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Geographical distribution and genetic relatedness of sympatrical thelytokous and arrhenotokous populations of the parasitoid *Venturia canescens* (Hymenoptera)

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Venturia canescens.

Abstract

Theory predicts that asexual reproduction has a competitive advantage over sexual reproduction because of the twofold cost of producing males. Few systems are suitable for directly testing this prediction. In the solitary parasitoid wasp *Venturia canescens* both arrhenotokously (sexual) and thelytokously (asexual) reproducing individuals occur sympatrically. We sampled 922 wasps from 22 localities along the coast of south-eastern France. Thelytokous wasps were less abundant (23%) than arrhenotokous wasps and were almost always found in sympatry with arrhenotokous ones. An analysis of genetic relatedness using amplified fragment length polymorphism (AFLP) markers showed the existence of a widespread thelytokous clone. In addition, a few thelytokous individuals were found to be closely related to arrhenotokous ones and vice versa. These data suggest the occurrence of occasional gene flow between both reproductive modes and/or recurrent origin of thelytokous clones from coexisting arrhenotokous populations in the area. The results are discussed in the context of the paradox of sex.

Introduction

The evolutionary significance of sexual vs. asexual reproduction is one of the central and unsolved enigmas in evolutionary biology. Whereas most organisms reproduce sexually, i.e. fusion of a male and a female gamete, others have asexual reproduction, i.e. females developing from unfertilized eggs and reproducing without males. This gives asexuals a twofold reproductive advantage, known as the twofold cost of sex (Maynard Smith, 1978). The resistance of sexual populations against invasions of asexual forms is generally poorly understood. Theoretically, a gene for asexual reproduction would rapidly spread in a sexual population and eventually reach fixation. The widespread occurrence of sex must be promoted by factors sufficiently counterbalancing the costs of sex. Several mechanisms have been proposed to explain the persistence of sexual reproduction including resistance to parasites (Lively, 1987; Kondrashov, 1993; Hurst & Peck, 1996; Peck *et al.*, 1998) and avoidance of mutation accumulation (Kondrashov, 1988; Kirkpatrick & Jenkins, 1989; Barton & Charlesworth, 1998). Also niche differentiation has been proposed as a mechanism which would favour sexual reproduction as sexuals should have a higher adaptive potential (Bell, 1985; Gould & Gould, 1989). However, a satisfactory explanation for the maintenance of sex has not yet been found and more studies are needed to test the validity of different hypotheses (West *et al.*, 1999). Organisms in which both sexual and asexual reproduction occur are particularly suitable for such a study (Corley & Moore, 1999).

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In the Hymenoptera sexual reproduction is defined as arrhenotoky (haploid unfertilized eggs develop into males, diploid fertilized eggs into females) and asexual reproduction as thelytoky (unfertilized eggs develop into diploid females). In accordance with these definitions we will use arrhenotoky to refer to sexual reproduction and thelytoky to refer to asexual reproduction of the parasitoid species studied here. Arrhenotoky is considered to be the ancestral reproductive mode. However, thelytoky occurs widespread and apparently has evolved independently many times (Cook, 1993). Reversal of thelytoky to arrhenotoky is considered less likely because thelytokous lineages appear to loose their ability to reproduce arrhenotokously over time (Williams, 1975; Silva & Stouthamer, 1996). This is believed to be the result of the accumulation of mutations in genes involved in sexual functions (e.g. female courtship, male fertility) in the absence of selection. In the Ichneumonid wasp *Venturia canescens* (Gravenhorst) both arrhenotokous and thelytokous reproduction can be found (Beukeboom *et al*., 1999). Thelytoky in *V. canescens* is obligate and not caused by *Wolbachia* bacteria. Therefore it is likely to have a genetical basis (Beukeboom & Pijnacker, 2000). Arrhenotokous and thelytokous females are both diploid and morphologically indistinguishable (K. Zwakhals, pers. comm.).

In the south of France, and also in many other places in south of Europe (Schneider, unpublished), both modes of reproduction often occur sympatrically; wasps of both kinds can be trapped in the same bait. This raises intriguing questions as how frequent and under what conditions thelytoky arises. Do thelytokous wasps replace the arrhenotokous ones? If not, what are the costs of thelytoky? The distribution and frequency of thelytoky can be considered to be the result of three main processes: the rate of appearance of thelytokous lines, clone survival and their spread by dispersal. The strength of these processes will be reflected in the geographical distribution of thelytoky and the genetic structure of sympatric populations of both modes. For example, if thelytoky arises frequently, with high clonal survival (at least in the order of several generations) and high dispersal rates, thelytokous wasps will be found almost everywhere, with many different clones and a low relatedness with local arrhenotokous populations. Alternatively, if the appearance of a thelytokous line is a rare event in *Venturia* and survival is high, one would expect to find one or just a few widespread clones with thelytokous wasps being more related to each other than to most of the arrhenotokous wasps. On the other hand, if the rate of appearance of thelytoky is high with low survival rates and low migration, one expects to find many different clones that show high relatedness with the sympatric arrhenotokous wasps. Clearly, many other and intermediate scenarios are conceivable, each generating particular patterns of geographical distribution and genetic structure.

