in the Côte d'Azur (France) with nuclear and mitochondrial markers. Both reproductive modes coexist in the field, although arrhenotokous wasps were more abundant than thelytokous ones. Analysis of the genetic structure of the populations revealed one widespread thelytokous lineage and a few rare ones with high genetic similarity to arrhenotokous individuals. Mitochondrial markers revealed that both reproductive modes are very dissimilar. In a later study, Schneider (2003) confirmed these results with wasps collected from a 500 km transect along the Rhône Valley in Southern France.

The results of Schneider *et al.* (2002; 2003) and Schneider (2003) suggest that gene flow between arrhenotokous males and thelytokous females is possible not only in the field but also under controlled conditions in the laboratory. From our experience we know that such crosses are not easy to repeat in the laboratory. With this study we aim to revise the genetic diversity of arrhenotokous and thelytokous wasps using individuals from the same populations as the ones used in Schneider *et al.* (2002). This time we use microsatellites as nuclear markers which are very suitable for intraspecific studies and are very reliable. Sequence data on a partial sequence of the mitochondrial *COI* gene will provide detailed information on the haplotype differences between both reproductive modes. We further discuss the origin and maintenance of thelytoky versus arrhenotoky.

Materials and methods

Field sampling

In the autumn of 1997 and 1998 and in the summer of 2003 wasps were collected between Cannes and St. Rafael along the Côte d'Azur in Southern France as described by Schneider *et al.* (2002) and one site in Spain (San Juan de Alicante). Briefly, baits with 3rd -4th instar larvae of the moth *Ephestia kuehniella* were placed in trees at 1-2 m height. Baits were exposed during 48 hours and then taken to the lab. Baits were checked daily for wasp emergence and emerging females were allowed to lay eggs individually over night to establish their reproductive mode. Females were subsequently stored at -80^{o}C for further analysis. Wasps were reared on larvae of the moth *E. kuehniella* at 25^{o}C in constant light.

Reproductive mode determination

Thelytokous females produce only females whereas arrhenotokous females produce only males if they are virgin or males and females if they have been inseminated. If a female produced at least seven females and no males in the F_1, she was scored as thelytokous. If the number of daughters in an all-female progeny was fewer than seven, the F_1 were bred to confirm their thelytokous mode. If males appeared among the offspring of a female, the female was scored as arrhenotokous.

DNA extraction and nuclear analysis

Genomic DNA was extracted from the abdomen of 22 arrhenotokous and 7 thelytokous females (Table 5.1) using a standard proteinase K/salt-chloroform protocol (Sambrook *et al.*, 1989). Presence and quality of DNA was checked on a 1% agarose gel.

Fifteen microsatellite markers isolated from *V. canescens* were used for genotyping 29 individuals (26 individuals from the same populations as the ones used in Schneider *et al.* (2002), two individuals collected in 1997 but not included in Schneider *et al.* (2002) and one wasp collected in Spain). These markers are described in Chapter III of this thesis and were selected based on reliability in amplification and variability. Approximately 5ng of template genomic DNA was amplified in 10 µl reaction mix 1X PCR buffer magnesium free (Promega) with 2.5 mM $MgCl_2$, 0.2 mM dNTPs (Roche), 0.2 µM of each primer and 0.4 units of Taq

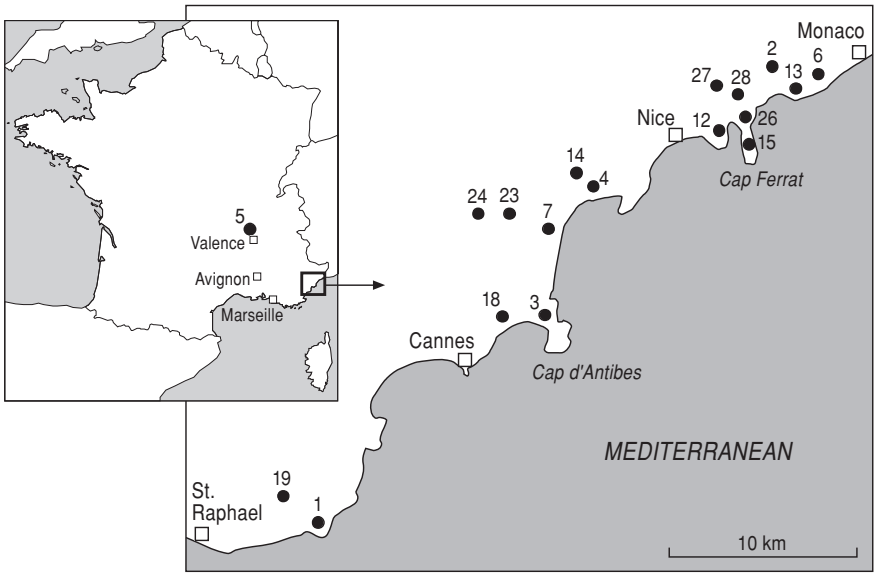

Figure 5.1. Collection sites in south-eastern France. Location abbreviations are indicated in Table 5.1. Modified after Beukeboom *et al.* (1999).

