Are symbiotic bacteria involved in vine weevil adaptation ability to different environments?

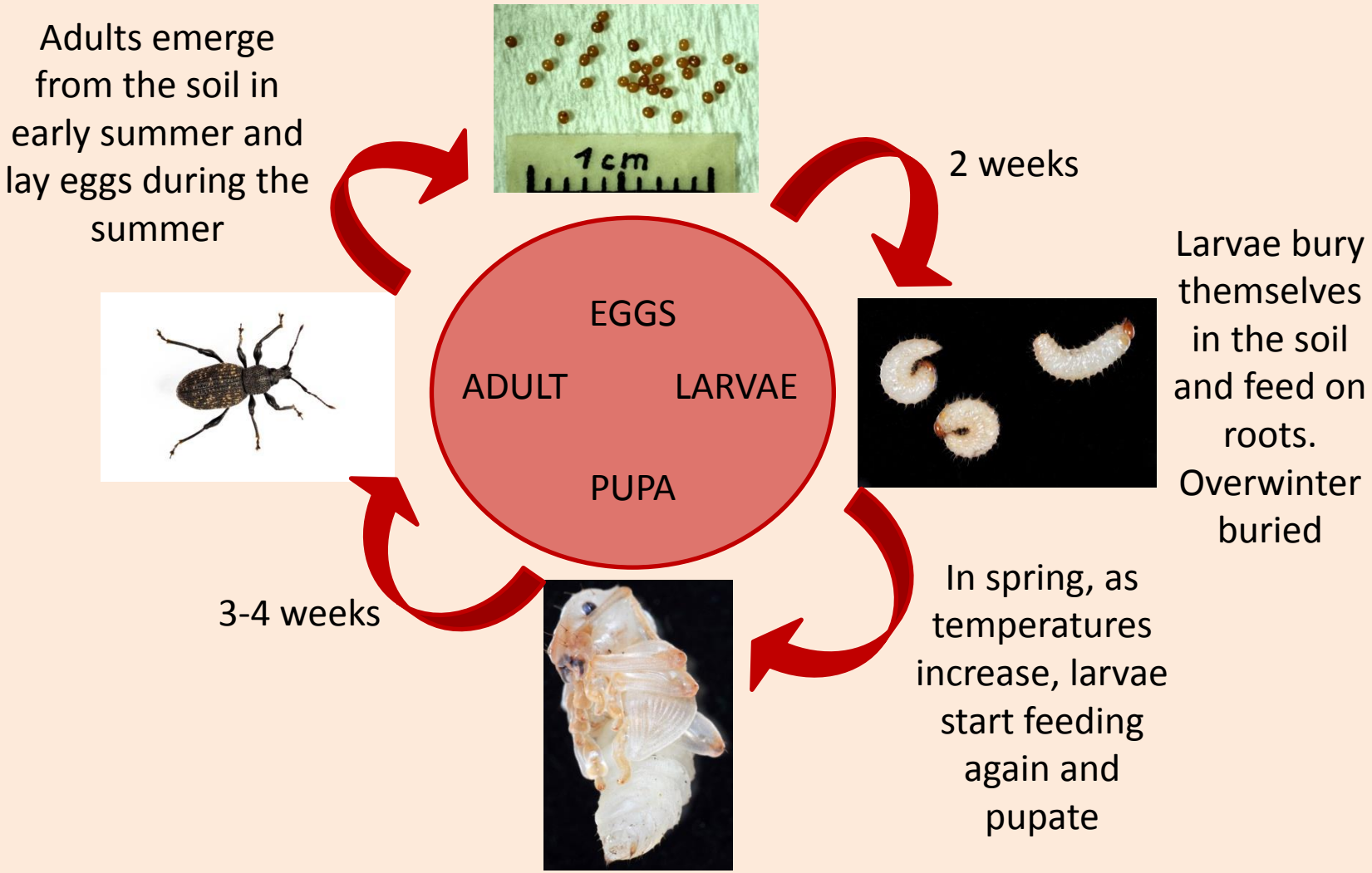
PILAR MORERA MARGARIT
James Hutton Institute & Harper Adams University