In order to gain insight in the origin of thelytokous populations and their co-occurrence with arrhenotokous wasps in the field, we studied their distribution and genetic relatedness along a 60-km coastal stretch. Although *V. canescens* is a mobile insect, it is quite unlikely that it is able to move over such a distance within one generation. This is the first study that analyses the distribution and genetic structure of sympatric arrhenotokous and not-*Wolbachia* induced, thelytokous parasitoids in the field.

Materials and methods

Field sampling

During the autumns of 1997–99 wasps were collected between Cannes and St Rafael along the coast of the Côte d'Azur in southern France. Baits consisted of a cotton screen bag (approximately 20 × 20 cm), containing third instar larvae of the Mediterranean flour moth *Ephestia kuehniella*, Zeller (Lepidoptera: Phycitidae). They were closed with a plastic clip and suspended from trees and shrubs at 1–2 m height. A plastic cup was placed upside down over the bait to protect it from rain. Before the baits were used they were left in the host culture medium (semolina) for 24 h to increase their attraction by impregnation with kairomones produced by the host larvae. The baits have been shown to be equally attractive to both arrhenotokous and thelytokous wasps in dispersal experiments in the field (I. Amat, pers. comm.).

In October 1997, 11 sites were sampled, in October 1998, 14 sites and in July and October 1999, 22 sites (Fig. 1). We used 10 baits per site with a distance between the baits of at least 10 m. The baits were left out for 48 h during which adult females could visit them and lay eggs in the host larvae through the screen bag. The average distance between sites was about 3 km. We consider it impossible that females could travel between sites during this period of 48 h. After the baits were collected, they were taken into the laboratory where they were checked daily for emerging wasps. Each emerging female (F_1) was set up as an isofemale line in order to determine her reproductive mode. After 24 h of exposure to hosts the F_1 females were killed and stored at –80 °C for later DNA analysis. If only females (at least five) occurred in the offspring the F_1 mother was scored as thelytokous, if only males or males and females occurred in the offspring the F_1 mother was scored as an arrhenotokous female. Males and females could occur in the F_1 when females mated with males emerging from the same trap before they were given hosts. If an emerging female produced only few daughters (≤5) these daughters were bred one more generation to ascertain that they were thelytokous. Note that wasps emerging from one bait could be siblings because a single visiting female may have oviposited in more than one host larva.

