Barcoding pour étudier les forces sélection/dérive génétique dans des populations de puceron

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Aphis gossypii :

- able to colonize more than 600 host plants,major pest on cucurbits
- a sap feeder and efficient plant viruses vector
- mainly asexual reproduction insect
- overlapping generations

- Restrictions of insecticide use promote aphid resistant cultivars deployment.

- The cluster ***Vat*** in melon confers resistance to infestations and also inhibits plant infections by non-persistent viruses transmitted by *A. gossypii.*
- The cluster ***Vat*** has been introduced into commercial lines that have strong success in southern France.

Evolution Forces

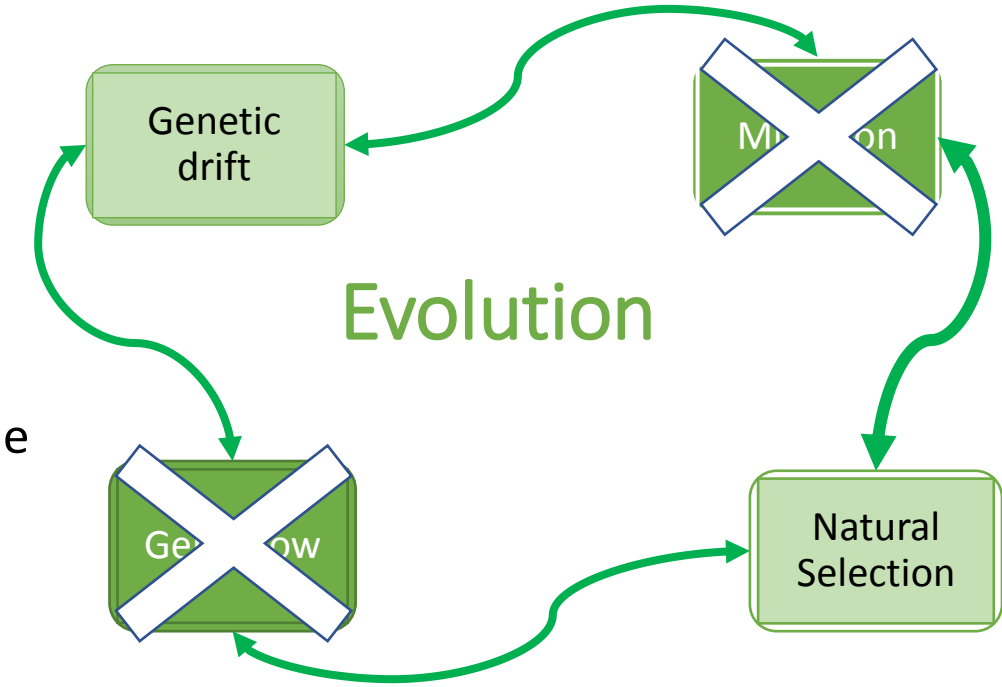
- *A. gossypii* populations has evolved to overcome ***Vat*** resistance.

C6 and CUC1 clones overcome ***Vat*** resistance in different manners.

Resistance (ETI) not triggered, ETI triggered but overcome

- In populations, four forces occur => which clones extinct or continue in nature.

- Laboratory conditions allow to study only the effects of **drift** and **selection** within the populations.



Which overcoming system is the most efficient ?

May drift occur in populations of clones overcoming resistance ?

Develop a technique to determine aphid clone frequencies in a population.

- Low cost
- Accurate

Investigate selection and (drift) occurrence in aphid populations.

