Characterization of the genetic basis of resistance to (bio)pesticides in Cydia pomonella

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Rencontres scientifiques de l'Anses

Anses scientific and doctoral days

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anses

CHARACTERIZATION OF THE GENETIC BASIS OF RESISTANCE TO (BIO)PESTICIDES IN *CYDIA POMONELLA*

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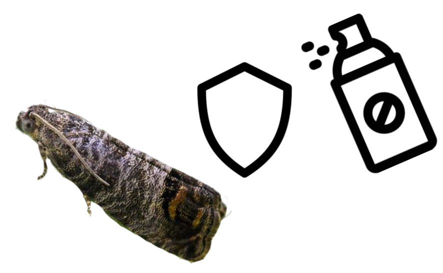
BACKGROUND



Codling moth is developing resistances to pesticides

Emergence of resistances in field → Current methods of detection (bioassays) have drawbacks : cost, accuracy, duration (~1 year) → Need to develop more effective tools !

Type of resistance	Genetic architecture and mechanisms
Target-site resistance	Simple and known
Detoxification	Complex and mostly unknown

State of the art of the types of resistance in *C. pomonella*

Ph.D. Objectives :

- Identify and complete the knowledge of the genetic basis of resistance in *Cydia pomonella*
- Develop a toolkit based on molecular techniques
- Proof of concept of the usability of the toolkit for monitoring in-field resistances

METHODS

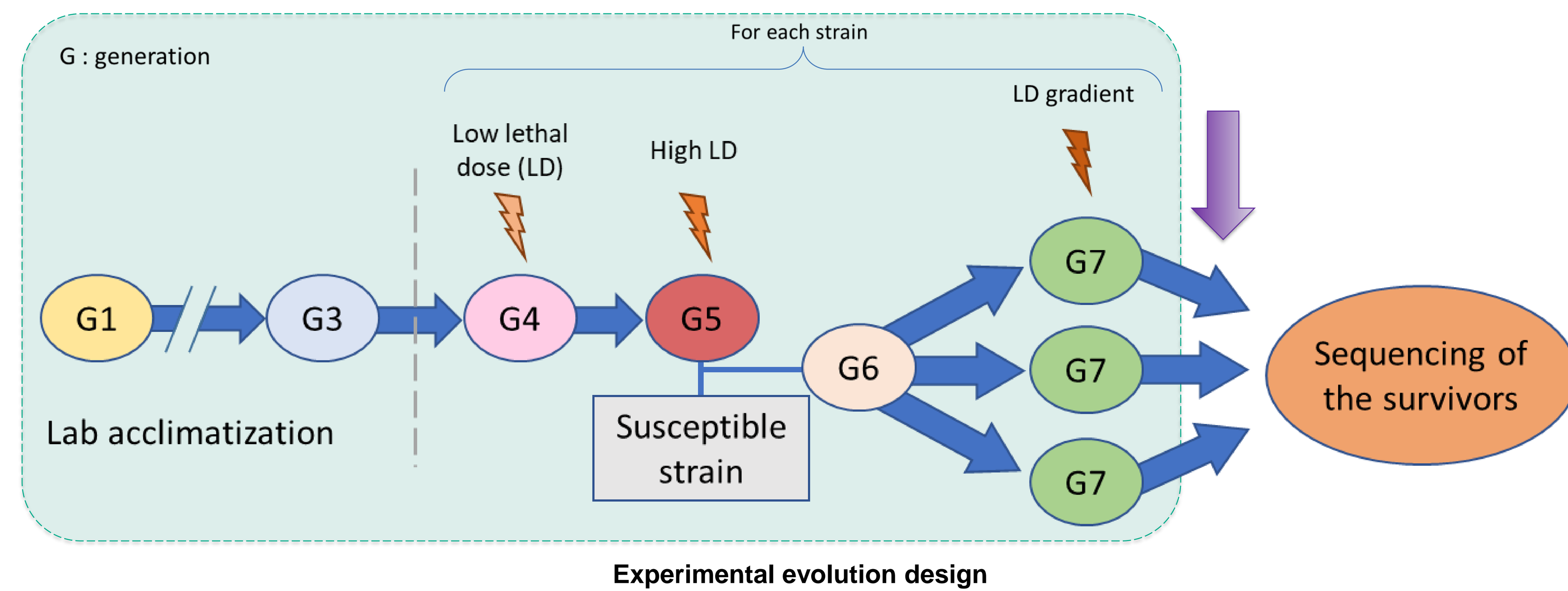
One population was sampled in Cavaillon (PACA, FR) and characterized as resistant to different pesticides, with resistance ratios of 4.9 for spinosad, 8.2 for deltamethrin, 2.1 for chlorantraniliprole compared to a reference susceptible strain.
An 'evolve and resequence' experiment was conducted, designed to detect genomic signatures of resistance from standing genetic variation (present in our multi-resistant population).