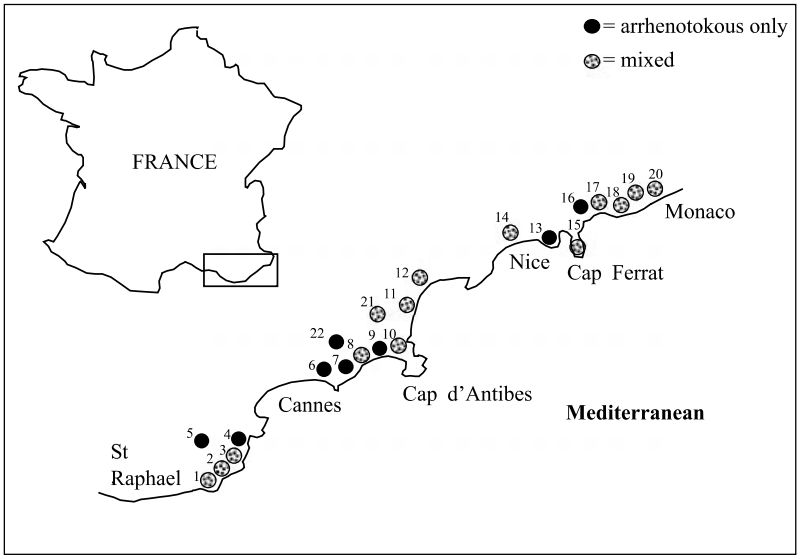

Fig. 1 Reproductive modes (arrhenotokous only or mixed arrhenotokous/thelytokous) at collection sites of *Venturia canescens* in south-eastern France. Localities are: 1 = Cap du Dramont (CdD), 2 = Agay, 3 = Anthéor (Anth), 4 = La Trajas (LT), 5 = Saint Guitte (StG), 6 = La Napoule (LN), 7 = La Croix de Gardes (LCdG), 8 = Vallauris (Val), 9 = Juan le Pins (JlP), 10 = Cap d'Antibes I.N.R.A. station (CdAI), 11 = La Brague (LB), 12 = Biot, 13 = Nice, 14 = Panoramèr (Pm) 15 = Mont Boron (MtB), 16 = Mont Vinagrier (MtV), 17 = Mont Gros (MtG), 18 = Villefranche (Vf), 19 = Eze, 20 = Saint Laurent (StL), 21 = Valbonne (Valb), 22 = la Vallée Verte (VV).

Therefore, from baits producing more than one wasp of either mode only one thelytokous and/or one arrhenotokous wasp was included in our analysis. Wasps were reared at 25 °C and 16:8 (L:D) on fourth instar *E. kuehniella* caterpillars which were raised on semolina.

DNA extraction and AFLP analysis

Wasp abdomen were used for DNA extraction using a Nucleon BACC2 kit according to the manufacturer's protocol (Amersham, Buckinghamshire, UK). The DNA was precipitated in ice-cold absolute ethanol, the pellet washed with 70% ethanol, air dried for 5 min and the DNA dissolved in 25 μL of sterile water. DNA extraction was checked on a 0.8% agarose gel and DNA concentration determined by spectrometry.

The amplified fragment length polymorphism (AFLP) technique (Vos *et al.*, 1995) is considered a reliable and powerful tool for the evaluation of genetic variability (Roa *et al.*, 1997; Lerceteau & Szmidt, 1999). The technique is based on the detection of genomic restriction fragments by polymerase chain reaction (PCR) amplification. The number of fragments detected in a single reaction can be tuned by selection of specific primer sets. The AFLPs have the advantage of requiring no *a priori* investment in terms of sequence analysis, primer synthesis or DNA probes characterization. AFLP products are reliable indicators of homology at least within species, they are locus-specific and stable across populations (Van den Berg *et al.*, 1997).

The AFLP procedure involves three main steps. (i) Restriction of the DNA and ligation of adapters. For each sample, approximately 250 ng of DNA was digested with four units of Mse/EcoRI restriction enzyme in a reaction volume of 20 μL. In the same reaction, ligation was performed with six units of DNA ligase and 100 ng μL^{-1} adapters. After incubation at 37 °C for 2 h, samples were transferred to a 65 °C water bath for 5 min in order to inactivate the DNA ligase. Incomplete digestion of genomic DNA can lead to false polymorphism in AFLP profiles. Therefore complete digestion was confirmed by running each sample on an agarose gel. We also checked whether each enzyme in the absence of the other led to complete digestion. (ii) Pre-amplification PCR: 2.5 μL of the restriction–ligation product was combined with 17.5 μL of preamplification primer solution, using the AFLP core mix (Perkin Elmer, Warrington, UK). The primers used were Eco+A and Mse+C. PCR consisted of 20 cycles of 94 °C for 1 s, 56 °C for 30 s, and 72 °C for 2 min terminated by a single step of 60 °C for 30 min using a MJ-PCR thermocycler (MJ Research, Landeraag, The Netherlands). After checking for the presence of a smear by agarose electrophoresis, the pre-amplification mixture was diluted 1:19 with TRIS-EDTA(TE) buffer. (iii) Selective amplification: primers that match the known adapter sequence plus three selective nucleotides were used to reduce the number of amplified fragments. Two primer combinations were identified as yielding many polymorphic fragments, Mse-CAC and the fluorescently labelled Eco RI primers Eco-ACC and Eco-AAC. A touchdown PCR reaction was used with one cycle of 94 °C for 2 min, 65 °C for 30 s and 72 °C for 2 min followed by 23 cycles in which the annealing temperature was reduced with 1 °C steps to 56 °C, again followed by a single step of 60 °C for 30 min.

For gel electrophoresis 2 μL of the selective amplification was added to a 3-μL mixture of formamide, loading buffer, and size standard (GeneScan 1000 Rox, Perkin

Elmer). The amplified labelled fragments were analysed on 5% Long Ranger polyacrylamide gels (BMA Bio Products, Denmark), using an ABI Prism 377 automated genetic analysis system (Perkin Elmer). Data were processed using ABI GeneScan Analysis 3.1 software (Perkin Elmer). Each sample was manually checked for correct aligning of the size standard and when necessary aligned by hand. Data were subsequently imported into Genographer (Benham, 1999) (http://hordeum. oscs.montana.edu/genographer) and AFLP profiles scored for the presence/absence of fragments between 50 and 500 bp. Reproducibility was tested for 10 individuals by repeating the complete AFLP procedure and found to be high (98%).