1) Strategy

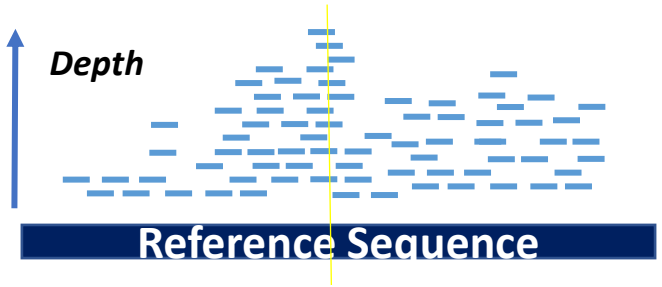
2) Amplicon design

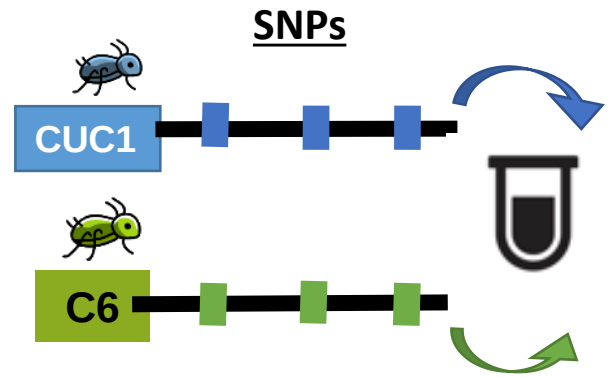
3) Sequencing

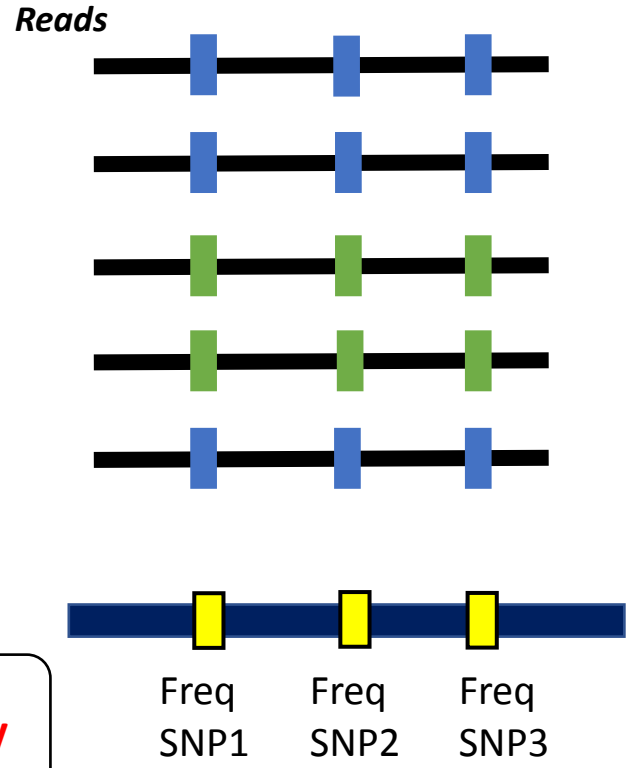
4) Abacus

Barcoding tools

1. Design DNA amplicons with multiples SNPs to distinguish CUC1 and C6 clones from a reference transcriptome.
2. Sequence amplicons by Illumina from samples that contain known frequencies of CUC1/C6 aphids
3. Verify coverage, amplicons and SNPs positions. Determine C6 read frequencies.
4. Verify reliability of observed C6 frequencies (Abacus).







Mean = Observed clone frequency

1) Strategy

2) Amplicon design

3) Sequencing

4) Abacus

Amplicon characteristics

- At least 3 SNPs homozygous for clones CUC1 and C6.
- Highly conserved regions for primer design.
- Amplicon length limited to Illumina read length (150 bp).
- Minimum expected coverage per aphid 10 X
- Not in melon

Limitations

- No reference genome (350 Mb)
- Not possible to verify amplicons obtained by Illumina before biological tests

Search for homozygous SNPs in a reference transcriptome from *A. gossypii* heads (33813 contigs)
Samstools, VarScan , IGV

