PROC. EXPER. & APPL. ENTOMOL., N.E.V. AMSTERDAM, Vol. 11, 2000

DISTRIBUTION OF A VLP-PROTEIN POLYMORPHISM IN SEXUAL AND ASEXUAL *VENTURIA CANESCENS* POPULATIONS (HYMENOPTERA)

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Keywords: genotype frequencies, parasitoid, *Venturia canescens*, virus-like particle

Summary

In the south of France both sexual and asexual forms of the parasitoid *Venturia canescens* (Gravenhorst) occur sympatrically. Two different alleles coding for a virus-like particle (VLP) protein have been described in *V. canescens*. These alleles differ in the presence or absence of an insertion of 54 bp. The two alleles code for two different proteins that are involved in the process of avoidance of encapsulation of the parasitoid egg by the host. Wasps of three different genotypes can be found in the field in sexuals: homozygous individuals with the 54 bp insertion, homozygous individuals without the insertion and heterozygous individuals that have both alleles. In asexuals only homozygous individuals were found. The genotype frequencies of asexuals and sexuals differ from each other and there are differences between local populations of each reproductive mode.

INTRODUCTION

While most organisms reproduce sexually, some have asexual reproduction. The evolutionary significance of sexual versus asexual reproduction is one of the central and unsolved enigmas in evolutionary biology. The resistance of sexual populations against invasions of asexual forms is generally poorly understood. The difficulty has been to find counterbalancing advantages of sexual reproduction when, theoretically, the costs of reproducing sexually are twofold compared to asexual reproduction at a 0.5 sex ratio (Maynard Smith 1978). Clearly, if sex is so costly the widespread occurrence of it must be promoted by factors sufficiently counterbalancing the costs of sex.

Venturia canescens (Gravenhorst) (Hymenoptera: Ichneumonidae) is a solitary endoparasitoid of pyralid moths (Salt 1975). Its mode of reproduction has been reported as both arrhenotokous (sexually), i.e. females developing from diploid, fertilized eggs and males developing from haploid unfertilized eggs, and thelytokous (asexually), i.e. females developing from diploid, unfertilized eggs. In the south-east of France (see Fig. 1) both modes of reproduction occur sympatrically; wasps of different kind can be trapped within the same tree (Beukeboom *et al.* 1998). Although in some parasitoid species *Wolbachia* bacteria cause thelytoky through gamete duplication, this is not the case in *V. canescens* (R. Stouthamer pers. com, R. Butcher, pers. com.) and it is likely that thelytoky in *V. canescens* is related to some genetical factor.

V. canescens attacks different host species and in some of these the defensive response of the host is very effective, *i.e.* the egg or early instar larva becomes encapsulated (Salt 1976). In some hosts, however, *V. canescens* is capable of avoiding encapsulation by making the egg 'chemically invisible' for the host's immune system (Feddersen *et al.* 1986). The surface of the parasitoid egg contains a coating of 'virus-like particles' (VLP's) in which at least four different proteins are involved. One of these VLP proteins, the 40 kDa protein (p40), has been cloned and sequenced (Theopold *et al.* 1994). Hellers *et al.* (1996) found two allelic variants in the p40-coding gene in thelytokous populations in Australia that differ in the presence or absence of a tandem repeated sequence of 54 bp. In parallel with the two allelic variants, two different VLP proteins exist. We will call the VLP p40 gene with insertion "+" and the one without "–" , the two homozygous genotypes "++" and "––" and the heterozygote "+–".

Although the precise function of the p40 protein in the avoidance of encapsulation is still unknown, its polymorphism may play a role in the coexistence of sexual and asexual populations of *V. canescens*. As the cytological mechanism of oogenesis leads to increasing homozygosity in thelytokous strains (Beukeboom & Pijnacker, in prep.), arrhenotokous populations may experience an advantage when heterozygotes are able to exploit a broader host range. As a starting-point of a research project on the role of VLP polymorphism in the coexistence of thelytokous and arrhenotokous modes of reproduction in *V. canescens* we here present the results of a study on VLP allele frequencies in field populations in the south of France.