Table 5.1. Summary of samples used in this study. Indicated is the transect location number from Figure 5.1, location name, collection year, reproductive mode, sample name and in which analysis the sample was used. A = arrhenotokous, T = thelytokous.

Transect location Figure 5.1	Location	Collection Year	Mode	Sample	Micro-satellite data	Mito-chondrial data
Côte d'Azur (France)						
2	Belvre l'Èze	1997	A	Bl'E A 97	+	+
4	Cagnes sur Mer	1997	A	CsM A 97	+	+
3	Cap d'Antibes INRA	1997	A	Cd'AI A 97	+	+
6	Eze	1997	A	Eze A 98	+	+
5	Gothéron INRA	1997	A	Got A 97	+	+
12	Mont Boron	1997	A	MtB A 97	+	+
14	Panoramèr	1997	A	Pmer A 97	+	+
		1997	A	PdC A 97	+	+
15	Saint Jan	1997	A	StJ A 97	+	+
13	Saint Laurent	1997	A	StL A 97	+	+
		1997	A	SV A 97	+	+
18	Vallauris	1997	A	Vala A 98	+	+
26	Villefranche	1997	A	VF A 98	+	+
1	Anthéor	1998	A	Anth A 98	+	+
7	La Brague	1998	A	LB A 98	+	+
23	La Vallée Verte	1998	A	VV A 98	-	+
27	Mont Gros	1998	A	MtG A 98	+	-
28	Mont Vinagrier	1998	A	MtV A 98	+	-
14	Panoramèr	1998	A	Pm A 98	+	+
19	Saint Guitte	1998	A	StG A 98	+	+
3	Antibes Botanique	2003	A	Ant Bot A 03	+	+
12	Mont Boron	2003	A	MtB A 03	+	+
1	Anthéor	1997	T	Anth T 97	+	+
14	Panoramèr	1997	T	Pmer T 97	+	+
18	Vallauris	1997	T	Val T 97	+	+
3	Antibes Botanique	2003	T	Ant Bot T 03	+	+
12	Mont Boron	2003	T	MtB T 03	+	+
24	Valbonne	2003	T	Valb T 03	+	+
Costa Blanca (Spain)						
	San Juan de Alicante	2003	T	SJ T 03	+	+

polymerase (Promega). Forward primer of each primer pair were labeled with a fluorescent dye (HEX, FAM or NED). The PCR profile was: 1 cycle of 3 min at 94ºC followed by 35 cycles of 1 min at 94ºC, 30 sec at the primer specific annealing temperature (see Table 3.3 in Chapter III), 1 min at 72ºC and a final cycle of 7 min at 72ºC. Reactions were carried out in a GeneAmp 9700 PCR machine from Applied Biosystems. PCR products were resolved by an 8% denaturing acrylamide electrophoresis on an ABI prism 377 automatic sequencer. Allele sizes were determined using Rox-500 size standard. The size of the fragments was calculated using GeneScan 3.1 software (Applied Biosystems).

The genetic diversity of microsatellite markers was quantified per reproductive mode using number of alleles, heterozygosity, allelic richness and allele frequencies observed in each group using Fstat (Goudet, 2001). To determine whether there was a significant difference between the genetic diversity of the two reproductive modes, we performed a Wilcoxon matched pairs test to compare the observed heterozygosities and a t-test to compare the allelic richness between both reproductive modes using Statistica (StatSoft, OK, USA). Nei's standard genetic distance *DS* (Nei, 1987) was used to construct a UPGMA tree using POPULATIONS 1.2.30 (available at: http://bioinformatics.org/~tryphon/populations/).

Table 5.2. Overview of variation at 15 microsatellites for each reproductive mode. Given are allele size range, number of alleles (N_A), number of females tested (N_{ind}), observed heterozygosity (H_O) and allelic richness (A) per locus and reproductive mode.

