Identification of wild and domestic bees by non-destructive molecular methods

Magalie Pichon [1]; Mélodie Ollivier[2]; Elisa Simon[3]; Emmanuelle Labarthe[3]; Alain Vignal[3]; Christophe Klopp[4]; Annie Ouin[2]; Kamila Canale Tabet[3]

1 : UMR Dynafor, INRAE, 31326, Castanet-Tolosan, France
2 : INP-ENSAT, Avenue de l'Agrobiopole - BP 32607 - Auzeville-Tolosane 31326 CASTANET-TOLOSAN Cedex
3 : GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France
4: MIAT, INRAE, 31326, Castanet-Tolosan, France

Corresponding authors :
magalie.pichon@inrae.fr
kamila.tabet@inrae.fr

There are more than 20,000 species of wild bees in the world and 967 in France playing a crucial function in the pollination of wild and cultivated plants. **Bees identification is a fundamental** step carried out with the help of identification keys or by molecular techniques such as barcoding and metabarcoding. **Non-lethal identification of pollinators has become a major issue**. In this project, we test a **DNA extraction protocol from traces** (hairs, excretions, etc.) left by bees on foraged flowers. Strawberry flowers were exposed for 3 days in natural conditions or in insect-proof boxes after manual introduction of wild bees. The use of degenerate oligonucleotides of a **16S mini barcode** (200-250bps) allowed us to **obtain fragments which have been sequenced.**

Environmental samples

4 bees
Captured with nets
Photographed
Identified

Introduced in cage A

2 *Antophora plumipes*, 1 *Lasioglossum sp*, 1 *Andrena sp*

|	A	B	C
Exposition conditions	Exposure to the 4 bees captured	Open air exposition	No insecte contact
Time exposition	3 days	3 days	3 days
Strawberry plants	4	4	4
flowers number harvested / plant	10	10	10

DNA extraction

Strawberry flowers were incubated in lysis buffer for 3 hours at 56°C and DNA extraction from insect traces was performed with the Qiagen DNeasy Blood & Tissue Kit (Ref. 69504).

16S region amplification
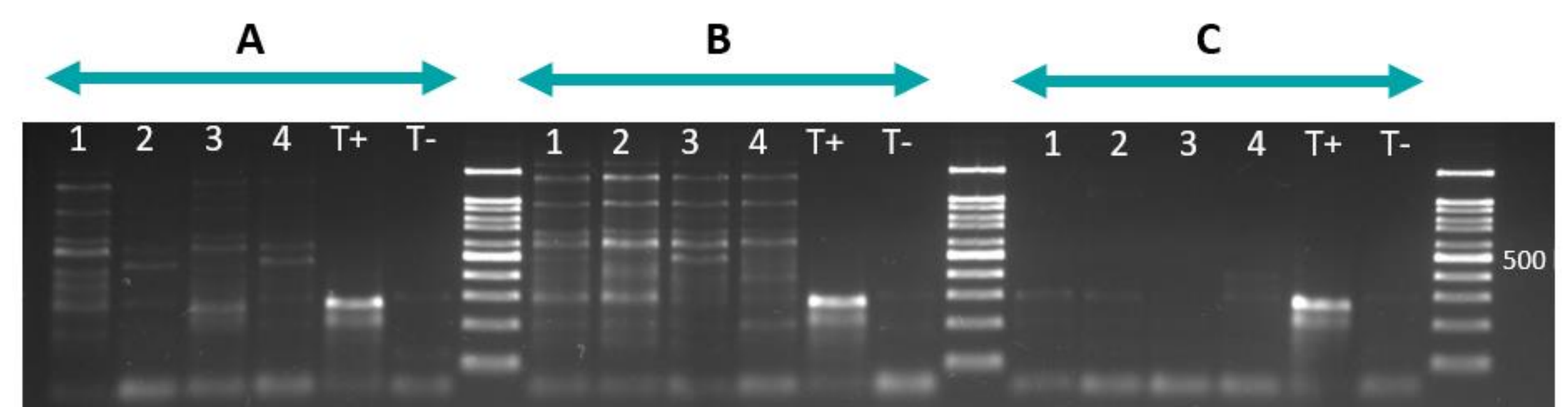
PCR were performed with 16S primers modified from Clarke et al., 2014. Four individual flowers (1-4), were tested for each of the three conditions (A-C). DNA extracted from *Antophora plumipes* leg was used as positive control (T+)