It consists of several steps:

A) Selecting pesticide resistant strains through an experimental evolution

B) Phenotyping to assess the resistance level of the strains

C) RNAseq to identify the differential expression and variants between reference genome and strains

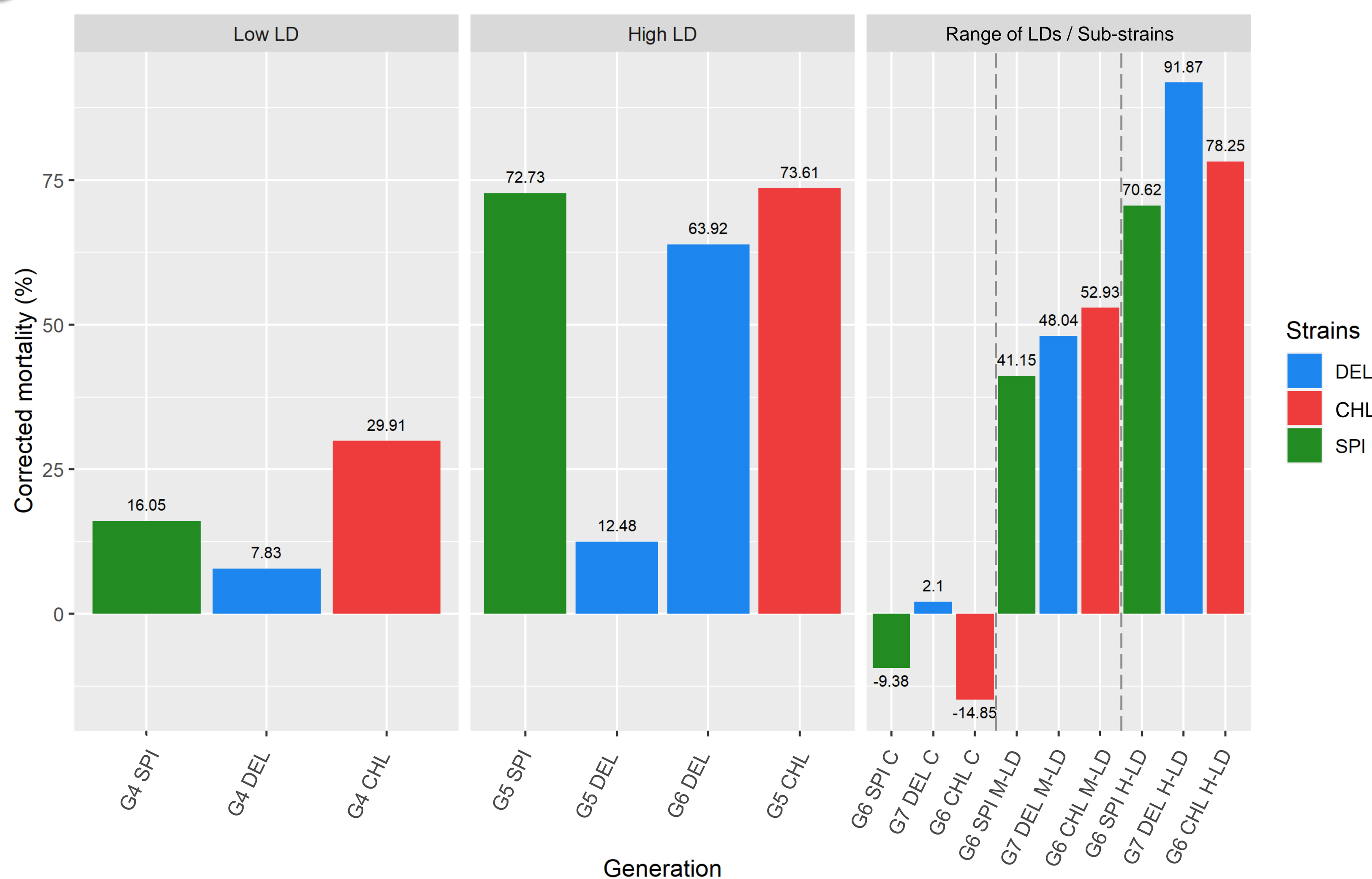


Experimental evolution design

3 strains were selected :
Spinosad-resistant (SPI), Deltamethrin-resistant (DEL) and Chlorantraniliprole-resistant (CHL).