The James Hutton Institute

Harper Adams University

Vine weevil life cycle

Otiorhynchus sulcatus Fabricius (Coleoptera: Curculionidae)



Vine weevil distribution

Moorhouse *et al.* 1992 & Lundmark *et al.* 2010

More than 150 recognised host plants

ORNAMENTALS

WEEVIL DAMAGE COSTS THE UK

SOFT FRUIT NURSERY CROPS STOCKS

SOFT FRUIT £10M/year

Taxus

ORNAMENTALS £24M/year

Rhododendron

Rubus

ASEXUAL REPRODUCTION PROVIDES LOW GENETIC VARIABILITY

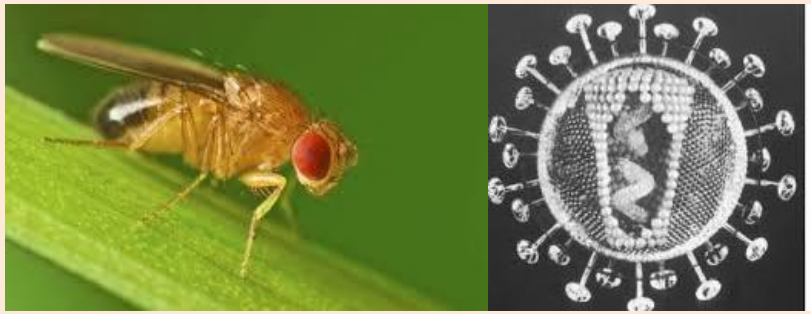
HOW COULD THEY ADAPT TO DIFFERENT ECOLOGICAL NICHES?

Adaptation through bacteria?

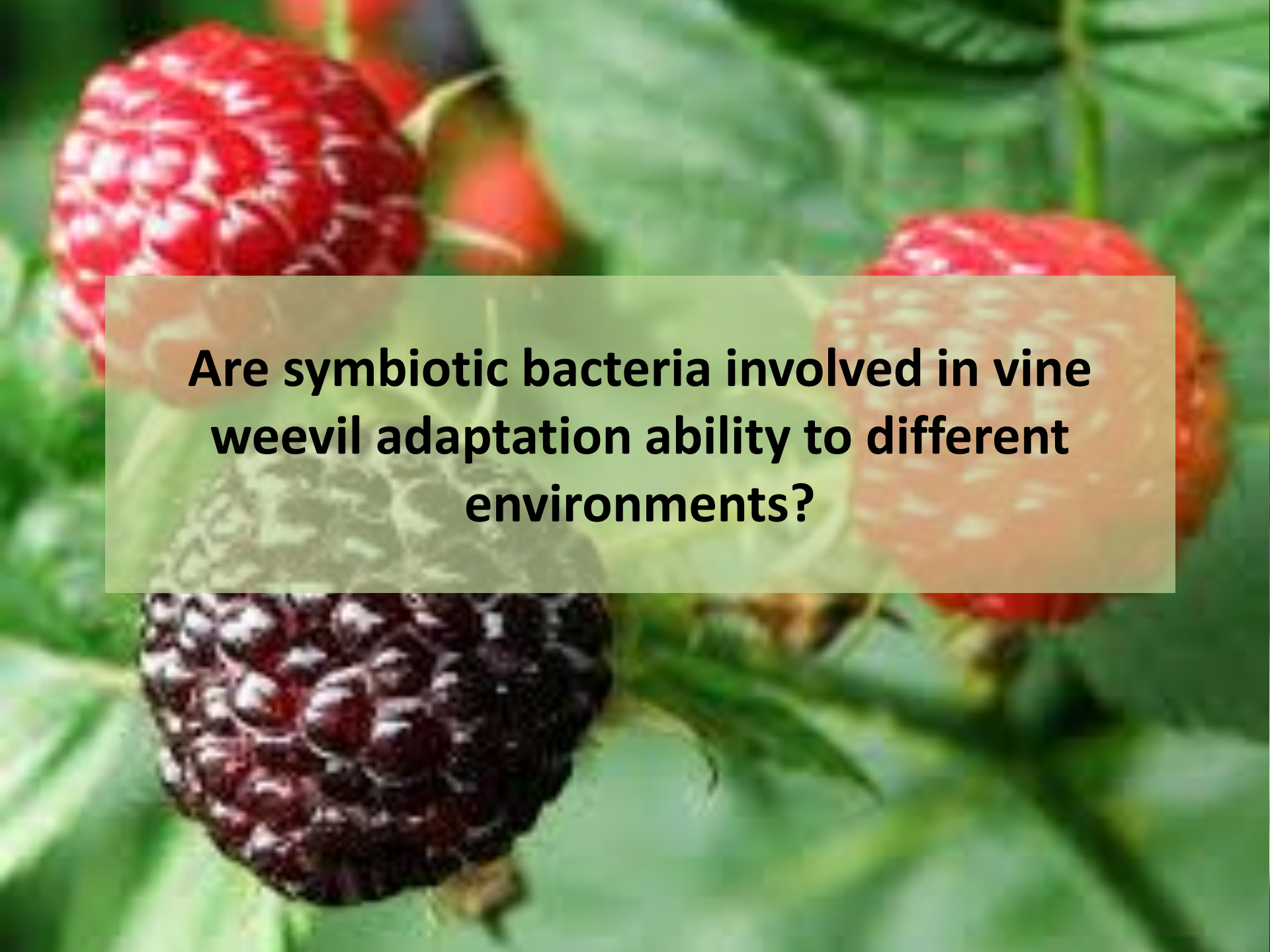
Acyrthosiphon pisum (pea aphid)