Data analysis

In Genographer we used the thumbnail option to visually score the presence and absence of bands. 0/1 Matrices were obtained for each primer and used in TREECON (TREECON for Windows Version 1.3b, University of Antwerp, Belgium) (Van de Peer & De Wachter, 1994) to calculate genetic distances using the Nei–Li distance coefficient. The advantage of using Nei–Li distance estimates is that they use only the shared presence of a band (assuming homology) (Nei & Li, 1979). A band can be absent for many reasons (substitution, deletion, insertion, restriction site absent, etc.). For each primer pair, the number of polymorphic and monomorphic bands was determined. For statistical analyses STATISTICA 5.5 (1999 Edition, Tulsa, USA) was used. A Neighbour Joining (NJ) tree was constructed based on genetic distances in TREECON.

Results

Geographical distribution

Over all 3 years a total of 922 wasps were collected; 23% were thelytokous and 77% arrhenotokous (Fig. 1, Table 1). Arrhenotokous wasps were more abundant than thelytokous ones in all years and found at many more sites than thelytokous wasps (Table 2). Neither of the two modes seemed to be restricted to a particular part of the sampling area. Thelytokous wasps were almost exclusively found in sympatry with arrhenotokous ones. There are two exceptions. During the second sampling period in October 1999 only thelytokous wasps were found at two places (Pm and CdD). However, these places yielded arrhenotokous wasps during the previous sampling period in the same year. Weather conditions during the second sampling period in 1999 were bad and very low numbers of wasps were obtained.

Table 1 Number of thelytokous and arrhenotokous wasps collected over 3 years.

	Thelytokous	Arrhenotokous	Total
1997	61 (23%)	207 (77%)	268
1998	86 (20%)	351 (80%)	437
1999	55 (25%)	162 (75%)	217
All years	202 (23%)	720 (77%)	922

Once an arrhenotokous population was found at a particular site it was almost always found again in subsequent samplings at the same site. Leaving aside the somewhat unrepresentative second sampling in 1999, there were 27 occasions in 1997 and 1998 where an arrhenotokous population was found. In 25 of these an arrhenotokous population was also found in the following years. The two exceptions are Valb and VV in 1999. As for the thelytokous populations the picture is completely different. Out of the nine thelytokous populations found in 1997 and 1998 only one (CdD) was found to have thelytokous wasps in the following year. Arrhenotokous populations, hence, seem to be fairly stable, whereas thelytokous ones are not.

As for the origin of the thelytokous populations these patterns suggest two possible scenarios. First, thelytoky arises frequently in arrhenotokous populations, but generally becomes extinct within the same year. Alternatively, there is a stable thelytokous source population from which wasps spread into the field, but this rarely leads to persistent colonization (see Discussion). The first scenario predicts a high relatedness between sympatric arrhenotokous and thelytokous populations, the second a higher relatedness among thelytokous than between thelytokous and arrhenotokous populations.

Genetic relatedness between reproductive modes

Using two primer combinations, 108 AFLP fragments could reliable be scored. Of these, 83 (77%) were polymorphic in more than one individual. The monomorphic markers and the markers that were present or absent in only one sample were excluded from the analysis. A total of 76 wasps collected in the first sampling of 1999 were analysed; 55 arrhenotokous and 21 thelytokous. Both primer combinations gave similar estimates of genetic similarity and data were therefore pooled. The NJ tree based on the combined genetic distance matrices is shown in Fig. 2. Three arrhenotokous wasps collected in Spain were used as outgroup. Most thelytokous wasps (15) cluster more or less together in the top of the tree, indicating that the genetical distances are smaller among these thelytokous than between thelytokous and arrhenotokous wasps. The remaining six thelytokous individuals appear among arrhenotokous wasps elsewhere in the tree. One of these (82 StL) is so extremely different from all others that it heavily affects all conclusions (Fig. 2). We therefore discard it from the subsequent analyses.

To test whether the observed pattern deviated from a random distribution of thelytoky in the tree, the distri-

Table 2 Reproductive modes of wasps at sampling localities over a 3-year period, and number of individuals used per locality for the AFLP analysis.