Locus	Allele size	Arrhenotokous				Thelytokous			
		N_A	N_{ind}	H_O	A	N_A	N_{ind}	H_O	A
*Vcan*061	178-194	5	11	0.45	3.154	2	5	0.00	1.867
*Vcan*062	250-266	3	7	0.43	2.662	2	6	0.00	1.773
*Vcan*063	174-186	4	19	0.53	2.342	1	7	0.00	1
*Vcan*064	280-295	5	13	0.38	2.292	2	3	0.00	2
*Vcan*065	200-236	11	21	0.86	4.709	2	7	0.00	1.692
*Vcan*066	240-260	5	21	0.67	2.852	2	7	0.00	1.692
*Vcan*067	139-160	6	16	0.44	3.975	2	7	0.00	1.692
*Vcan*069	217-229	5	18	0.50	2.408	2	7	0.14	1.429
*Vcan*070	212-230	5	19	0.37	2.665	2	6	0.00	1.97
*Vcan*071	228-246	6	10	0.20	3.583	2	3	0.33	2
*Vcan*097	140-152	3	20	0.40	2.126	2	7	0.00	1.692
*Vcan*109	189-193	3	19	0.63	2.528	1	7	0.00	1
*Vcan*110	172-174	2	17	0.00	1.559	1	6	0.00	1
*Vcan*112	143-161	4	21	0.33	2.155	2	7	0.14	1.429
*Vcan*114	237-251	4	17	0.59	2.589	1	6	0.00	1
Mean over loci		4.73		0.45	2.77	1.73		0.04	1.55

The genetic diversity per individual was calculated testing the heterozygosity per individual (number heterozygous markers/number of markers amplified) using an angular transformation of proportions (Table 5.3). We compared the mean heterozygosity per reproductive mode with a two sample *t*-test using the statistical package Statistx 4.0 analytical software.

Mitochondrial DNA analysis

A 449-bp fragment of the mitochondrial Cytochrome Oxidase I gene (*COI*) was amplified by PCR with primers *COI Vcan* F 5′-GGTTTGGCTCTATTGGGATAA-3′ and *COI Vcan* R 5′-AAAATGTTGAGGGAAAAATGTTAGA-3′. PCR reactions were carried out in a 25µl reaction volume containing approximately 5 ng of DNA, 1X PCR buffer magnesium free (Promega), 2.5 mM $MgCl_2$, 0.2mM dNTPs (Roche), 0.2µM of each primer and 0.4 units of Taq polymerase (Promega). The cycling

Table 5.3. Genetic diversity per individual. The number of markers amplified for each individual (*M*), the number of heterozygous markers (*H*) and the heterozygosity per individual as (*H*)/(*M*) are indicated.

Arrhenotokous				Thelytokous			
Sample	*M*	*H*	*H/M*	Sample	*M*	*H*	*H/M*
Bl'E A 97	9	5	0.56	Anth T 97	13	0	0.00
CsM A 97	11	4	0.36	Pmer T 97	12	0	0.00
Cd'AI A 97	12	8	0.67	Val T 97	13	2	0.15
Eze A 98	12	4	0.33	Ant Bot T 03	13	0	0.00
Got A 97	12	4	0.33	MtB T 03	13	1	0.08
MtB A 97	11	4	0.36	Valb T 03	12	0	0.00
Pmer A 97	10	5	0.50	SJ T 03	13	0	0.00
PdC A 97	12	7	0.58				
StJ A 97	13	6	0.46				
StL A 97	11	7	0.64				
SV A 97	13	7	0.54				
Vala A 98	13	6	0.46				
VF A 98	11	5	0.45				
Anth A 98	12	8	0.67				
LB A 98	14	4	0.29				
VV A 98	13	7	0.54				
MtG A 98	10	3	0.30				
MtV A 98	10	5	0.50				
Pm A 98	13	7	0.54				
StG A 98	-	-	-				
Ant Bot S 03	13	4	0.31				
MtB A 03	13	6	0.46				

conditions were 1 minute denaturation at 94°C, followed by 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C, and 1 minute and 30 seconds extension at 72°C, ending with a final extension at 72°C for 5 minutes. PCR products were purified with isopropanol and sequenced in one direction using primer *COI Vcan* F with fluorescent Big Dye terminator (Applied Biosystems, Warrington, UK) on a 377 DNA sequencer from Applied Biosystems.

Mitochondrial sequences were aligned using ClustalX (Thompson *et al.*, 1997) and haplotype diversity was calculated using DnaSP version 4.10 (Rozas *et al.*, 2003). A graphical network indicating the haplotype relations was constructed using a statistical parsimony method calculated with the software TCS version 1.21 (Clement *et al.*, 2000). A genetic distance tree was constructed using software package PHYLIP version 3.6 (Felsenstein, 2004).