36 amplicon candidates

Search for highly conserved regions bordering SNPs
Primer3, Blast

18 amplicon candidates

Sanger sequencing from genomic DNA
ChromasBlast on melon

7 amplicon candidates

Primer design for Illumina sequencing
Primer3, Blast

10 amplicon candidates

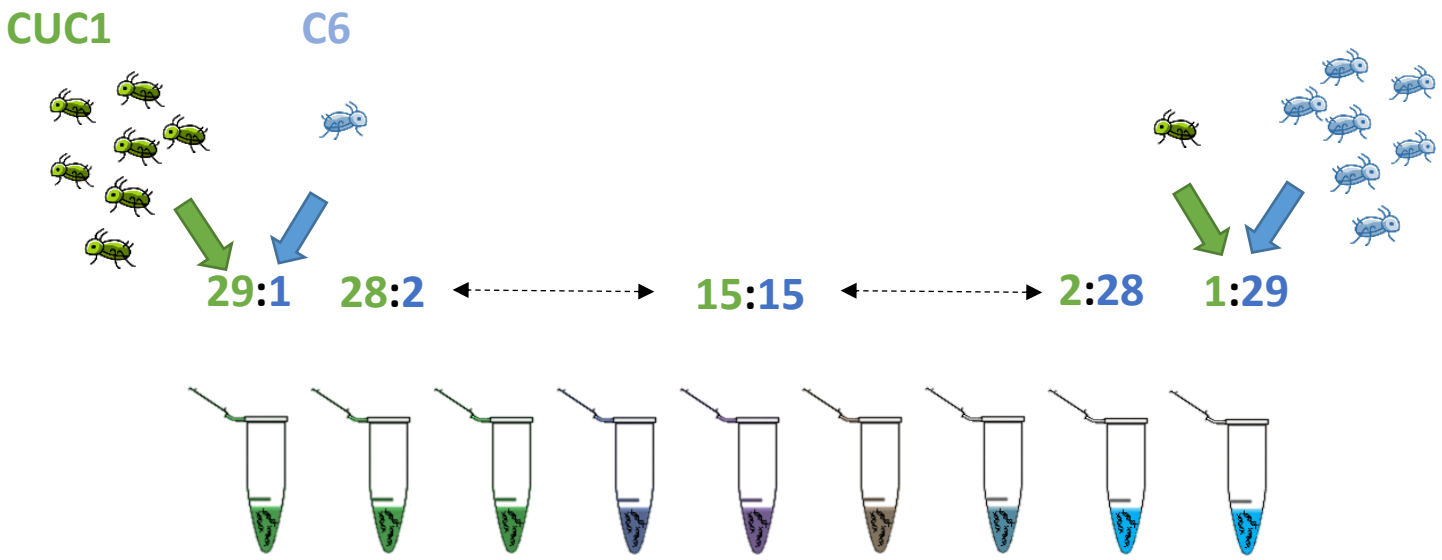
2 amplicons

Strategy

Amplicon design

Sequencing

Abacus



