FINAL REPORT
SHORT-TERM SCIENTIFIC MISSION (STSM)
COST ACTION FA1404

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Host: Dr Lise Roy, Centre for Evolutionary and Functional Ecology (UMR CEFE 5175), France
Period of stay: 6 -10 July 2015

Purpose of the visit

This short STSM took place within the framework of an ongoing project in collaboration with the Host Institution (Dr Lise Roy) on the genetics of *Dermanyssus gallinae* and the closely related soil/litter mites resistant to pyrethroid and carbamate. It aimed to identify and molecularly characterize the target genes of the main acaricides in order to be able to detect mutations on a given target that confer the resistance to pyrethroid and carbamate previously recorded in *D. gallinae* populations using some bioassays.

Description of the work carried out during the visit

Firstly, a review was made of the available information in literature, in order to have a picture of current information about Acari and more distantly related arthropods (mainly insects) in relation to the two target genes under scrutiny, their known mutations and associated resistance phenotypes. The literature reports many mutations associated with acaricide resistance to pyrethroid and carbamate in several species belonging to the Acarina Order, specifically over a large part of the Voltage Gated Sodium Channel (VGSC), and on Acetylcolinesterase (AChE) coding genes for pyrethoids and carbamate, respectively. In particular, VGSC gene mutations are reported in some species in the Tetranychidae, Varroidae, Sarcoptidae and Ixodidae families (mainly in domains II and III), whereas AChE gene mutations are reported in some species of the Tetranychidae and Ixodidae families.

Based on this preliminary work, it was decided to focus the activity firstly on the VGSC gene.

Seventy-three sequences of the whole VGSC gene from the following species of Acarina were downloaded from the Nucleotide-Gen Bank: *Tetranychicus urticae, Tetranychicus cinnabaris, Tetranychicus evansi* and *Panonichus citri* (Tetranychidae)*, Varroa destructor* (Varroidae), *Sarcoptes scabei*, (Sarcoptidae), *Rhipicephalus microplus, Rhipicephalus anulatus* and *Ixodes scapularis* (Ixodidae). Some confusing batches (i.e. shotguns from Illumina) were excluded, and then the sequences were aligned using the MAFFT program (E-INS-i parameter).

Subsequently, each trans-membrane domain (from domain I to IV) was delineated using the Seaview program (**van Leewen et al., 2010**), and the *Varroa destructor* (the most closely related mite to *Dermanyssus*) longest VGSC sequence (about 9,000 bp) was selected as the main reference.

In order to design primer pairs able to amplify the overlapping fragments, almost the entire VGSC sequence (from domain II to IV) was divided into three different parts using Primer BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and PriFi (http://cgi-www.cs.au.dk/cgi-chili/PriFi/main) programs. For primer BLAST, sequence no. AY259834 (*Varroa destructor*-VGSC) was used as the reference sequence and comparisons with sequences from the RefSeq database were allowed. For PriFi, multi-sequence alignments as established from the Genbank tick and mite sequences (either all included, or only Parasistiformes – i.e. ticks and *Varroa*) were submitted as an input

The best obtained primers were then inserted into the whole alignment to check whether they matched within a good conserved region (as estimated from the above-listed species sequences) to allow expectation of the most likely successful PCR amplification.

Description of the main results obtained

A total of 26 primer pairs were retained. Primer BLAST allowed the design of 16 primer pairs (10 for domains II and IV and 6 for domain III) (**Figure 1**) and PriFi allowed 10 primer pairs (2 for domain II, 8 for domain IV, none in domain III). However, none of the primer pairs were considered acceptable after alignment, due to very great variability in the targeted regions.

Future collaboration with host institution

Future collaboration will involve the design of new primer pairs using different criteria and different methods, selection of the most appropriate primer pairs and performance of PCR tests on wild type *D. gallinae* specimens in order to obtain the whole sequence of the VGSC gene. Once the gene has been molecularly characterized, *D. gallinae* populations previously identified as having low and high susceptibility to pyrethroid (**Marangi et al. 2009)** will be tested in order to identify the mutations related to pyrethroid resistance.

References

Van Leeuwen T, Vontas J, Tsagkarakou A, Dermauw W, Tirry L. 2010. Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: A review. Insect Biochemistry and Molecular Biology 40:563e572.

Marangi M, Cafiero MA, Capelli G, Camarda A, Sparagano OA, Giangaspero A. 2009. Evaluation of the poultry red mite, *Dermanyssus gallinae* (Acari: Dermanyssidae) susceptibility to some acaricides in field populations from Italy. Experimental & Applied Acarology, 48:11–18.