Results

Microsatellite analysis

Twenty-two arrhenotokous and seven thelytokous individuals were screened with 15 polymorphic microsatellites. Sample VV S 98 was excluded from the analysis because it only amplified for one marker. Table 5.2 summarizes the number of alleles, number of individuals amplified, observed heterozygosity and allelic richness per marker and reproductive mode; allele frequencies are given in the Appendix II. The number of alleles per locus varied from two to eleven in the arrhenotokous samples and from one to two in the thelytokous samples. The least polymorphic locus was *Vcan*110 with two alleles in the arrhenotokous samples and one in the thelytokous sample. *Vcan*065 was the most polymorphic locus with eleven alleles among the arrhenotokous wasps and seven among the thelytokous ones.

The observed heterozygosities of arrhenotokous and thelytokous females were significantly different (Wilcoxon matched pairs test, $N = 15$, $Z = 3.23$, $p = 0.0012$) as well as the allelic richnesses ($A_{arrh} = 2.77$, $A_{thely} = 1.55$, $t = 6.54$, $df = 14$, $p < 0.0001$). Although the number of females tested for each reproductive mode was different, this should not affect the average heterozygosity but only the variance (i.e. more extreme values in the thelytokous group). The mean proportion of heterozygous markers per individual was 0.468 ($SE = 0.001$, $N = 21$) in the arrhenotokous and 0.010 in the thelytokous ($SE = 0.004$, $N = 7$) sample. The difference in the heterozygosity level between the two reproductive modes was tested with a two sided *t*-test ($t = -11.30$, $df = 26$, $p < 0.0001$) which confirmed that thelytokous individuals are less heterozygous than arrhenotokous ones though three times as many arrhenotokous individuals were tested. This is also reflected in the total number of alleles observed in both modes: 81 alleles in the arrhenotokous group and 26 in the thelytokous one. Thus, proportionally the same number of alleles is present in both reproductive modes. There are five alleles that are unique for the thelytokous group.