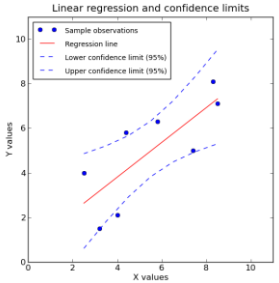
Abacus construction

Two replicats

DNA extraction, **Aphids + Plants**

Illumina sequencing

Linear regression : observed vs estimated frequencies



Strategy

Amplicon design

Sequencing

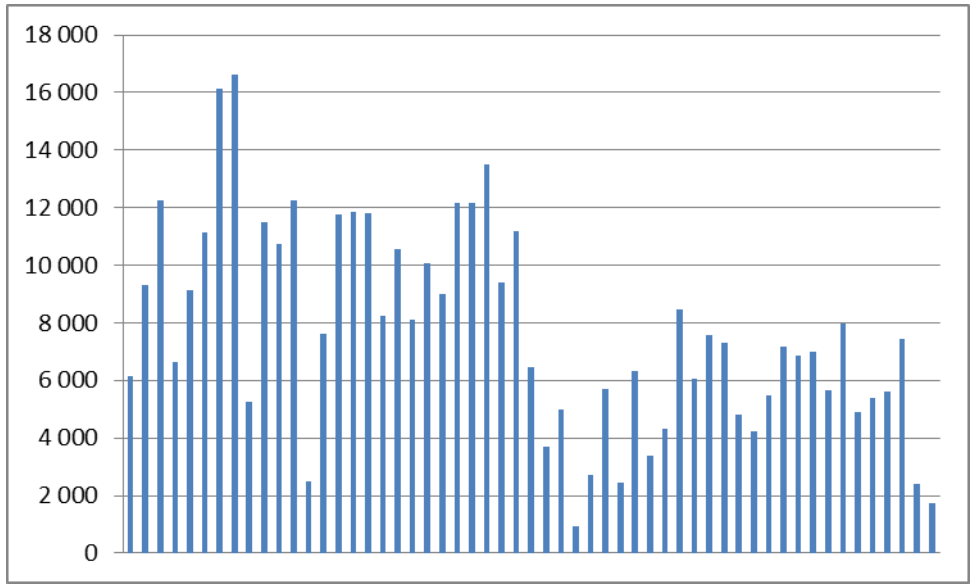
Abacus

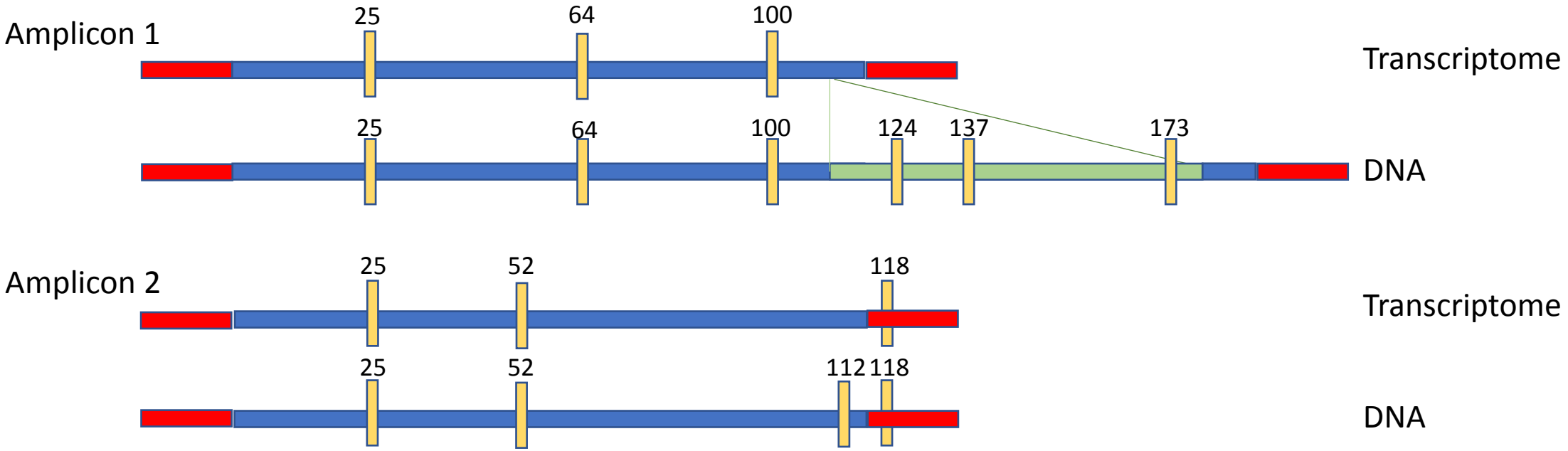
Illumina Miseq micro 2*150,

Raw data filter (Q>30)