Leptinotarsa decemlineata larvae (left) and beetle (right)

A. pisum being parasitized by *Aphidius ervi*



Drosophila melanogaster(left) and RNA virus (right)


Are symbiotic bacteria involved in vine weevil adaptation ability to different environments?

Vine weevil adults were collected in different locations in the UK and kept as separate lines

15 lines from different locations, different crops and non-crop environments

Adults feeding on strawberry leaves are kept in rooms under controlled conditions (18°C, 16:8 h L:D)

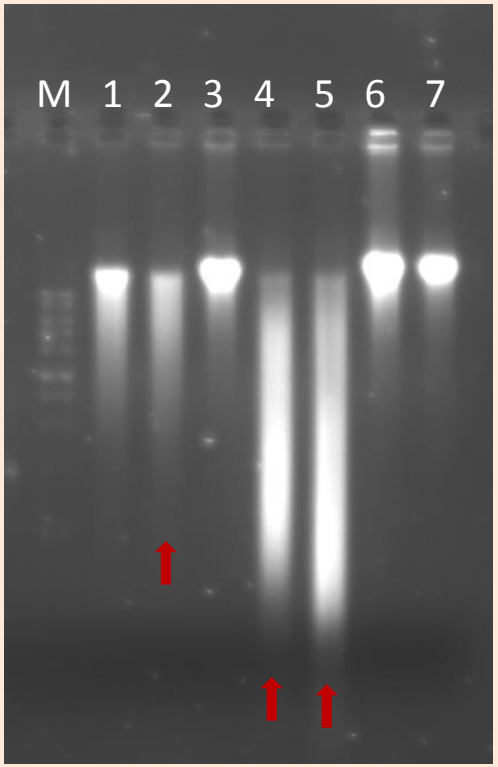
Adults directly frozen and kept at -40°C

1) DNA extraction

2) PCR with universal bacterial primers

3) Sequencing

DNA extraction from larva, adult and eggs using "**Dneasy Blood & Tissue**" kit (Qiagen)