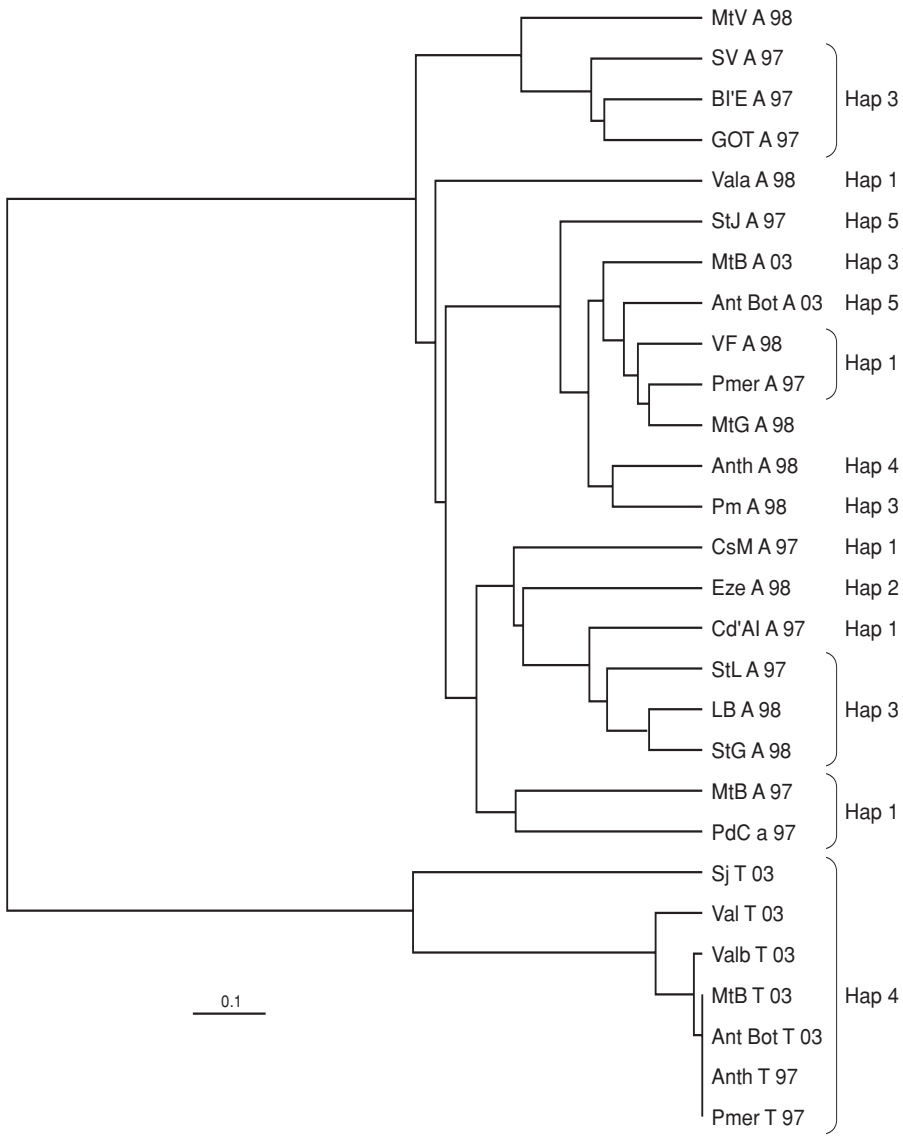

Figure 5.2. Unrooted neighbour joining tree based on Nei's standard genetic distance DS (Nei, 1987) between individuals calculated on the basis of 14 polymorphic microsatellites. Colours indicate the haplotype of each individual. Location abbreviations are indicated in Table 5.1.

We compared the location of the polymorphic and non-polymorphic markers of the thelytokous samples on the linkage map presented in Chapter IV of this thesis. Of the 15 microsatellites, two were not mapped. Of the eight polymorphic markers, four are located on the tip of a linkage group, whereas the other four have a more central position; three of the non-polymorphic microsatellites are located

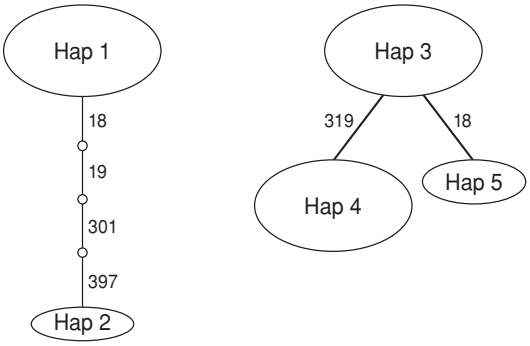

Figure 5.3. Haplotype network based on the 449bp mitochondrial *COI* sequence. Labels in circles represent particular haplotypes, numbers along lines the nucleotide positions in the sequence that changed. The size of the circles reflects the haplotype frequency.

on the tip of a linkage group and two were central. Thus, we did not see a clear correlation between the polymorphism of the markers and their location on the linkage groups, as might be expected from the mode of diploidy restoration.

The genetic distance analysis (Figure 5.2) indicated a clear differentiation between both reproductive modes but did not show clustering of arrhenotokous individuals according to the mitochondrial haplotype or geographical origin.

Mitochondrial DNA analysis

Twenty-seven samples out of 29 were successfully sequenced (for samples MtG A 98 and MtV A 98 no sequence was obtained). Six single polymorphisms were found in the *COI* gene among the samples and reproductive modes of *V. canescens* (Table 5.4). Among the 27 individuals (20 arrhenotokous and 7 thelytokous, see Table 5.1) we found five haplotypes (Figure 3) with a haplotype diversity (Hd) of 0.75. The haplotypes are represented in two networks in Figure 5.1 and in a phylogenetic analysis (Figure 5.4): haplotypes 1 and 2 (Hap 1 and Hap 2) were quite similar to each other differing in four base pairs. Hap 1 was found in seven indi-

Table 5.4. Mutation positions (bp) of mitochondrial haplotypes.

	Position					
Haplotype	018	019	020	301	397	419
Hap 1	T	-	C	-	A	A
Hap 2	A	-	-	G	G	A
Hap 3	T	A	T	-	A	A
Hap 4	T	A	T	-	A	G
Hap 5	C	A	T	-	A	A