Number of reads for 30 aphids:
Median 7287
Min 924,
Max 16629

Amplicon 2 more efficient



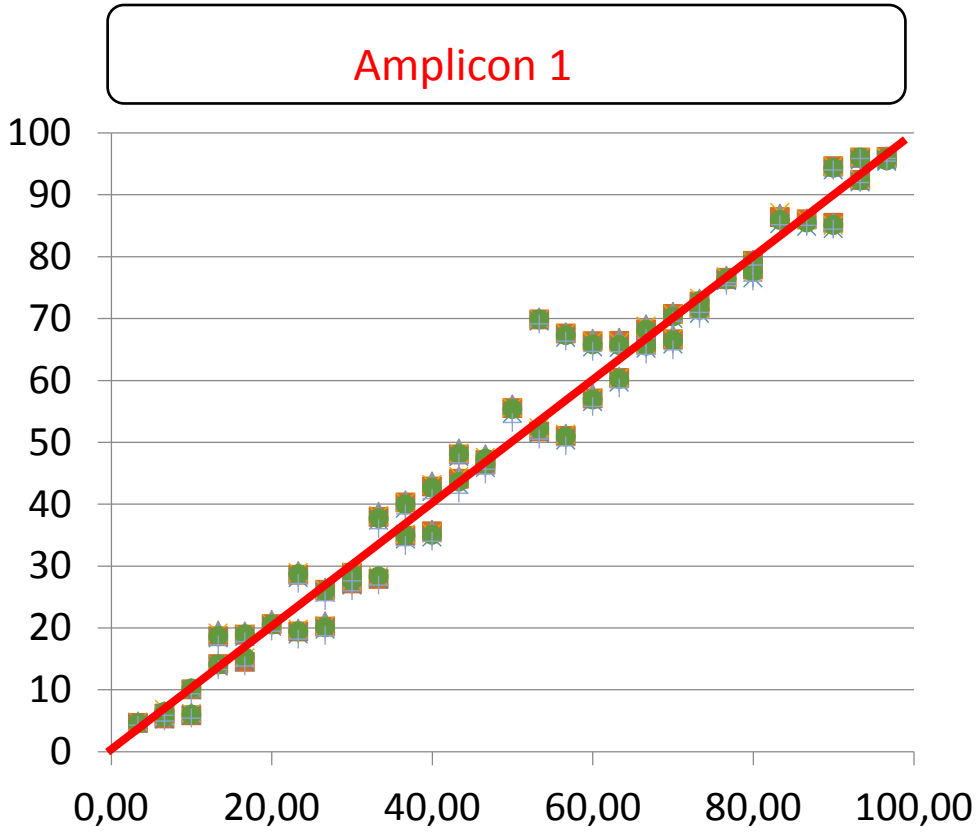


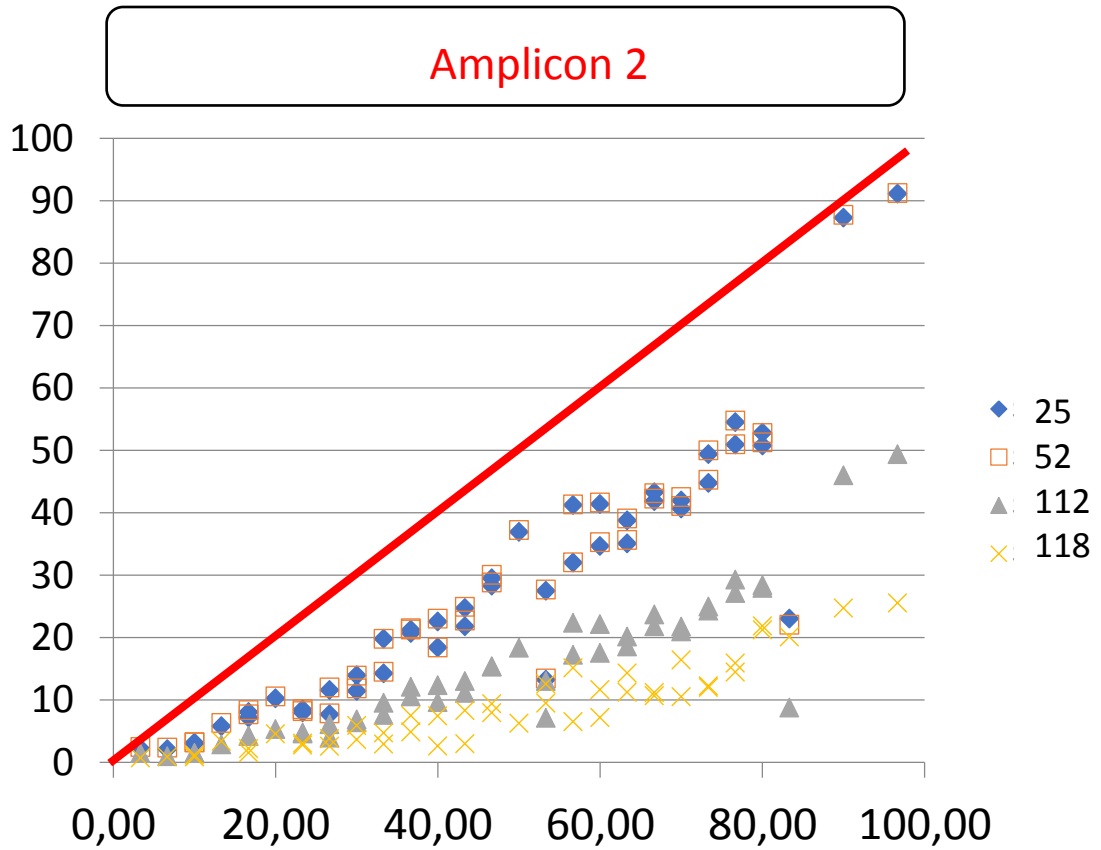
Strategy

Amplicon design

Sequencing

Abacus





Heterospecificity => Unexpected results

Accuracy of Amplicon 1

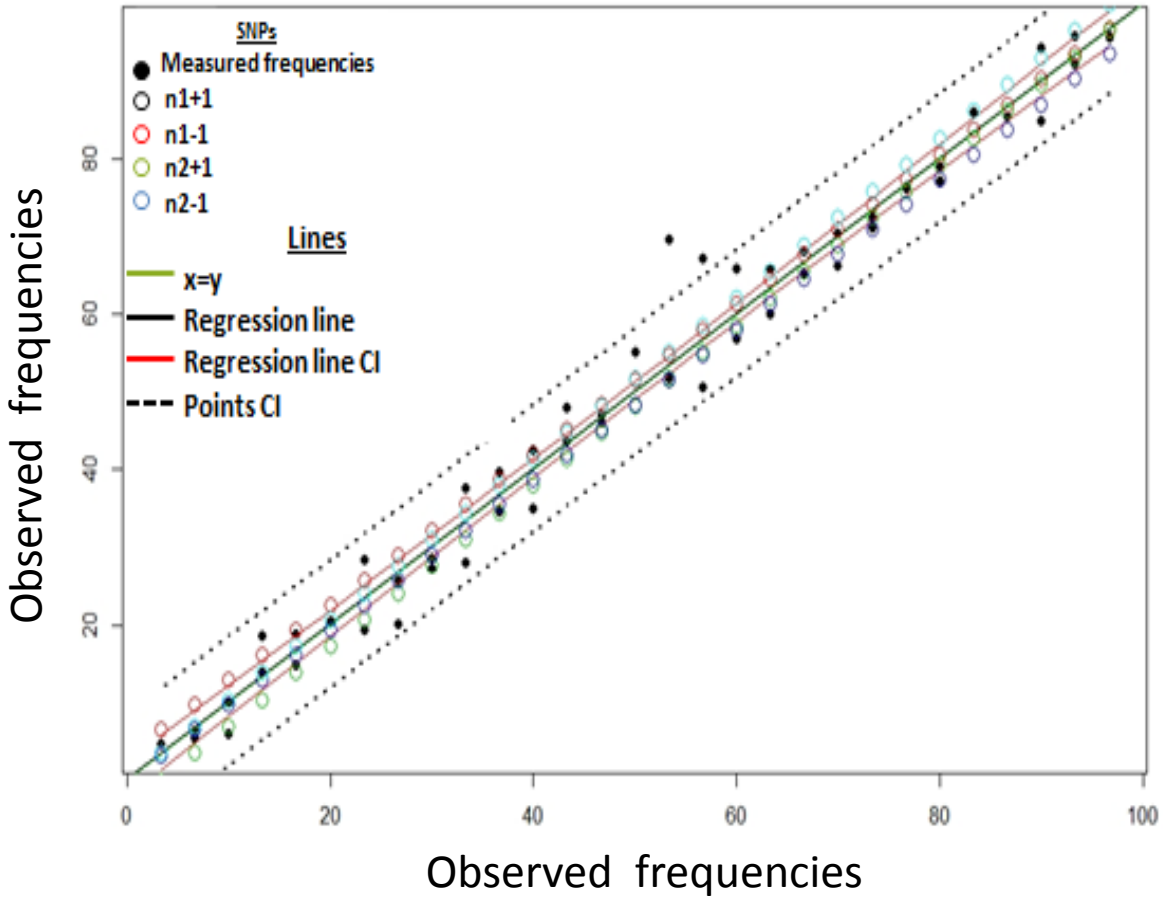
Confidence band at 95% shows accuracy ± 5%

Only two values were outside of the confidence interval of predicted point.

Error test in tubes manipulation does not explain values outside interval.

A vérifier
Avec des cohortes de pucerons d'âges différents
Avec des mélanges de 100-500 pucerons

Amplicon 1 used to analyze the biological tests