					Individuals used in AFLP analysis from July 1999	
Locality	1997 October	1998 October	1999 July	1999 October	Arrhenotokous	Thelytokous
CdD	ns	A + T	A + T	T	2	4
Agay	ns	A + T	A	no F_2	3	0
Anth	A + T	A	A + T	A + T	6	7
LT	A	ns	A	A	4	0
StG	ns	A	A	no F_1	1	0
LN	ns	ns	A	no F_2	4	0
LCdG	A	ns	A	no F_1	3	0
Val	A + T	A	A	no F_1	1	0
JlP	ns	ns	A	A	5	0
CdAl	A + T	A	A + T	no F_1	1	3
LB	A + T	A	A + T	no F_2	4	1
Biot	ns	A + T	A	no F_1	2	0
Nice	A	ns	A	no F_2	2	0
Pm	A + T	A	A	T	2	0
MtB	A	A + T	A	A	2	0
MtV	ns	A	A	A	1	0
MtG	ns	A	A + T	A	2	1
Vf	ns	A	A + T	A	3	1
Eze	A	A	A + T	A	2	1
StL	A	ns	A + T	no F_2	5	3
Valb	ns	A + T	no F_1	no F_1		
VV	ns	A	no F_1	no F_1		
Total A	11	16	20	8		
Total T	5	6	7	3		

A = Arrhenotokous, T = thelytokous, no F_1 = no wasps emerged, no F_2 = undetermined reproductive mode of emerged wasps, ns = not sampled. Locality numbers are explained in the legend of Fig. 1.

bution of mean genetical distances among thelytokous wasps, under the null hypothesis of random distribution of thelytoky, was simulated. In the matrix of the genetical distances of all 75 wasps, 20 of them were randomly allotted to be thelytokous, whereas the remaining ones were allotted arrhenotokous. Each run the mean genetical distance between the thelytokous wasps was calculated. After 10 000 runs the cumulative distribution of distances under the null hypothesis could be constructed and compared with the observed mean distance. The probability level was highly significant ($P < 0.001$), from which we conclude that the distribution of thelytokous wasps indeed indicates real clustering. However, as can be seen in Table 1, at some sampling sites many more thelytokous wasps were caught than in others. If these wasps are genetically closer because of common ancestry this could skew the pattern in Fig. 2 towards clustering. Therefore, the above exercise was repeated 1000 times, but now with only one randomly chosen arrhenotokous and thelytokous wasp per sampling site at which more than one wasp of a mode was caught. Under the null hypothesis that thelytoky is randomly distributed, a uniform distribution of probability levels of the mean distances in these subsamples is then expected (horizontal line in Fig. 3). The actual distribution of *P*-levels in Fig. 3 is clearly skewed towards the left ($X^2_9 = 3630$, $P < 0.0001$) with 41% being less than 0.05 (bootstrapped probability $P < 0.001$). This shows that the thelytokous wasps also cluster when only one wasp per mode per site is taken into account.

There were five thelytokous wasps (excluding the exceptional 82StL) that did not cluster with the other thelytokous ones. They are spread over different branches of the tree and there is no univocal pattern in similarity to their sympatrics (Fig. 2): three of them (72Anth, 73Anth and 32Vf) have sympatric arrhenotokous counterparts relatively close in the tree (64Anth, 86Anth and 39Vf, respectively), whereas the closest sympatric arrhenotokous counterparts of the two others (22Eze and 30CdD) are in completely different branches of the tree (50Eze and 6CdD).

The mean genetic distance between all thelytokous wasps was 17.63 (SE = 0.31) and between all arrhenotokous wasps 21.22 (SE = 0.14) (*t*-test (assuming unequal variance), $t_{268} = 10.67$, $P < 0.001$). This shows that genetical variation within thelytokous wasps is smaller than within arrhenotokous wasps. The mean genetic distance of all pairwise comparisons between arrhenotokous and thelytokous wasps was 21.55 (SE = 0.14). This value is only slightly and not significantly larger than the pairwise comparisons within arrhenotokous wasps [*t*-test (assuming unequal