Within each strain, a gradient of LD was applied : Control (C), Medium LD (M-LD) and High LD (H-LD).

A OBSERVED MORTALITY IN REARING

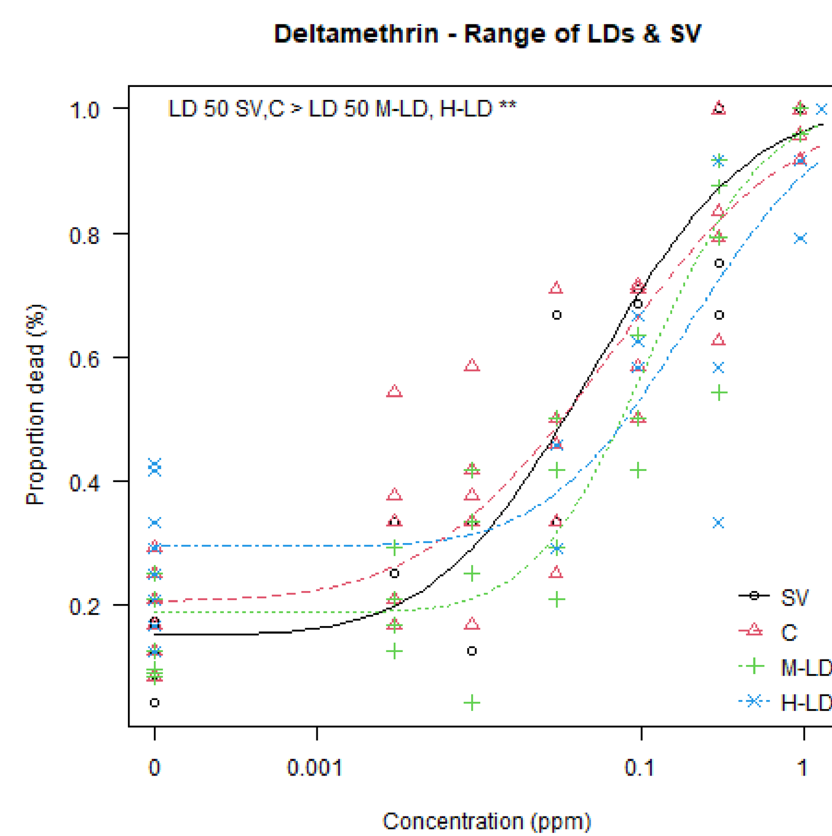


Corrected mortality (%) for each generation of selection of each of the strains created.

After acclimatization, different selection pressures were applied: except for one generation (G5 DEL) where another round of selection was needed, the observed mortality for all the strains was satisfying. Corrected mortality was obtained using Abbott's formula (Abbott, 1925).