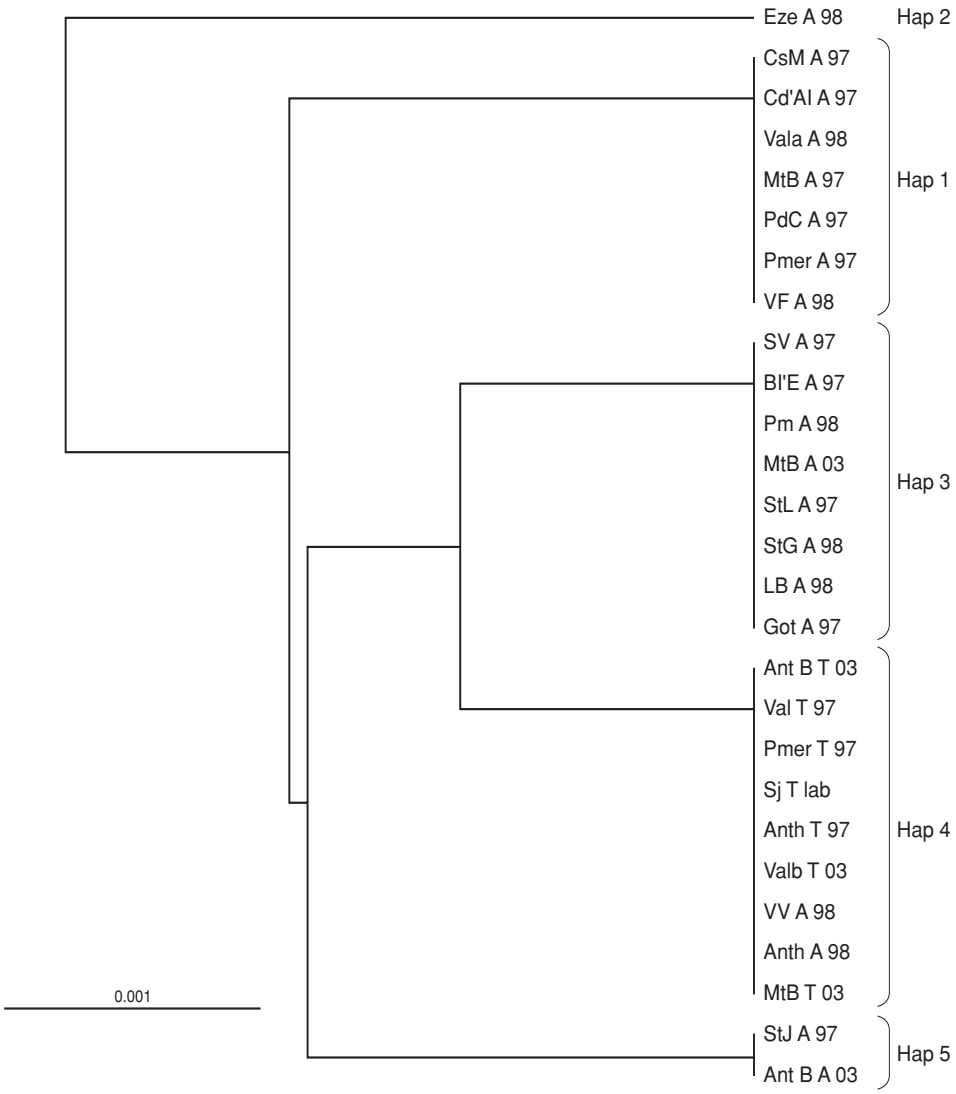

Figure 5.3. Haplotype network based on the 449bp mitochondrial *COI* sequence. Labels in circles represent particular haplotypes, numbers along lines the nucleotide positions in the sequence that changed. The size of the circles reflects the haplotype frequency.

viduals whereas Hap 2 represented a single individual. Haplotypes 3, 4 and 5 (Hap 3, Hap 4 and Hap 5) were very similar to each other differing in one base pair only. Hap 4 was the most common of the haplotypes (9 individuals), followed by Hap 3 (8 individuals) and Hap 5 represents 2 individuals. Hap 1, Hap 2, Hap 3 and Hap 5 were exclusive of arrhenotokous individuals whereas Hap 4 was the only haplotype present among the thelytokous wasps and occurred in two arrhenotokous samples (Table 5.5).

Table 5.5. Comparison of haplotypes described in this study and in Schneider *et al.* (2002). Location abbreviations are indicated in Table 5.1.

Sample	This study	Schneider *et al.* 2002	Sample	This study	Schneider *et al.* 2002
Cd'Al A 97	Hap 1	II	StG A 98	Hap 3	II
CsM A 97	Hap 1	II	MtB A 03	Hap 3	
MtB A 97	Hap 1	II	Anth A 98	Hap 4	II
PdC A 97	Hap 1		VV A 98	Hap 4	II
Pmer A 97	Hap 1	II	Anth T 97	Hap 4	I
Vala A 98	Hap 1	II	Pmer T 97	Hap 4	I
VF A 98	Hap 1	II	Val T 97	Hap 4	I
Eze A 98	Hap 2	II	Valb T 03	Hap 4	
Bl'E A 97	Hap 3	II	MtB T 03	Hap 4	
Got A 97	Hap 3	II	Ant Bot T 03	Hap 4	
StL A 97	Hap 3	II	SJ T 03	Hap 4	
SV A 97	Hap 3		StJ A 97	Hap 5	II
LB A 98	Hap 3	II	Ant Bot A 03	Hap 5	
Pm A 98	Hap 3	II			

Discussion

Using several parameters to measure genetic variation, this study has shown that thelytokous individuals are less heterozygous than arrhenotokous ones, although not necessarily less diverse. This supports the hypothesis of the genome homozygosation suggested by Beukeboom & Pijnacker (2000) as a consequence of the type of diploidy restoration that thelytokous eggs undergo (central fusion automictic parthenogenesis). Loci distal from the centromere will become more homozygous over generations than loci located near the centromere due to increased recombination chance of distal loci. We have looked for further support of this process by relating the genome position of loci to their homozygosity level. Unfortunately, no such a correlation was evident, polymorphic microsatellites as often had a central position as homozygous ones. This might be because our linkage map is not saturated with markers and the location of the markers is not definitive.