MATERIAL AND METHODS

Field sampling.
In October 1997 and September-October 1998 approximately 200 baits were put out at 25 sites in a 60 km coastal region of the Côte d'Azur (southeastern France) between St. Raphael and Monaco (see Fig. 1.) Baits consisted of small screen bags containing approximately 30 *Ephestia kuehniella* larvae in which 'wild' wasps could oviposit. Baits were suspended from trees (mostly figs) with a string and protected from rain by attaching a plastic container cup upside down to the string. They were collected after two days and transported to the laboratory (25°C) for emergence. All female offspring were individually placed on 20-30 *Ephestia* hosts to determine their reproductive mode. Females producing all-female progeny were scored as thelytokous and those with all-male or mixed broods as arrhenotokous. Of each bait one thelytokous and one arrhenotokous female (if present) was used for PCR analysis of the VLP p40 gene. In total 19 asexual and 102 sexual females were analyzed.

Establishment of allele frequencies.
To calculate the VLP genotype frequencies in the field PCR with p40 gene specific primers was carried out (Fig. 2) on genomic DNA of *V. canescens* according to the protocol described in Hellers *et al.* (1996).

RESULTS

The sampling sites that yielded *Venturia canescens* in 1997 and/or 1998 are shown in the top part of Fig. 1. These were analyzed for VLP's. Note that the sites sampled in 1997 are not all the same as the sites sampled in 1998. The figure shows 1) that sexual reproduction is the predominant mode, and 2) that the distribution of the reproductive modes can change from one year to the next.

For the analysis of the genotype frequencies we pooled the data for 1997 and 1998 and we divided the total area in three regions: north-east, central and south-west. In none of these regions heterozygous asexuals with respect to the VLP alleles were found, although the number of animals in the analysis was low (bottom part of Fig. 1). In the sexual populations heterozygous individuals were common and the genotype frequencies were significantly different between regions (χ^2=20.2, *df*=4, *P*=0.0004). The overall frequency of the '–' allele increased from 0.28 in the south-west region to 0.54 and 0.70 in the central and north-east regions respectively.