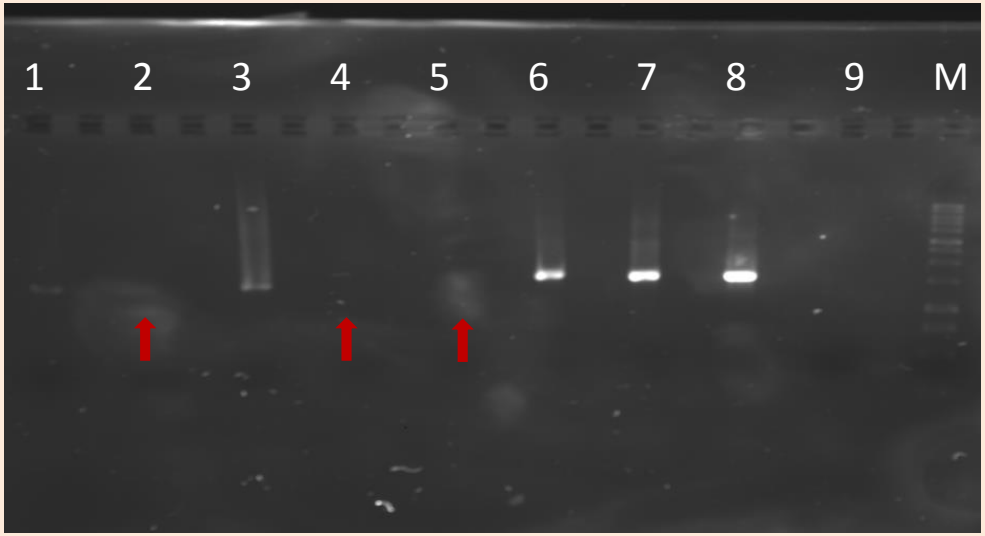
M. Molecular marker
1. Larva
2. Adult
3. Eggs
4. Adult treated with antibiotic
5. Adult treated with antibiotic
6. Eggs from adult treated with antibiotic
7. Eggs from adult treated with water

DNA concentration using nanodrop (ng/µL)

Adult	68.6
Adult treated with antibiotic	164.8
Adult treated with antibiotic	130.9

Highly degraded adult DNA

PCR for a bacterial universal sequence from DNA extracted with "**Dneasy Blood & Tissue**" kit (Qiagen)



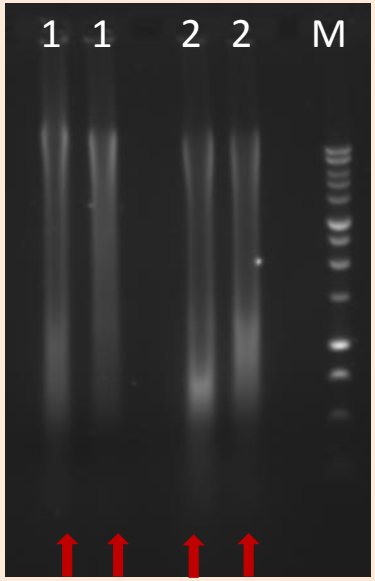
PCR using 27F and 1494R primers (1467 bp)

1. Larva
2. Adult
3. Eggs
4. Adult treated with antibiotic
5. Adult treated with antibiotic
6. Eggs laid by adult treated with antibiotic
7. Eggs laid by adult treated with water
8. *E. coli* DNA (+control)
9. Water (-control)

M. Molecular marker

- The bacterial universal sequence was amplified for larva and eggs
- The bacterial universal sequence could not be amplified on adult DNA due to the high DNA degradation