Develop a technique to determine aphid clone frequencies in a population.

- Low cost
- Accurate

Investigate selection and drift occurrence in aphid populations.

Biological essays

Infestations

Vat and non Vat plants

CUC1 and C6 clones at different departure conditions

N=12 - 24

Proportion

1/3

3/1

2/2

Density

1:1

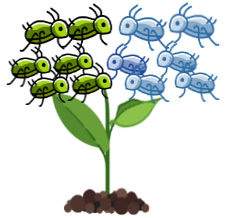
2:2

3:3

4:4

5:5

6:6

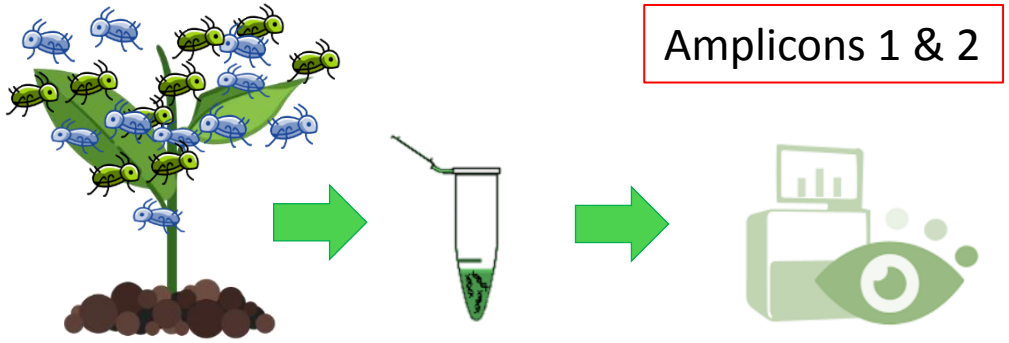


Sequencing

Estimating aphid populations
after 4 and 8 days on independent essays
N=4

Frequency analyses

Collecting aphid populations (with plants)
after 4 and 8 days for proportions
after 4 days for density