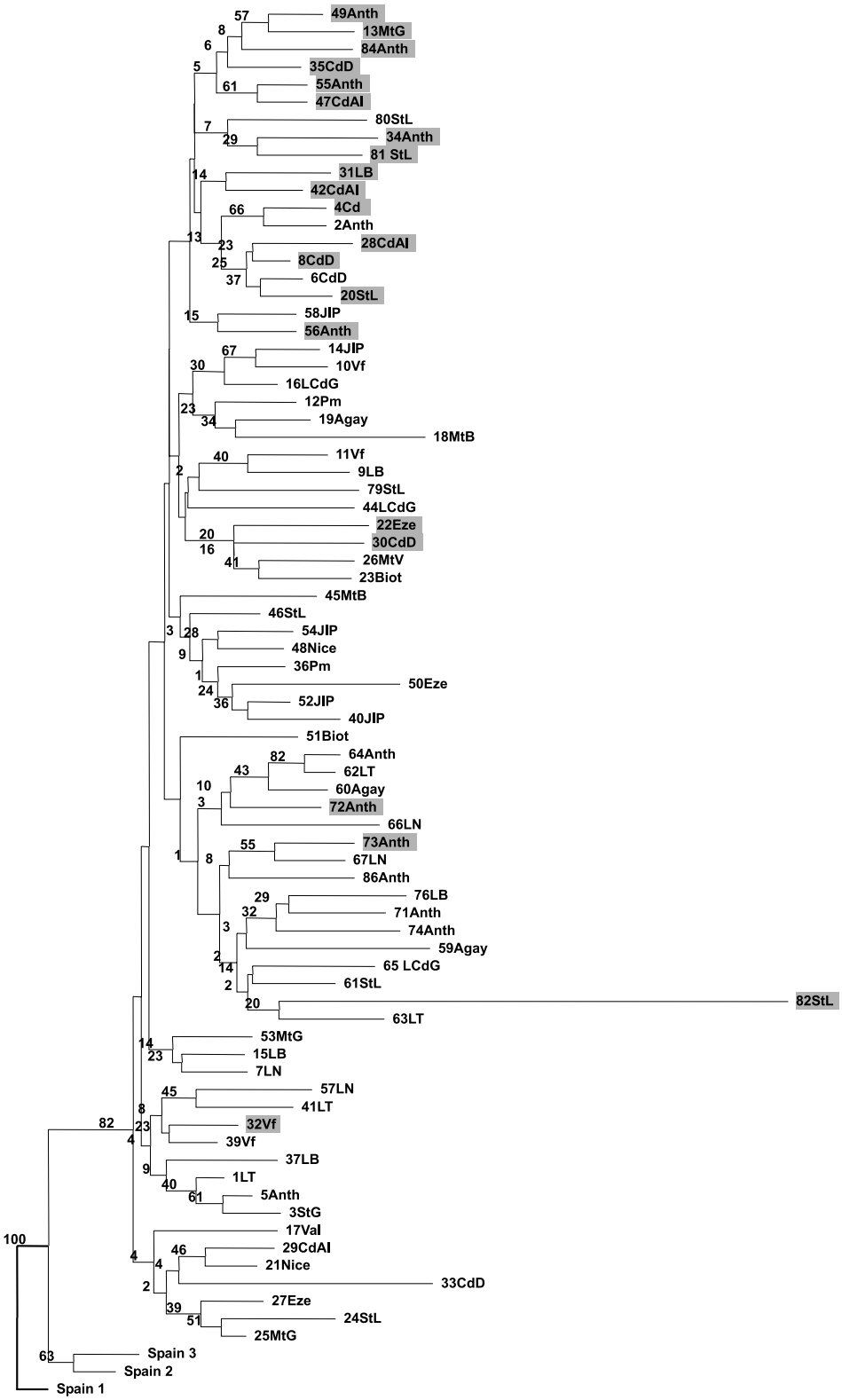

Fig. 2 Genetic distance Neighbour Joining tree of 55 arrhenotokous and 21 thelytokous wasps. Number at nodes represent bootstrap values, shaded boxes indicate thelytokous wasps.