DNA extraction from adult using **phenol: chloroform: isoamyl alcohol** protocol



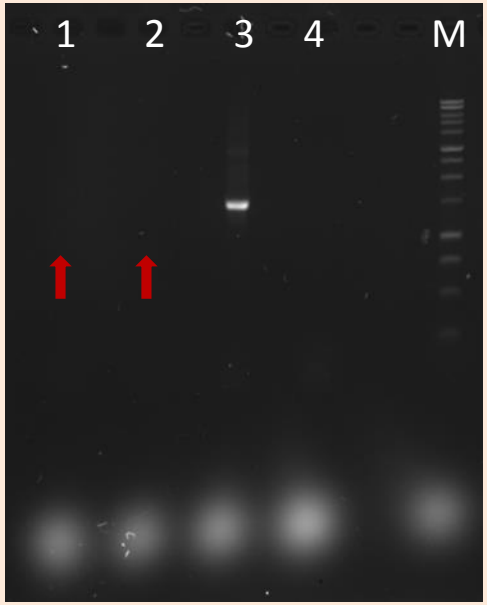
1. Adult (replicate 1)
2. Adult (replicate 2)
3. M. Molecular marker

DNA concentration using nanodrop (ng/µL)

Adult	1,104.3
Adult	1,282.3

Highly degraded adult DNA

PCR for a bacterial universal sequence from DNA extracted with using **phenol: chloroform: isoamyl alcohol** protocol



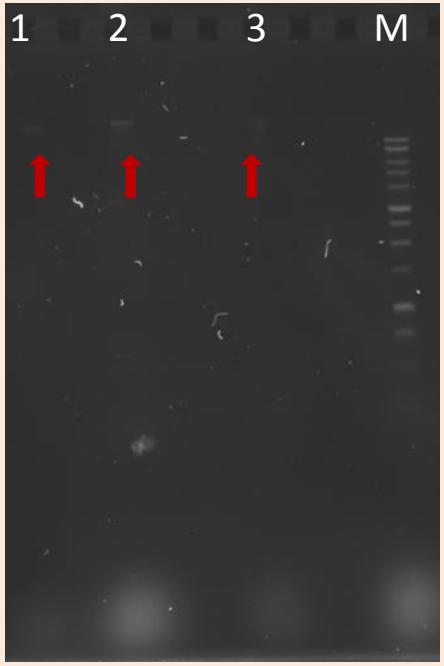
PCR using 27F and 1494R primers (1467 bp)

1. Adult (replicate 1)
2. Adult (replicate 2)
3. *E. coli* DNA (+control)
4. Water (-control)

M. Molecular marker

❖ The bacterial universal sequence could not be amplified on adult DNA due to the high DNA degradation

DNA extraction from adult using "**NucleoSpin**" kit (Macherey-Nagel)



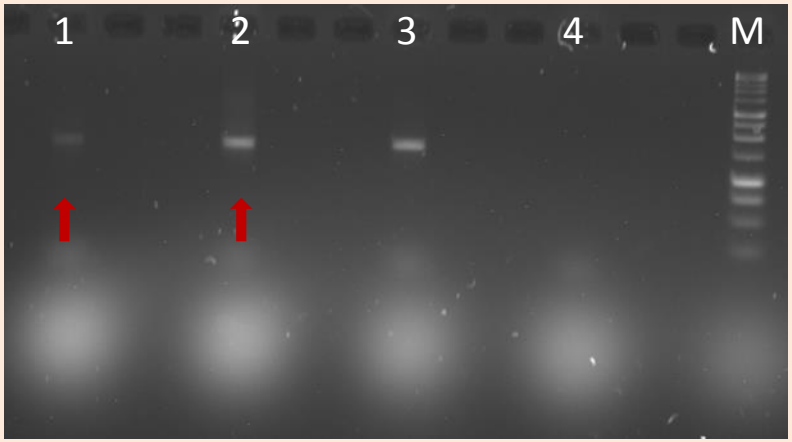
1. Adult 1st replicate (4µL)
2. Adult 1st replicate (8µL)
3. Adult 2nd replicate (4µL)
4. M. Molecular marker

DNA concentration using nanodrop (ng/µL)

Adult	21.4
Adult using the alternative step on the protocol	58.4

Good adult DNA quality

PCR for for a bacterial universal sequence from DNA extracted with "**NucleoSpin**" kit (Macherey-Nagel)



PCR using 27F and 1494R primers (1467 bp)