Amplicons 1 & 2

Selection vs Drift

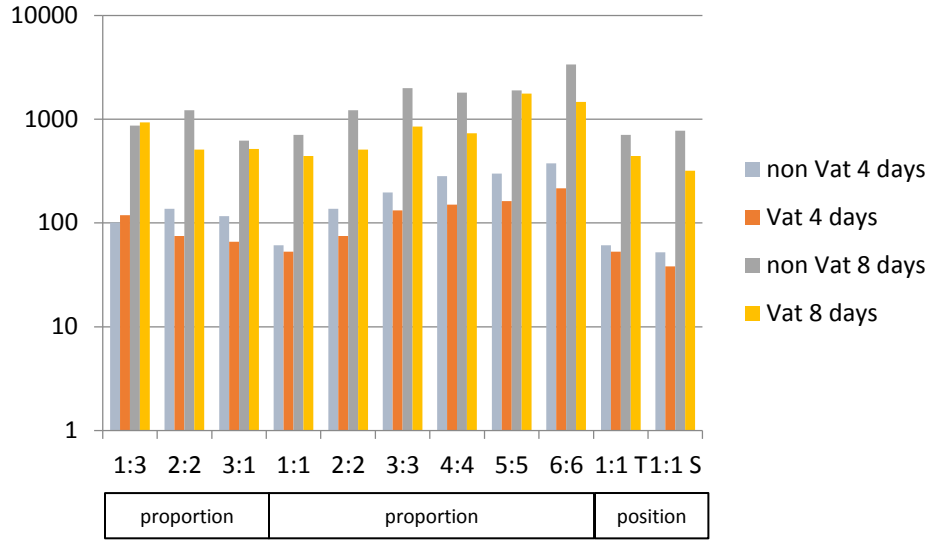
Biological essays

Number of aphids per plant

Estimated on independant essays

N=4

Little bit less aphids on Vat plants



Sequencing

323/324 samples with amplicons

Amplicon 1 :

Median 1565 reads

Min 49 reads

Max 24262 reads

IC SNP_{C6} frequency [0,08-1,33]

Coverage

Amplicon 1

146 samples with an estimated coverage >10

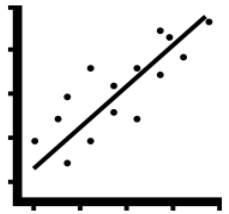
No samples collected after 8 days had a coverage > 10