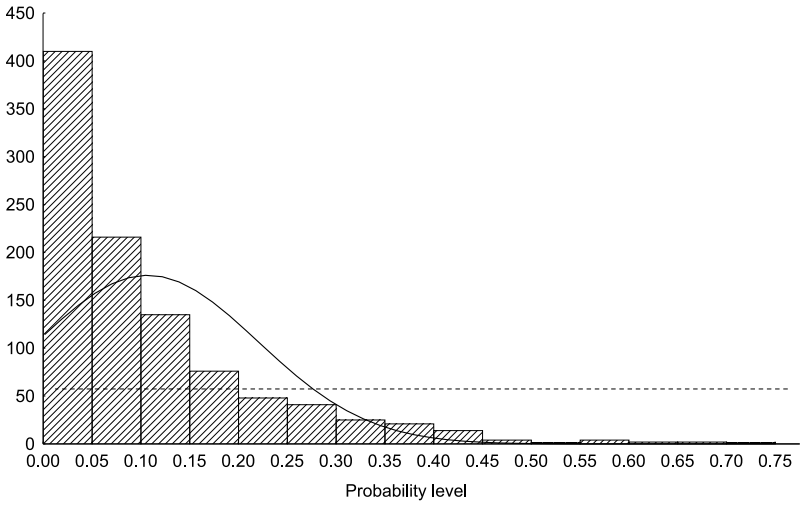

Fig. 3 Frequency distribution of probability levels of the mean genetical distance within thelytokous wasps when only one arrhenotokous and/or thelytokous wasp per sampling site is randomly drawn. The null hypothesis that thelytoky is randomly spread over the tree is indicated by the dotted line. The distribution is based on 1000 repeats (see text for further explanation).

variance), $t_{1485} = -1.74$, $P < 0.10$]. This implies that the genetic variation of the thelytokous wasps falls within the genetic variation of the arrhenotokous wasps.

A general problem with pairwise distance tests is that each genotype produces several pairwise distance estimates and that when sample groups differ in size, some genotypes are represented more frequently in the analysis than others. Therefore the significance of the *t*-test suffers from inflated degrees of freedom. As for the conclusion that genetical variation within thelytokous wasps is smaller than within arrhenotokous wasps we do not expect that this has led to an unjustified rejection of the null hypothesis as given the low probability level was in the order of 10–21. The conclusion that the genetic variation of the thelytokous wasps falls within the genetic variation of the arrhenotokous wasps is not affected by this problem, because the probability level was already larger than 0.05.

Genetic relatedness at the local level

We tested whether wasps collected within the same locality were genetically closer (smaller genetic distance) than wasps collected from different localities, and did this separately for arrhenotokous, thelytokous and both modes together. Arrhenotokous wasps within localities (for those where more than one wasp was available) were not more closely related than arrhenotokous wasps between localities (Kolgomorov–Smirnov test, $D = 0.026$, $P > 0.50$). In contrast, the relatedness among thelytokous wasps collected within a locality was significantly higher than among thelytokous wasps from different localities (Kolgomorov–Smirnov test, $D = 0.22$ $P < 0.025$). Note that all wasps of similar reproductive mode collected within one locality hypothetically could be the offspring of a single female. To test for local similarity between arrhenotokous and thelytokous wasps, we compared the pairwise genetic distance values of both reproductive modes. Relatedness between arrhenotokous and thelytokous wasps within localities is not different from between localities (Kolgomorov–Smirnov test, $D = 0.11$, $P > 0.05$). This indicates that genetic variation between both reproductive modes is smaller at the local scale than at the scale of the whole sampling area.

Discussion

The geographical analysis has shown that arrhenotoky is more widespread and persistent than thelytoky in the *V. canescens* populations in our sampling area. In the Côte d'Azur region, arrhenotokous wasps have already been noticed over 30 years ago (J. Daumal, INRA, Antibes, personal communication). Moreover, over the 3 years of our sampling, the relative abundance of populations of both reproductive modes remained equal, indicating no dramatic change in the overall frequency of arrhenotoky and thelytoky. The analysis of genetical structure of the populations in the study area showed that there is one widespread thelytokous clone and a few rare ones. The evidence for this conclusion is: (i) the significant clustering of thelytokous individuals in the tree, (ii) the low genetic distance among thelytokous individuals and (iii) the presence of six individuals that appear elsewhere in the tree (three together with sympatric arrhenotokous wasps, two separate and one very different from all others). The presence of a widespread thelytokous clone which may have arisen at least several generations ago, seems in contradiction with the indications for high extinction rates that we found for thelytokous populations in the field. This pattern can, however, be

explained by recurrent colonization from thelytokous source populations.

Venturia canescens is a widely used biological model in behavioural, population dynamical, genetical and physiological studies, and the earliest publications date back to the 1930s. Except for one publication (Beukeboom *et al*., 1999) it has always been reported as having obligate thelytokous reproduction. This is likely because of two reasons. First, most of these studies made use of a few commonly shared laboratory strains. The second reason is that many of the hosts of *Venturia* are stored product pests and the strains were generally collected from bakeries or granaries (Press *et al*., 1982; Cline *et al*., 1983; Driessen *et al*., 1995; Harvey & Vet, 1997; Bonsall & Hassell, 1998). These places provide a relatively constant environment and ample supply of pyralid hosts (Freeman, 1980; Goater, 1986; Harvey & Thomson, 1995). Under such conditions arrhenotokous populations are likely to be rapidly outcompeted by thelykous ones. Regular shipments of flour between mills and bakeries can spread a thelytokous strain rapidly over a large region once it has appeared. Therefore bakeries may serve as source populations from which thelytokous wasps can enter the environment.