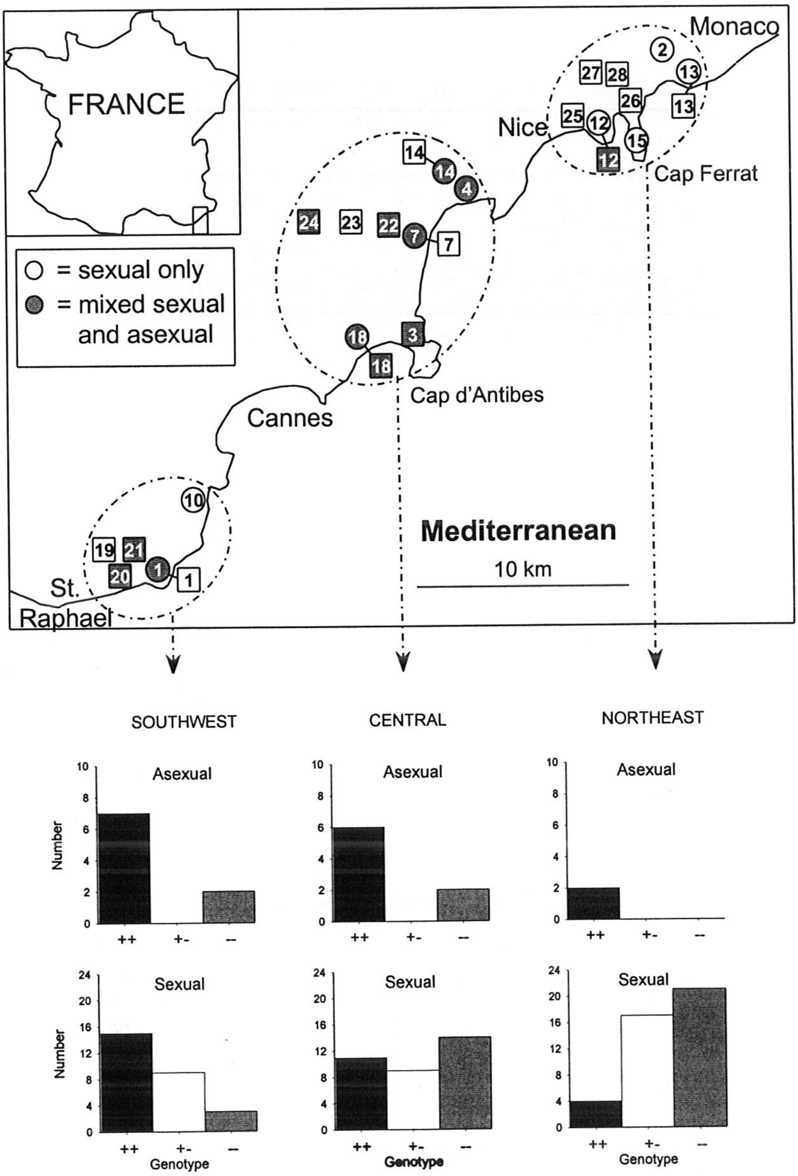

Fig. 1. Collection sites of *Venturia canescens* in southeastern France.
Shown are samples of 1997 (circles) and 1998 (squares) used for VLP analysis.
Localities are: 1 = Anthéor, 2 = Belvre l'Èze, 3 = INRA station at Cap d'Antibes, 4 = Cagnes sur Mer, 6 = Juan les Pins, 7 = La Brague, 8 = La Croix des Gardes, 10 = Le Trajas Supérieure, 12 = Mont Boron, 13 = St. Laurent, 14 = Camping Panoramer in Cagnes sur Mer, 15 = St. Jean- Cap-Ferrat in St. Jean village, 18 = Vallauris, 19 = St. Guitte, 20 = Cap de Dramont, 21 = Agay, 22 = Biot, 23 = La Vallée Verte, 24 = Valbonne, 25 = Nice, 26 = Villefranche, 27 = Mt. Gros, 28 = Mt. Vinaigrier.

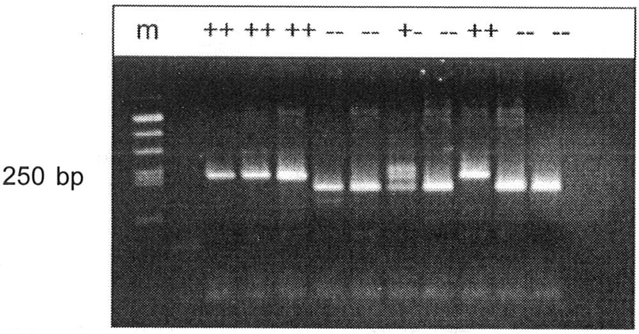

Figure 2. Detection of VLP alleles, ++ = homozygote with insertion, –– = homozygote without insertion, +/– = heterozygote, m = size standard

DISCUSSION

The results of this study show that there is geographical variation in the frequency of VLP alleles in natural populations of *V. canescens* in the south of France. Due to the low number of asexuals in the analysis, it is difficult to draw firm conclusions from the distribution of the VLP alleles in thelytokous populations. However, the complete absence of heterozygotes can be explained by the cytological mechanism for thelytokous oogenesis described by Beukeboom & Pijnacker (in prep.). This mechanism is genetically similar to central fusion and produces increasing homozygosity in succeeding generations except for those genes that are close to the centromere (Suomalainen *et al.* 1987). The exact location of the VLP gene on the genome is still unknown, but our results suggest that it is not likely to be located near to the centromere.

From laboratory observations it is known that males can court and inseminate thelytokous females (Andreae, unpublished). Whether the sperm can be functional in asexual females is not known yet, but (1) given the common presence of '-' alleles in the sexual populations, (2) the predominance of '+' alleles in the asexuals, and (3) the absence of heterozygotes in the asexuals these results suggest that if this sperm were functional, matings between males and thelytokous females are probably rare in the field, and that both modes of reproduction are (largely) genetically isolated.

Since the deletion in the '–' allele preserves the open reading frame of the VLP-protein coding gene, the two VLP proteins occur together in heterozygotes (Dongmei Li, pers. comm.). This might enable heterozygotes and hence sexual populations to exploit a broader host range, which in turn might provide an explanation for the sympatric coexistence of both modes of reproduction. A study of the survival of parasitoids of different VLP genotype is one of our future research aims.

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