B PHENOTYPING VIA INGESTION BIOASSAYS

Deltamethrin - Range of LDs & SV



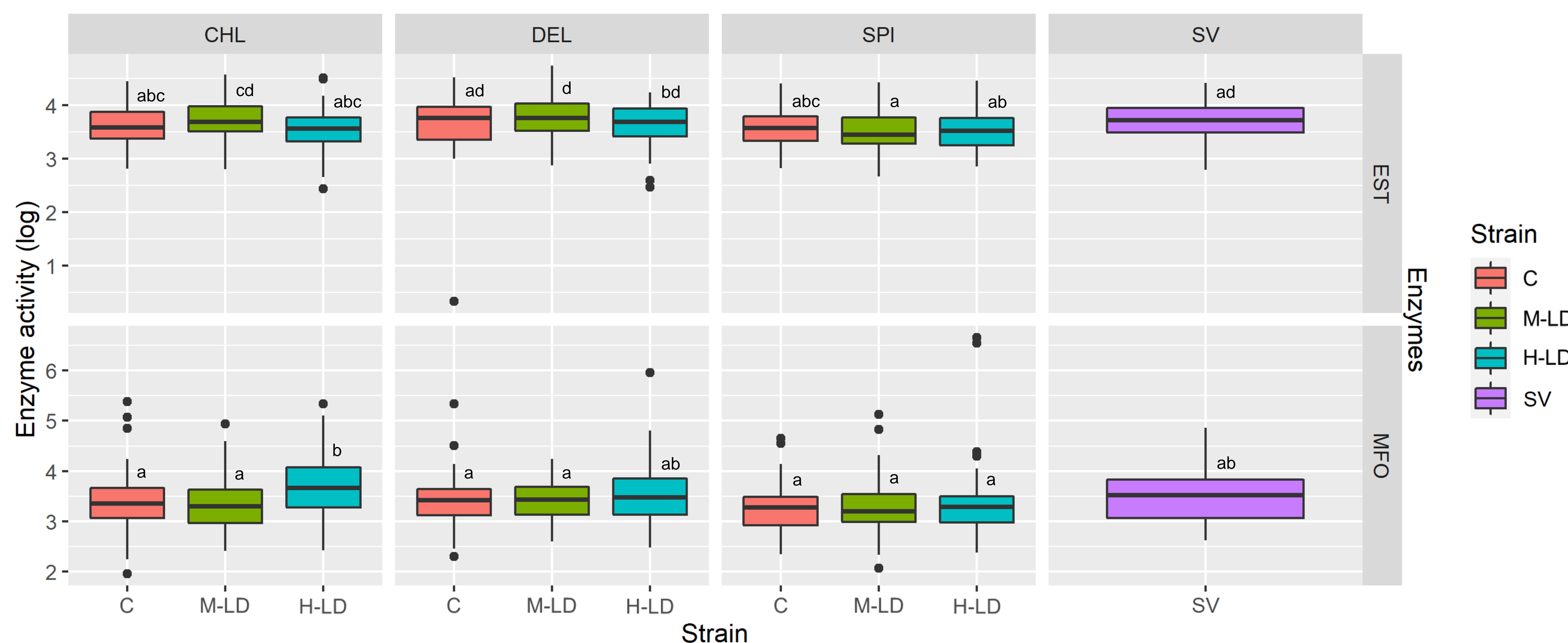
Dose-response curves obtained from bioassays of the 3 deltamethrin sub-strains and the susceptible reference strain, treated with different concentrations of deltamethrin.

Active substance / Strain	Control	Medium LD strain (M-LD)	High LD strain (H-LD)	Susceptible strain (SV)
Spinosad	5.66 ns (±1.08)	7.53 ns (±0.657)	7.35 ns (±0.853)	7.88 (±1.56)
Deltamethrin	0.064 ns (±0.013)	0.111 ** (±0.019)	0.175 ** (±0.04)	0.05 (±0.012)
Chlorantraniliprole	0.517 *** (±0.027)	0.823 ns (±0.062)	0.75 ns (±0.056)	0.955 (±0.089)

LD50 of active substances for the 9 sub-strains obtained and the reference susceptible strain. Significant effects compared to the susceptible strain values are noted with *, ** and *.**

Bioassays were conducted on neonates, in a microtiter plate filled with artificial medium and pesticide solution (Reyes et al., 2007).
Every sub-strain was tested with at least 6 concentrations for each active substance to establish dose-response curves and LD50.
The deltamethrin M-LD and H-LD strains are the only ones to have significantly higher LD50 than the SV strain, meaning a higher resistance to deltamethrin.

B PHENOTYPING VIA BIOCHEMICAL ASSAYS



Esterase (EST) and mixed-function oxidases (MFO) enzymatic activities for the 9 sub-strains obtained and the reference susceptible strain. Significant differences are noted with different letters (p < 0.05)

Enzymatic activity was obtained by absorbance measurement (using α-naphthyl acetate as substrate for ESTs) and fluorescence measurement (using 7-ethoxycoumarin O-deethylation for MFOs).
The strains are not significantly different from the susceptible strain, but the CHL H-LD strain shows a higher MFO activity than the other two CHL strains. GST (glutathione S-transferase) activity measurement was also conducted, but we found no significant difference in any strains.

C SEQUENCING IS IN PROGRESS

Conclusions and perspectives:

- Precise phenotype characterization is complex
- Inconsistency between an effective selection in rearing and the different phenotype measurements
- The obtained strains are not homogenous: phenotype measurements show variabilities within a strain (outliers in biochemical analyses)

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