In order to understand the simultaneous presence of these two reproductive modes information is required about amounts of genetic variation within populations and gene flow between them. We used an AFLP analysis to measure relatedness between arrhenotokous and thelytokous wasps, and to obtain an indication of genetic exchange between both modes. AFLPs are convenient markers (Robinson *et al*., 1999; Sharbel, 1999; Ritland, 2000) and particularly suitable for genetic diversity studies within species (Yan *et al*., 1999; Kreher *et al*., 2000; Sharbel *et al*., 2000; Van der Hulst *et al*., 2000). The main assumption in AFLP studies is that identical fragment sizes are homologous. We only used the shared presence of a band to calculate genetic distances. Our population genetic analysis focused on the comparison between arrhenotokous and thelytokous wasps. As thelytokous reproduction violates many of the assumptions of standard population genetic processes such as Hardy–Weinberg equilibrium and Mendelian segregation (Travis *et al*., 1996; Muller & LaReesa Wolfenbarger, 1999; Yan *et al*., 1999; Gaudeul *et al*., 2000) we could not estimate commonly used parameters such as heterozygosities and F_{ST} values. Six thelytokous wasps were found not to cluster with the others. One of them (82StL) was very different from all others. The exclusion of this individual from the analysis was based on its extreme high genetic distance values to any other individual in the analysis (arrhenotokous and thelytokous wasps). Perhaps the DNA quality was not appropriate (e.g. degradation), but we also cannot exclude a species misidentification (e.g. *Prestomeris* spp. and other parasitoid species also emerged from the baits). Two thelytokous individuals were collected at opposite extremes of the sampling area but cluster together along with arrhenotokous wasps from different parts of the sampling area. They could be old clones that survived over long time or may have migrated over a long distance. Although *V. canescens* known from field experiments were able to move over a 100 m a day (Desouhant *et al.,* unpublished), we doubt that the wasps can move over such a distance in one generation. Three thelytokous wasps clustered together with sympatric arrhenotokous wasps which could be the result of two nonmutually exclusive processes, i.e. recurrent origin of new thelytokous lines from local arrhenotokous ones and genetic exchange through matings between thelytokous females and arrhenotokous males. Indeed, we have evidence from laboratory experiments that both these processes are possible (Beukeboom *et al*., 1999; M.V. Schneider, K. Eunen & A. Selzner unpublished). As AFLP are dominant nuclear markers we cannot distinguish between both possible sources of genetic homogeneity between arrhenotokous and thelytokous wasps. We are currently developing mitochondrial DNA markers in order to quantify the number of clones in the sampling area. By comparing mitochondrial haplotypes with nuclear multilocus fingerprints we expect to gain more insight into the importance of the two processes in the field: the frequency of origin of thelytoky and sex between thelytokous and arrhenotokous wasps.

However, all this does not explain why thelytokous populations have not replaced arrhenotokous ones in the field. In contrast to the suggestion in the literature that *V. canescens* exclusively has obligate thelytokous reproduction, our current data show that arrhenotokous reproduction is more abundant in the field. We have evidence that arrhenotoky occurs widespread in a large part of the Mediterranean (unpublished results). Standard explanations of the paradox of sex may be invoked here (Hurst & Peck, 1996; Jokela *et al*., 1997; Peck *et al*., 1998; Peck & Waxman, 2000). For example, arrhenotokous wasps may have different ecological requirements, such as a wider tolerance to changes in abiotic factors and differences in host distribution and specificity (Gade & Parker, 1997; Jokela *et al*., 1997; Vrijenhoek, 1999). Detailed ecological data are necessary to verify this.

The system of *V. canescens* has great potential for empirical study of the evolution of sex paradox because it has the advantage that both modes of reproduction are obligate and not associated with differences in ploidy level (both are diploid), and that both occur within a single species in sympatry. As a first step towards understanding the simultaneous occurrence of thelytokous and arrhenotokous wasps we have determined the population genetic structure at a small geographical scale. Our data indicate that both reproductive modes are not necessarily genetically isolated in the field. This enlarges the evolution of sex paradox because thelytokous wasps may benefit from occasional sex without paying for the

twofold cost of producing males. However, the observed genetic similarity between thelytokous and arrhenotokous wasps could also partly be explained by recurrent origin of thelytokous clones from arrhenotokous wasps. Further investigation into the causes of genetic variability in both reproductive modes is required for a better understanding of their persistence.

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