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CHARACTERIZATION AND ECOLOGY OF MOSQUITO SPIROPLASMAS FROM ATLANTIC BIOTOPES IN FRANCE

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SUMMARY

Field studies on the ecology of mosquito spiroplasmas (Mollicutes) were carried out on a number of swampy biotopes along the Atlantic coast of France and the banks of the Loire River (Loire-Atlantique). *Spiroplasma sabaudiense* and the *Cantharis* spiroplasma were isolated from *Aedes detritus* and *Ae. caspius* during May and June 1988 but disappeared in August and September. These data confirm the results of previous surveys performed in Savoia, Northern Alps, among other species of mosquitoes. It is possible that mosquitoes acquire their spiroplasma infections during the spring from flowers following their emergence and not from their aquatic environment.

RÉSUMÉ : **Caractérisation et écologie des spiroplasmes de moustiques isolés à partir de biotopes atlantiques en France.**

L'écologie des spiroplasmes de moustiques (Mollicutes) a été étudiée dans un certain nombre de biotopes marécageux de la côte atlantique française et des bords de la Loire. *Spiroplasma sabaudiense* et le spiroplasme de *Cantharis* sp. ont été isolés à partir d'*Aedes detritus* et d'*Ae. caspius* pendant les mois de mai et juin 1988, mais ont disparu en août et septembre de la même année. Ces résultats confirment ceux qui ont été précédemment obtenus au cours d'enquêtes réalisées en Savoie, dans les Alpes du Nord, avec d'autres espèces de moustiques. Il est possible que les moustiques acquièrent au printemps leur infection à spiroplasme au contact de fleurs peu après leur émergence et non directement à partir de leur environnement.

INTRODUCTION

Spiroplasmas are helical, motile, wall-free prokaryotes (class Mollicutes) parasitizing plants, insects and ticks, and classified in groups according to their serological affinities (Tully *et al.*, 1987). These organisms were only recently identified from mosquitoes. The first isolation of a mosquito spiroplasma (msp) was reported from *Aedes sollicitans* collected in New Jersey (Slaff and Chen, 1982). *Ae. sollicitans* is a salt marsh mosquito which may act as a vector for a number of highly pathogenic arboviruses including Eastern Equine Encephalitis virus in USA (Karabatsos, 1985).

Since then new msp have been isolated from other mosquitoes collected in Savoia, France (Chastel *et al.*, 1985, 1987), Taïwan (Clark *et al.*, 1987) and Alabama, USA (Shaikh *et al.*, 1987).

At present, three species of msp have been fully described: *Spiroplasma culicicola,* group X, from USA (Hung *et al.*, 1987), *S. sabaudiense,* group XIII, from France (Abalain-Colloc *et al.*, 1987) and *S. taiwanense,* group XXII from the Far East (Abalain-Colloc *et al.*, 1988). Another species (or complex of serovars), the *Cantharis* spiroplasma, group XVI, is being described. It has been isolated in USA and France from *Cantharis* beetles, *Monobia* wasp, *Photinus firefly* and mosquitoes, and also from a flower, the thistle (*Circium* sp.) (Shaikh *et al.*, 1987; Chastel *et al.*, 1987, 1990). Many other isolates from mosquitoes remain insufficiently characterized but may represent new, undescribed species.

Up until now, ecological studies have been restricted to France, in Alpine biotopes of Savoia were at least 5 species of mosquitoes *(Aedes, Coquillettidia richiardii)* have been found infected by *S. sabaudiense* and/or the *Cantharis* spiroplasma (Chastel *et al.*, 1987, 1990).

In order to complete our data on msp ecology in France, field studies were extended to new mosquito species from swampy biotopes located along the Atlantic coast of France, in the vicinity of La Baule and Nantes.

MATERIAL AND METHODS

Collections of mosquitoes

Specimens were collected in the « Loire-Atlantique » district from the following locations:

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— May 1988: La Turballe, Le Bassin du Mes and Asserac, near La Baule (salt marshes);
— June 1988: La Turballe and Asserac;
— August 1988: La Turballe;
— September 1988: Passay l'Héronière and Passay-le-Plessis (Lac de Grand-Lieu) and Couëron along the banks of the Loire river, near Nantes.