The microsatellite survey has revealed five unique alleles in the thelytokous sample that are not present among the arrhenotokous individuals. One would expect that if thelytokous populations arise from arrhenotokous ones, individuals of both reproductive modes would share the same alleles. It is well known that microsatellites have high mutation rates (Schlötterer, 2000) and this is how new unique thelytokous alleles might appear. The observation of unique thelytokous

alleles might be related with the age of the thelytokous clone, indicating that this is an old clone which has developed its own alleles.

In this study we find five mitochondrial haplotypes that show a very accurate association with reproductive mode: thelytokous individuals have exclusively haplotype 4, whereas the other haplotypes are present only in arrhenotokous individuals. This clear differentiation between arrhenotokous and thelytokous mitochondrial types might suggest that each reproductive mode is a different species. However, an exception are two arrhenotokous individuals (VV A 98 and Anth A 98) that share haplotype 4 and are apparently not related to each other. A possible explanation is that there is one widespread thelytokous clone that originated from an arrhenotokous individual with haplotype 4. An alternative is that occasional sex occurs between both reproductive modes. This last explanation implies that arrhenotokous and thelytokous wasps belong to the same species. It is not surprising to see that several different haplotypes occurs in the arrhenotokous populations given that sex occurs among individuals of different populations that may carry different mitochondrial types. Schneider *et al.* (2003) found two haplotypes that exactly corresponded to each reproductive mode. The fact that we find more mitochondrial diversity than Schneider *et al.* (2003) is certainly due to the mothods used in each study: RFLPs have lower resolution than sequencing. In Schneider (2003), more mixing between arrhenotokous and thelytokous individuals is observed in the genetic analysis of field samples and she suggests that this pattern might be due to frequent occasional sex in the field between both reproductive modes. We find a very clear segregation of the two reproductive modes for both nuclear and mitochondrial markers which indicates that such gene flow is not so common in the field. This is consistent with our findings of arrhenotokous males inseminating thelytokous females, but not obtaining hybrid offspring.

In theory the system of *Venturia canescens* has a great potential for the study of the paradox of sex as stated by Schneider *et al.* (2002). However, we have shown in this study that our results do not completely support their findings and conclusions. The multiple events of occasional sex that they suggest to explain their results might be an artifact of the markers they used. We infer that AFLP markers used by Schneider *et al.* (2003) may overestimate similarities between both reproductive modes. This does not mean that *V. canescens* is a less interesting study model. As we will show in Chapter VI of this thesis, other approaches can be used to study the genetic basis of arrhenotoky and thelytoky in this system.

Acknowledgements

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Appendix I. Genotyping raw data for 15 microsatellites of the 29 individuals used in this study.