Figure 1: Primer pairs obtained by PRIMER BLAST for domains II to IV

http://www.ncbi.nlm.nih.gov/tools/primer-blast/primertool.cgi?ctg_time=1436463819&job_key=anGFtzm9kJmrp6-pzoed19Wqr8bGtbLD

Primer pair 1	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GTGGAGGTGCTGGCAAAAAG	Plus	20	840	859	59.97	55.00	4.00	0.00
Reverse primer	TGTCTTCCGACATTGGGGTG	Minus	20	1352	1333	59.96	55.00	4.00	0.00
Product length	513 Fp not good Rp **								

Primer pair 2	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CGAGTTTGTCTCTGCCTGGT	Plus	20	1107	1126	59.97	55.00	3.00	1.00
Reverse primer	CGACCATAGGATGCCGAGTC	Minus	20	1619	1600	60.04	60.00	3.00	3.00
Product length	513 Fp and Rp:too mismacth with Ixodex								

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ACGAAGGAGTCGCAGTTGAG	Plus	20	1466	1485	60.04	55.00	3.00	2.00
Reverse primer	AGTTTCCTGAGCGGAGAACG	Minus	20	1991	1972	60.04	55.00	4.00	2.00
Product length	526 Fp no complementary with Ixodes and other mites Rp not found								

Primer pair 4

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CGTCGCTATCGTACCTGGTC	Plus	20	103	122	60.04	60.00	5.00	3.00
Reverse primer	GCTCGTCCTTCCGAACTTCA	Minus	20	791	772	60.04	55.00	5.00	3.00
Product length	689: Fp good Rp no complementary								

Primer pair 5

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GACCGTCGCTATCGTACCTG	Plus	20	100	119	60.04	60.00	5.00	1.00
Reverse primer	TCGTCCTTCCGAACTTCACG	Minus	20	789	770	60.04	55.00	5.00	2.00
Product length	690: Fp * Rp not complementary								

Primer pair 6

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CAACGGAAAGGTTCGCAAGG	Plus	20	1087	1106	60.04	55.00	3.00	0.00
Reverse primer	AGTCTTCAGAATCCAGCCGC	Minus	20	1550	1531	60.11	55.00	5.00	3.00
Product length	464: Fp ** Rp not good								

Primer pair 7

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGGGGGTGTTGACCCAAAAA	Plus	20	234	253	59.96	50.00	4.00	1.00
Reverse primer	AGCTGCTTGCATCTCTCTGG	Minus	20	826	807	60.11	55.00	6.00	0.00
Product length	593 Fp **Rp not good								

Primer pair 8

	Sequence (5'->3')	Templat	Lengt	Star	Stop	Tm	GC%	Self	Self 3'

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CTCAGGAACACCTCCCGTTC	Plus	20	1293	1312	60.04	60.00	4.00	1.00
Reverse primer	ACAGCCAGCGAGACACTTTT	Minus	20	1792	1773	60.18	50.00	2.00	0.00
Product length	500 Fp **Rp not good								

Primer pair 9

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AAGACAACGGGGCCATCATT	Plus	20	1347	1366	59.96	50.00	4.00	2.00
Reverse primer	AACAGCCAGCGAGACACTTT	Minus	20	1793	1774	60.18	50.00	2.00	1.00
Product length	447 Fp * Rp not good								

Primer pair 10

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TCTCGCAGAAGCAGCTACAC	Plus	20	1394	1413	60.11	55.00	5.00	1.00
Reverse primer	AAACAGCCAGCGAGACACTT	Minus	20	1794	1775	60.18	50.00	2.00	0.00
Product length	401 Fp* Rp not good								

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GTAAGCCTGGCCGCATCTAA	Plus	20	111	130	60.18	55.00	5.00	1.00
Reverse primer	CAAAGTCGAGCCAACACCAC	Minus	20	693	674	59.69	55.00	4.00	0.00

Internal oligo   Plus

Product length 583 Fp not good Rp good

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC %	Self complementarity	Self 3' complementarity
Forward primer	GCGAACCTCCAAACCTCGAT	Plus	20	52	71	60.39	55.00	4.00	2.00
Reverse primer	TGATCAGCCACTGACGTTGT	Minus	20	573	554	59.61	50.00	6.00	2.00
Internal oligo		Plus							
Product length	522 Fp not good Rp not so much								

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC %	Self complementarity	Self 3' complementarity
Forward primer	CTCATCATCCCTGAGCTGCC	Plus	20	333	352	60.25	60.00	4.00	1.00
Reverse primer	TTACCGGCTCCTAGCCATGT	Minus	20	748	729	60.69	55.00	5.00	3.00
Internal oligo		Plus							
Product length	416 Fp not good Rp not good								