Mosquitoes were collected by aspiration from human or animal bait or by using an entomological sweep net in vegetation. After anaesthesia the specimens were identified, put individually in plastic tubes and frozen in dry ice during their transportation to the laboratory of Parasitology in Nantes. Here they were stored at −70° C. In addition, a number of nymphs were removed from « Le Bassin du Mes » (May 9, 1988) and allowed to emerge as imagos in the laboratory. They were then processed in the same manner as imagos caught directly in the field.

Subsequently, all specimens were transferred in dry ice to the virus laboratory in Brest and processed by msp assays.

A total of 1,067 mosquitoes specimens were tested for msp and other mycoplasmas.

Isolation and characterization of msp

The classical SP4 medium (Tully *et al.*, 1977) containing Penicillin, 100 000 U/ml (SP4), was used throughout the study for both isolation an characterization procedures of msp.

Each mosquito was processed individually. First, an external washing was performed in 2 ml of SP4 and the washing product was removed and filtered through a 0.45 μm Millipore membrane. The same specimen was then carefully ground in a chilled mortar with 2 ml of SP4 and the suspension was filtered at 0.45 μm. Both filtered washing products and extracts, undiluted and diluted 1 : 10, were incubated at 30° C in SP4—for ten days in plastic culture tubes. The acidification of the medium was indicative of the possible development of a mollicute. Positive culture was confirmed by dark field microscopy showing the presence of helical organisms or the once blind passaged.

The isolates were cloned on solid SP4 medium and helical microorganisms were identified using the deformation test and hyperimmune rabbit sera prepared against reference spiroplasma strains, according to Williamson, Whitcomb and Tully (1978).

The identification of non-helical mycoplasmas (probable acholeplasmas) isolated from mosquitoes during this study was not attempted.

RESULTS

The results of msp isolation attempts from 1,067 specimens are presented in *table I. Aedes detritus, Ae. caspius* and *Anopheles maculipennis* comprised the bulk of mosquito species examined, while « miscellaneous » included *Culiseta annulata, C. subochrea, C. morsitans, Culex pipiens, Anopheles claviger, Aedes vexans, Aedes* sp.

Eleven isolates of msp were obtained: one of *S. sabaudiense* from an *Aedes detritus* female caught in May 1988 and 10 of *Cantharis* spiroplasma from 8 *Ae. detritus* females and one from both a male and a female of *Ae. caspius,* all caught in June 1988. No msp was isolated from mosquitoes collected in August and September 1988, including 424 specimens of *An. maculipennis.* Furthermore, two non-helical mycoplasmas were obtained in June from females of both *Ae. detritus* and *Ae. caspius.*

All isolates came from filtered extracts of mosquitoes and not from the corresponding washing products.

No isolation occurred from 70 imagos of both males or females of *Ae. detritus, Ae. caspius* and *Culex pipiens* processed after emergence of nymphs in the laboratory.

DISCUSSION

From biotopes located in the « Loire-Atlantique » district, no spiroplasma other than those previously found in Savoia, France, was recovered, *i. e. S. sabaudiense* and the *Cantharis* spiroplasma.

However, the prevalence of spiroplasmas in mosquitoes could appear quite different in the two areas. Only one isolate of *S. sabaudiense* was obtained from 356 *Ae. detritus* caught in the field, showing an « actual isolation rate» (AIR) of 1 : 356 or 0.28 %, far from the AIR of the same spiroplasma in *Aedes sticticus* in Savoia, 11 : 41 or 26.8 %

TABLE I. — *Mosquito spiroplasmas isolated in 1988.*

		May 1988 (Sp. sabaudiense)		*June 1988 (Cantharis spiroplasma)*	*August 1988*	*September 1988*	
		Catching	*After emerging*	*Catching*			*Total*
Aedes detritus	♂	0/38	0/5	—	—	—	0/43 *
	♀	1/37	0/23	8/205	0/57	0/19	9/341
Aedes caspius	♂	—	0/28	1/1	—	—	1/29
	♀	—	0/13	1/53	0/95	0/41	1/202
An. maculipennis	♀	—	—	—	—	0/424	0/424
Miscellaneous	♂	0/1	—	0/1	—	0/1	0/3
	♀	—	0/1	0/8	—	0/16	0/25
Total		1/76	0/70	10/268	0/152	0/501	*11/1,067*

* Number of isolated spiroplasmas/number of tested mosquitoes.