Indv	Location	*Vcan*61	*Vcan*62	*Vcan*63	*Vcan*64	*Vcan*65	*Vcan*66	*Vcan*67	*Vcan*69	*Vcan*70	*Vcan*71	*Vcan*97	*Vcan*109	*Vcan*110	*Vcan*112	*Vcan*114
Arrhenotokous																
1	Bl'E	0	254	182184	286	230233	240252	0	223226	224	0	142	0	0	143149	0
2	StJ	0	0	182	286	209218	248	142154	226	221224	0	140	189	0	143	241251
3	Cd'Al	0	250	182184	280286	227236	240244	0	220226	224	228234	140	0	172	143149	247251
4	StL	0	250266	182	286295	233	240244	157	0	224	0	140	189191	172	143	247
5	CsM	184	0	0	286	224233	244	157	226	215	0	140142	189191	172	143149	247
6	SV	0	0	174184	286	215230	252260	151	226	224	0	140142	189	0	149	241
7	GOT	0	0	0	280286	212230	240244	151	0	224	0	142	191	172	149161	237241
8	Mt.B	188190	0	182	283289	218221	240	154160	223229	0	234	140	191193	0	143149	241
9	PdC	188190	0	182184	286289	209233	240	142	226229	0	234	140	189191	174	143	247
10	Pmer	0	0	182	286	212227	240244	0	226	221224	0	140144	189193	172	143149	241247
11	Eze	0	250266	182184	0	218	240244	142160	217226	224227	237	140142	191	172	143	247
12	LB	178	250266	182	0	221227	240244	157	223226	224	0	140144	191	172	143	241247
13	MtG	188	0	182	0	209212	240244	157	226	0	237243	0	189191	172	143	241247
14	MtV	188190	0	182	0	209218	240252	139	220226	224	0	140142	191193	174	147161	247251
15	Anth	188	0	182186	286	230	244	142	226	212221	234	140142	189191	172	143	241
16	Pm	190	0	182186	286	218230	240244	142154	226	212221	0	140	189191	172	143	241247
17	StG	0	0	182	0	212227	240244	0	0	221224	234	140	191	172	143	241
18	Vala	0	0	182186	0	215224	240260	0	223226	221	246	140142	191	172	143	0
19	VF	190194	250	182186	0	209227	240244	154157	226	221224	246	140	189193	172	143	0
20	VV	0	0	0	0	0	0	0	0	221	0	0	0	0	0	0
21	Anth	188	0	182184	286	212227	240	139154	226	221	240	140	191193	172	143	0
22	MtB	188190	266	182	0	200227	244	154160	223226	224	0	140	189191	172	143	241247
Thelytokous																
23	Anth	0	256	182	289	221	248	157	229	0	231	142	193	172	161	243
24	Pmer	0	256	182	289	221	248	157	229	227	0	142	193	0	161	243
25	Val	188	256	182	286	221	248	157	229	230	231243	142	193	172	159161	243
26	Anth	188	256	182	0	221	248	157	229	230	0	142	193	172	161	243
27	Valb	188	256	182	0	221	248	157	223229	230	0	142	193	172	161	243
28	MtB	188	0	182	0	221	248	157	229	230	0	142	193	172	161	243
29	SJ	190	250	182	0	215	244	151	229	227	243	152	193	172	161	0

Appendix II. Observed allele frequencies in arrhenotokous and thelytokous females for 15 microsatellites. N designates the number of individuals tested of each reproductive mode.

Locus	Alleles	Arrhenotokous	Thelytokous
*Vcan*61	N	11	5
	178	0.091	0
	184	0.091	0
	188	0.455	0.8
	190	0.318	0.2
	194	0.045	0
*Vcan*62	N	7	6
	250	0.5	0.167
	254	0.143	0
	256	0	0.833
	266	0.357	0
*Vcan*63	N	19	7
	174	0.026	0
	182	0.711	1
	184	0.158	0
	186	0.105	0
*Vcan*64	N	13	3
	280	0.077	0
	283	0.038	0
	286	0.769	0.333
	289	0.077	0.667
	295	0.038	0
*Vcan*65	N	21	7
	200	0.024	0
	209	0.119	0
	212	0.119	0
	215	0.048	0.143
	218	0.143	0
	221	0.048	0.857
	224	0.048	0
	227	0.167	0
	230	0.143	0
	233	0.119	0
	236	0.024	0
Vcan66	N	21	7
	240	0.452	0
	244	0.381	0.143
	248	0.048	0.857
	252	0.071	0
	260	0.048	0
Vcan67	N	16	7
	139	0.094	0
	142	0.219	0
	151	0.125	0.143
	154	0.188	0
	157	0.281	0.857
	160	0.094	0
*Vcan*69	N	18	7
	217	0.028	0
	220	0.056	0
	223	0.139	0.071
	226	0.722	0
	229	0.056	0.929
*Vcan*70	N	19	6
	212	0.053	0
	215	0.053	0
	221	0.316	0
	224	0.553	0
	227	0.026	0.333
	230	0	0.667
*Vcan*71	N	10	3
	228	0.05	0
	231	0	0.5
	234	0.45	0
	237	0.15	0
	240	0.1	0
	243	0.05	0.5
	246	0.2	0
*Vcan*97	N	20	7
	140	0.7	0
	142	0.25	0.857
	144	0.05	0
	152	0	0.143
*Vcan*109	N	19	7
	189	0.342	0
	191	0.526	0
	193	0.132	1
*Vcan*110	N	17	6
	172	0.882	1
	174	0.118	0
*Vcan*112	N	21	7
	143	0.738	0
	147	0.024	0
	149	0.19	0
	159	0	0.071
	161	0.048	0.929
*Vcan*114	N	17	6
	237	0.029	0
	241	0.441	0
	243	0	1
	247	0.441	0
	251	0.088	0