Primer pair 4

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC %	Self complementarity	Self 3' complementarity
Forward primer	CGAACCTCCAAACCTCGATCA	Plus	21	53	73	60.07	52.38	4.00	2.00
Reverse primer	GCTTGCGACCGGTACAATTT	Minus	20	457	438	59.48	50.00	6.00	3.00
Internal oligo		Plus							
Product length	405 Fp not good Rp not good								

Primer pair 5

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC %	Self complementarity	Self 3' complementarity
Forward	GGCTTGACGTCTGTTCGTT	Plus	21	27	47	60.0	52.3	6.00	0.00

								Self complementarity	Self 3' complementarity
d primer	TC				7	8			
Reverse primer	GGCAAAAAGTGGCTGGGAAT	Minus	20	431	412	59.31	50.00	2.00	2.00
Internal oligo		Plus							
Product length	405 Fp not good Rp not good								

Primer pair 6

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GTTGATGGCAAACTGGAGGG	Plus	20	162	181	59.11	55.00	3.00	0.00
Reverse primer	CAGCCACTGACGTTGTTTCA	Minus	20	569	550	58.99	50.00	4.00	1.00
Internal oligo		Plus							
Product length	408 Fp not good Rp could be								

http://www.ncbi.nlm.nih.gov/tools/primer-blast/primertool.cgi?ctg_time=1436523809&job_key=eWKNqSOjioe1qrGk0IqD2sunscvYuKzO

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CGTCTTTACGGCAGAATGCG	Plus	20	220	239	59.97	55.00	3.00	2.00
Reverse primer	TGTGGCCTGCGAGTAGTTTT	Minus	20	847	828	59.89	50.00	4.00	0.00
Product length	628 Fp good Rp good								

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AACCCGCAAAGGCTATTCCA	Plus	20	27	46	59.96	50.00	3.00	1.00
Reverse primer	TTGACTAGGCGCAGAACTCG	Minus	20	426	407	60.11	55.00	4.00	2.00
Product length	400 Fp good Rp good								

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AAAGGCTATTCCACGGCCAA	Plus	20	34	53	59.96	50.00	4.00	0.00
Reverse primer	TCGACGCCATACCGATGTTT	Minus	20	579	560	59.83	50.00	4.00	0.00
Product length	546 Fp good Rp good								

Primer pair 4

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CGAGTTCTGCGCCTAGTCAA	Plus	20	407	426	60.11	55.00	4.00	1.00
Reverse primer	AGCGCGATCAGCTTGTACTT	Minus	20	1023	1004	60.11	50.00	4.00	0.00
Product length	617 Fp not so many good Rp good								

Primer pair 5

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CGACCTGACGACGAACAGAA	Plus	20	79	98	60.04	55.00	3.00	0.00
Reverse primer	CAAGATCATGGACTGGCCGA	Minus	20	622	603	59.82	55.00	6.00	2.00
Product length	544 Fp not good Rp not good								

Primer pair 6

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GCGAGACCGAAGGAAACTGT	Plus	20	717	736	60.32	55.00	3.00	3.00
Reverse primer	GTACTCGCCCTTTGACCTCC	Minus	20	1261	1242	60.11	60.00	4.00	0.00
Product length	545 Fp not found Rp not good								

Primer pair 7

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CGTGAAACATCGGTATGGCG	Plus	20	556	575	59.70	55.00	3.00	2.00
Reverse primer	AGGAGGGTGCTCGAGATAGG	Minus	20	1194	1175	60.18	60.00	6.00	0.00
Product length	639 Fp could be Rp not good								

Primer pair 8

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AGAAGAGCCGCTGCAGATAC	Plus	20	973	992	59.90	55.00	6.00	2.00
Reverse primer	TCATACTTGCGCGTCAGACC	Minus	20	1402	1383	60.46	55.00	5.00	3.00
Product length	430 Fp ok Rp not good								

Primer pair 9

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TTGCCGTCTTTACGGCAGAA	Plus	20	216	235	60.25	50.00	8.00	2.00
Reverse primer	CTGTGGCCTGCGAGTAGTTT	Minus	20	848	829	60.32	55.00	4.00	0.00
Product length	633 Fp ok Rp ok								

Primer pair 10

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CTACTCGCAGGCCACAGAAG	Plus	20	832	851	60.46	60.00	4.00	2.00
Reverse primer	ACGACAATGGCGGTATCCTG	Minus	20	1311	1292	60.18	55.00	5.00	2.00
Product length	480 Fp ok Rp not good								