MiSeq sequencing

16 S minibarcode
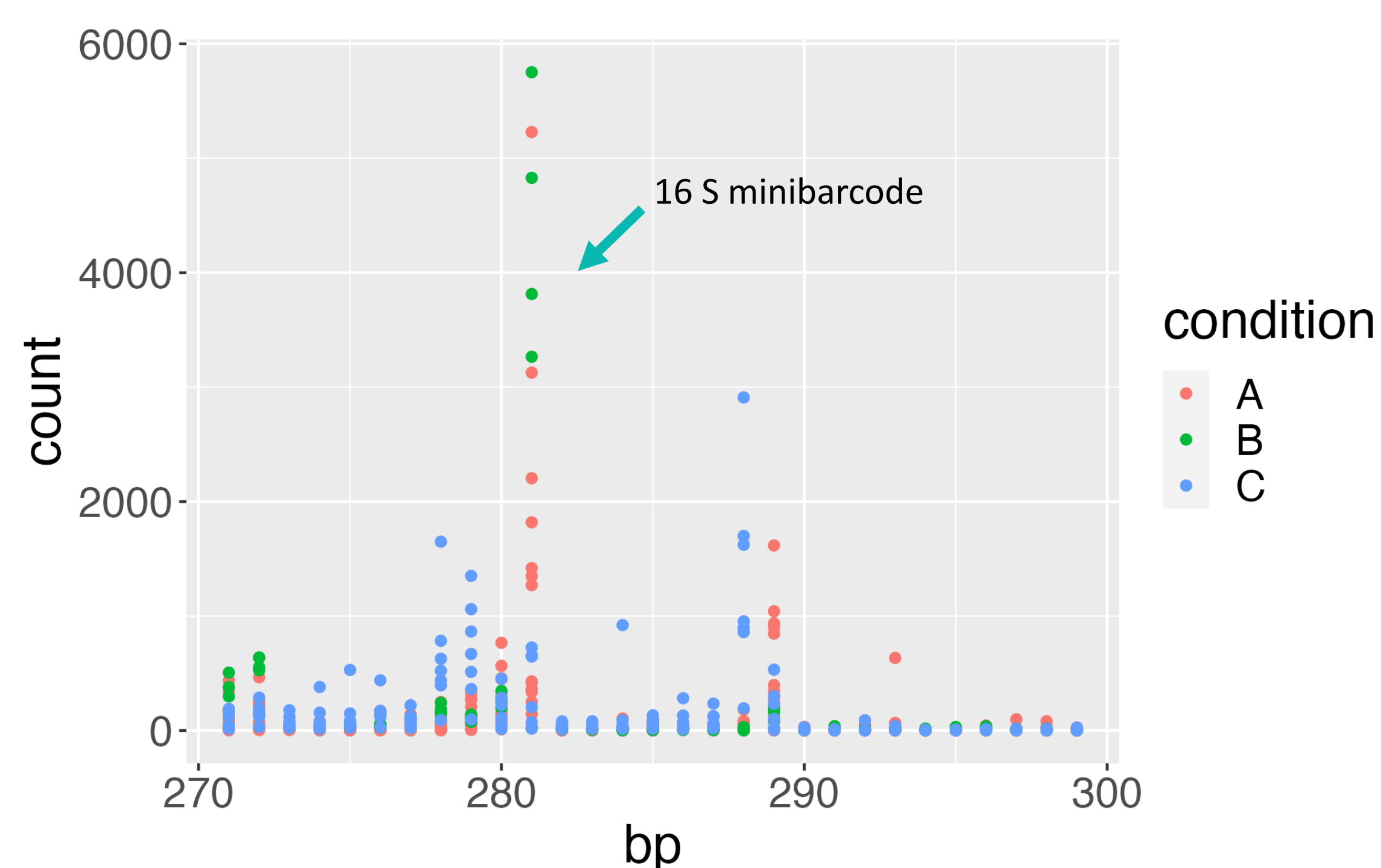
Length distribution of Illumina sequences merged with Flash (Magoc and L. Salzberg; 2011)

Sequences analysis

Arthropods

	Match number
Seladonia	120
Lasioglossum	109
Apis	23
Andrena	17
Bombus	13
Nomada	5
Eucera	2
Halictus	2
Sphecodes	1
Drosophila	22
Philantus	4
Lanthanomeli	4
Holopyga sp	1

Bees
Others

Pathogens

	Match number
Aureobasidium pullulans	51
Zychaea mexicana	91
Corynebacterium striatum	112
Craterium leucocephalum	15
Cryptococcus neoformans	632

Plants

	Match number
Geum urbanum	7
Fragaria vesca	100
Fragaria x ananassa	447
Comarum palustre	157
Quercus robur	11
Potentilla anserina	11

Blast results of merged sequenced for one flower from condition A. Mammalian sequences mainly *Homo sapiens* and *Oryctolagus cuniculus*, were removed. Analysis of others flowers is underway.

References :
• Clarke LJ, Soubrier J, Weyrich LS, Cooper A. Environmental metabarcodes for insects: in sillico PCR reveals potential for taxonomic bias. Mol Ecol Resour. 2014 Nov;14(6):1160-70.
• Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011 Nov 1;27(21):2957-63.

Conclusions/Perspectives

➢ In a context of wild pollinator decline, it is challenging to develop non-destructive protocols to study plant pollinator interactions.
➢ Wild flower eDNA is a powerful tool to obtain information on pollinator communities.
➢ Our preliminary results using 16S barcode allow detecting PCR products from strawberry flowers DNA.
➢ Future experiments should be performed to complete these data , such as testing others families of plant with different floral morphology , adding short or long tongue bee families in insect-proof tents etc…