(Chastel *et al.*, 1990). The difference is highly significant ($x^2 = 79.03$; $p < 0.001$).

On the contrary, the *Cantharis* spiroplasma was isolated from two species of mosquitoes, *Ae. detritus* (AIR = 8/356 or 2.25 %) and *Ae. caspius* (AIR = 2/190 or 1.05 %). These two mosquito species represent new hosts for the *Cantharis* spiroplasma which have been isolated previously from *Aedes cantans, Ae. cinereus, Ae. sticticus, Ae. rusticus* and *Coquillettidia richiardii* in Savoia (Chastel *et al.*, 1987, 1990 and unpublished results) and from other mosquitoes in Alabama (Shaikh *et al.*, 1987).

An important fact already established in Savoia (Chastel *et al.*, 1990) is that all isolates were obtained from filtered extracts of mosquitoes and not from the corresponding washing products. This clearly indicates that these organisms originate from the internal tissues of the mosquitoes and not from their integuments (external contamination).

Another interesting finding was the first observation of a msp isolated from a male mosquito. During ecological studies of our group in Savoia (1985-1988) spiroplasma was never isolated from a male mosquito in spite of testing 1,257 specimens (unpubl. results).

On the other hand, no isolations were made from 70 imagos following emergence in the laboratory of wild caught nymphs, confirming our previous observations made in Northern Alps (Chastel *et al.*, 1987). This reinforces our opinion that msp are probably acquired in nature by mosquitoes, including females (Magnarelli, 1978), after feeding on the nectar of flowers and not from the water at breeding sites before or during the emergence of imagos (Chastel *et al.*, 1990). Moreover, there is no proof that spiroplasmas may survive in surface waters at least in their helical form; congenital (vertical) transmission also appears improbable because no msp was isolated in Savoia from 500 *Ae. sticticus* larvae (Chastel *et al.*, 1990).

Concerning the population dynamics of msp, the present study also confirmed a number of published or unpublished data established in Savoia: msp circulate broadly from May-June to August and disappear rapidly in August-September. This represents another argument in favour of the hypothesis that msp are acquired in nature by mosquito populations from flowers during the summer. This may represent the natural cycle of *Cantharis* spiroplasma (Chastel *et al.*, 1990).

The mechanisms of acquiring the msp by mosquito, of spreading the spiroplasmas among mosquitoes and of invading the mosquito body remains unknown, although the digestive route is the most probable mode of invasion as in other insects. To date, we have no explanation for the disappearance of msp in the late summer, nor for the manner in which msp survive during fall and winter (in insects, plants, cool- or warm-blooded vertebrates?).

Another fascinating problem about msp concerns the potential effects of the infection on the physiology and life-span of mosquitoes. Since of the first description of a msp *(S. culicicola)* from *Ae. sollicitans* by Slaff and Chen (1982), it was postulated that the spiroplasma could possibly modify the vector competence of this mosquito for Eastern Equine Encephalitis virus. This is also true for other mosquito species, other viruses and other pathogens as well. The inoculation of msp into mosquitoes significantly reduced their survival time and this may lead to their field use in mosquito control (unpublished results).

In Europe, *Ae. detritus* is not known as an arbovirus vector but at least three arboviruses, Tahyna, Issyl-Kul and Isfahan, have been already isolated from *Ae. caspius* in the Palearctic region (Karabatsos, 1985). However, the low AIR of the *Cantharis* spiroplasma found during this study (0.9 %), if confirmed from other areas, may prevent any significant role of this msp on the vector competence of *Ae. caspius* (for Tahyna virus, for instance).

Thus, msp appear as very interesting organisms, parasiting many different species of mosquitoes worldwide and the study of their ecology deserves attention, especially in view of their potential as biological control agents directed against mosquito vectors.

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