1. Adult
2. Adult
3. *E. coli* DNA (+control)
4. Water (-control)

M. Molecular marker

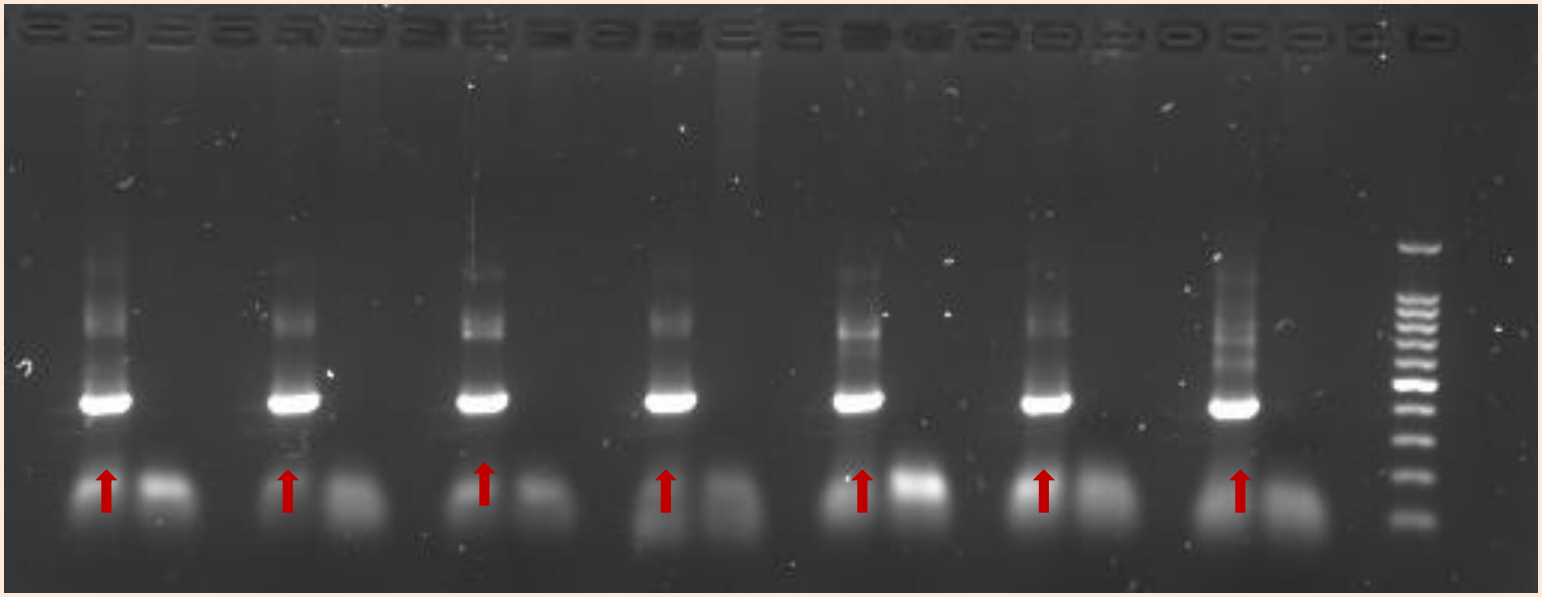
- The bacterial universal sequence could be amplified for adults
- It can be concluded that adults harbour bacteria

1) DNA extraction

2) PCR with universal bacterial primers

3) Sequencing

We have amplified a specific bacteria universal sequence from DNA extracted from different vine weevil populations



Pairs: left amplified sample and right the corresponding negative control

1) DNA extraction

2) PCR with universal bacterial primers

3) Sequencing

Waiting for the sequencing results …

Sequencing results

- Do different vine weevil populations harbour different bacteria?
    - Identify bacterial species harboured by different vine weevil populations and the their relative abundances

- **Next:** Do these bacteria help to defend from natural enemies?
    - **SSCR grant proposal 2017:** Do vine weevil larvae produce antimicrobial substances to avoid colonization by the entomopathogenic fungus *Metarhizium brunneum* (*anisopliae*)?

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Any questions?