Frequency analyses

Biological essays

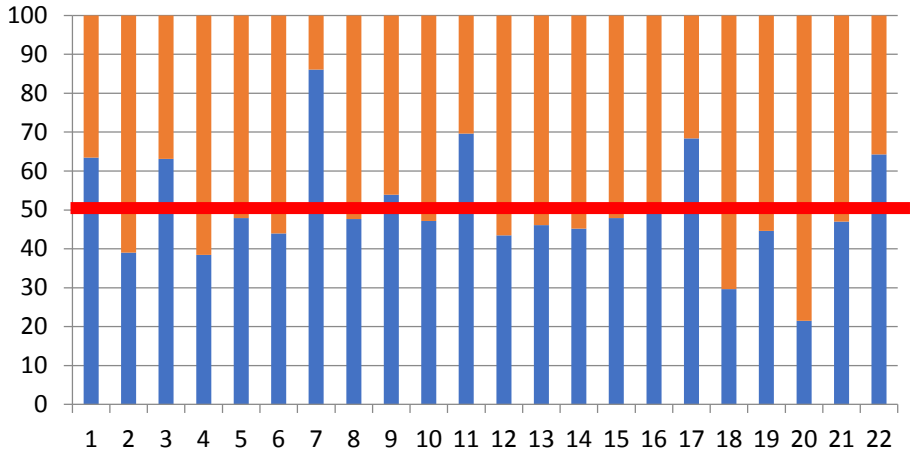
Sequencing

Frequency analyses

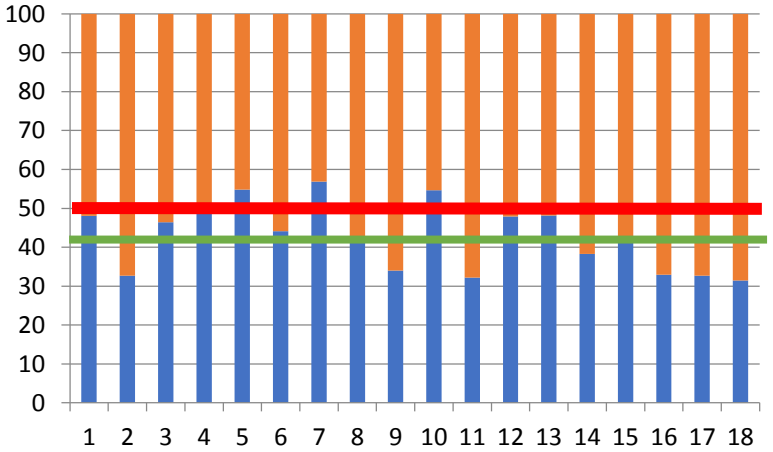


C6 CUC1

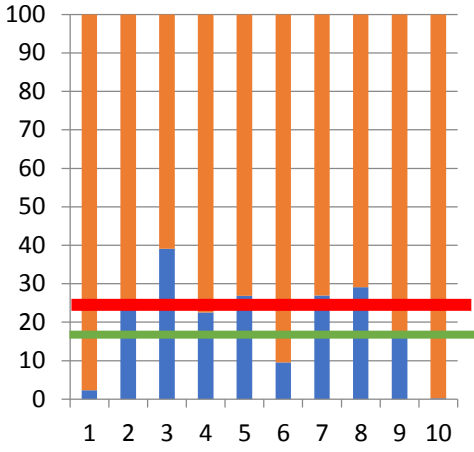
1-1 non-Vat plants



2-2 non-Vat plants



3-1 non-Vat plants



No significant selection

Drift ?

Drift ?

Selection vs Drift

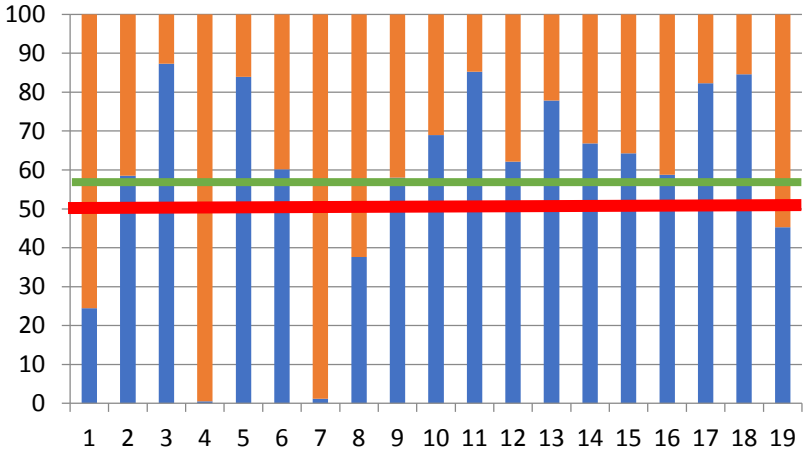
Biological essays

Sequencing

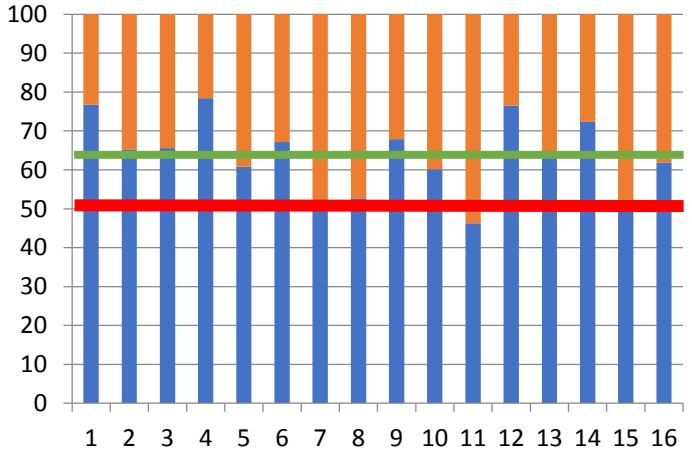
Frequency analyses

CUC1 C6

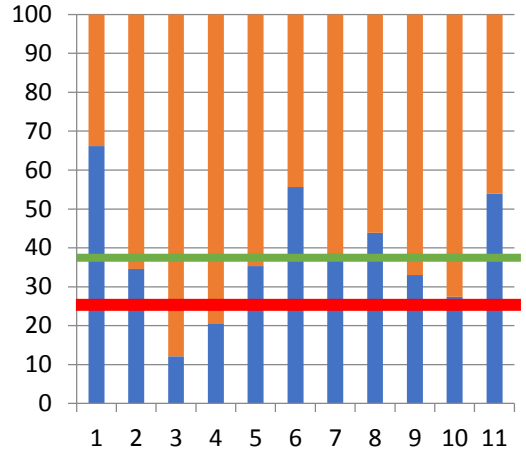
1-1 Vat plants



2-2 Vat plants



3-1 Vat plants



No significant selection

Drift ?

Selection in favor of C6 (p<0.05)

Drift ?

- Built an abacus with large cohorts reared on non-Vat and Vat plants
- Amplicon 1 will be sufficient to inferC6 frequency
  - if amplified alone
  - in 7 days population
- Amplicon 1 can be used to study 3 other clones

- Selection was favorable to C6 on resistant plants (ETI not triggered).
- Drift may occur at low departure conditions on non-Vat and Vat plants
- More analysis is required to assess the effects of drift