Novel applications for monitoring and management codling moth resistance

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INTRODUCTION

The codling moth (CM) (*Cydia pomonella* L.) is the most important apple pest in Croatia and Europe. It is a highly controlled species. Intensive application of chemical based insecticide treatments for the control of CM has led to resistance development. It's a serious and growing problem in apple production and there is a need to explore and implement novel methods. The combined use of single nucleotide polymorphisms (SNPs) and geometric morphometrics to detect resistant variants is a novel approach where morphological traits can provide additional information about underlying population genetics, and morphology can retain useful information about genetic structure.

Figure 1. Codling moth adult

AIM
to find a reliable pattern of difference related to resistance type using population genetic and geometric morphometric approach.

MATERIALS AND METHODS

Adult male *C. pomonella* samples were collected in 2017 and 2018 in the apple orchards in continental part of Croatia. The same individuals were used both for the genetic and morphometric analysis.

- Organic orchards populations
- 94 CM individuals
- Integrated orchards populations
- Suseptible population
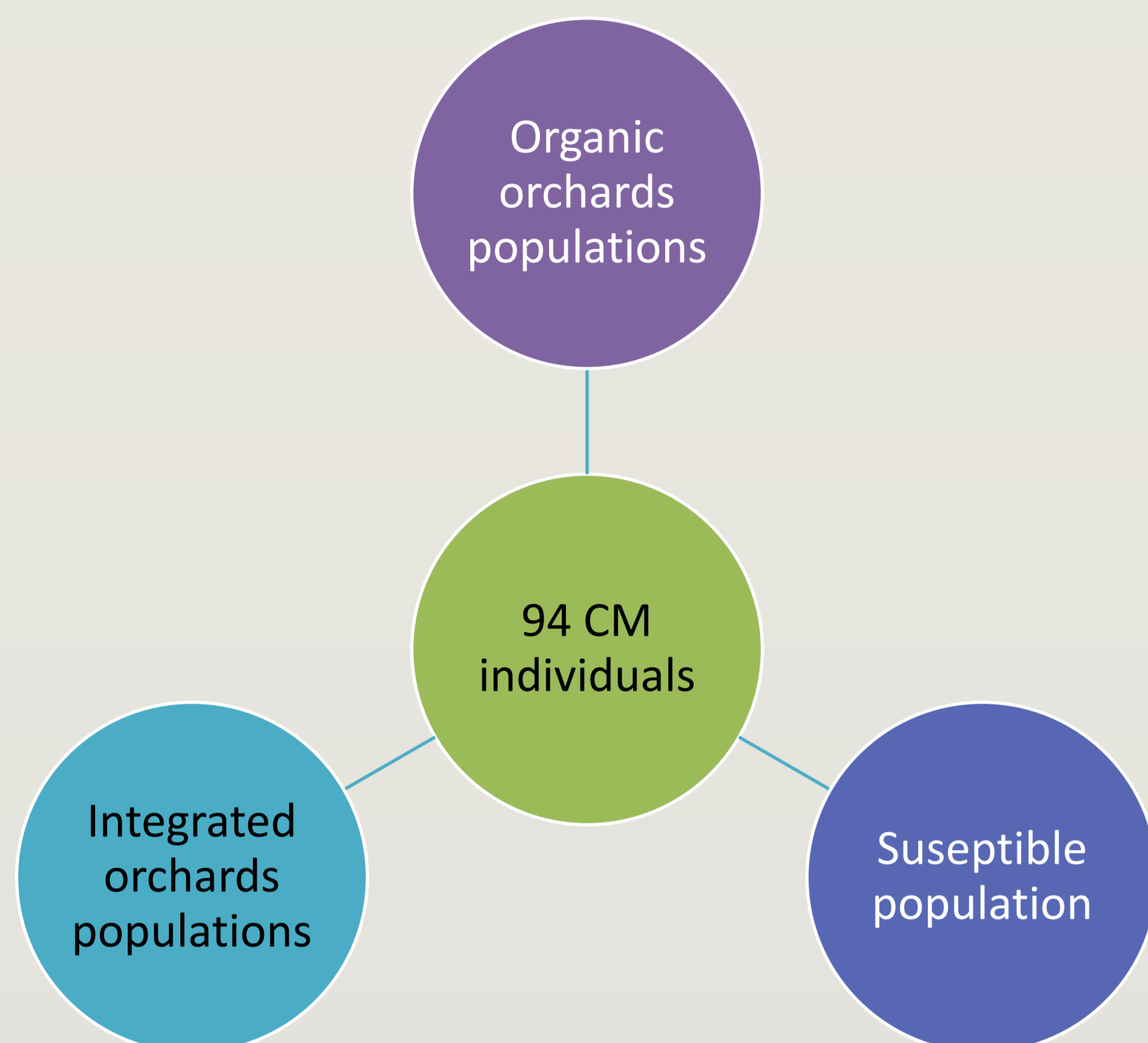
Single nucleotide polymorphisms (SNPs)

DNA extracted from the whole body tissue of 94 adult CM using the 'Qiagen DNeasy Blood and Tissue Kit'. After quality control samples were sent for genotyping to Diversity Array Technology Pty Ltd (DArT, Canberra, Australia).
All population genetic data analyses were undertaken using the coding environment in R using the R packages adegenet v2.1.3 and dartR v1.1.11 . After quality filtering of the SNP data, we retained 4,165 SNPs for further data analysis.
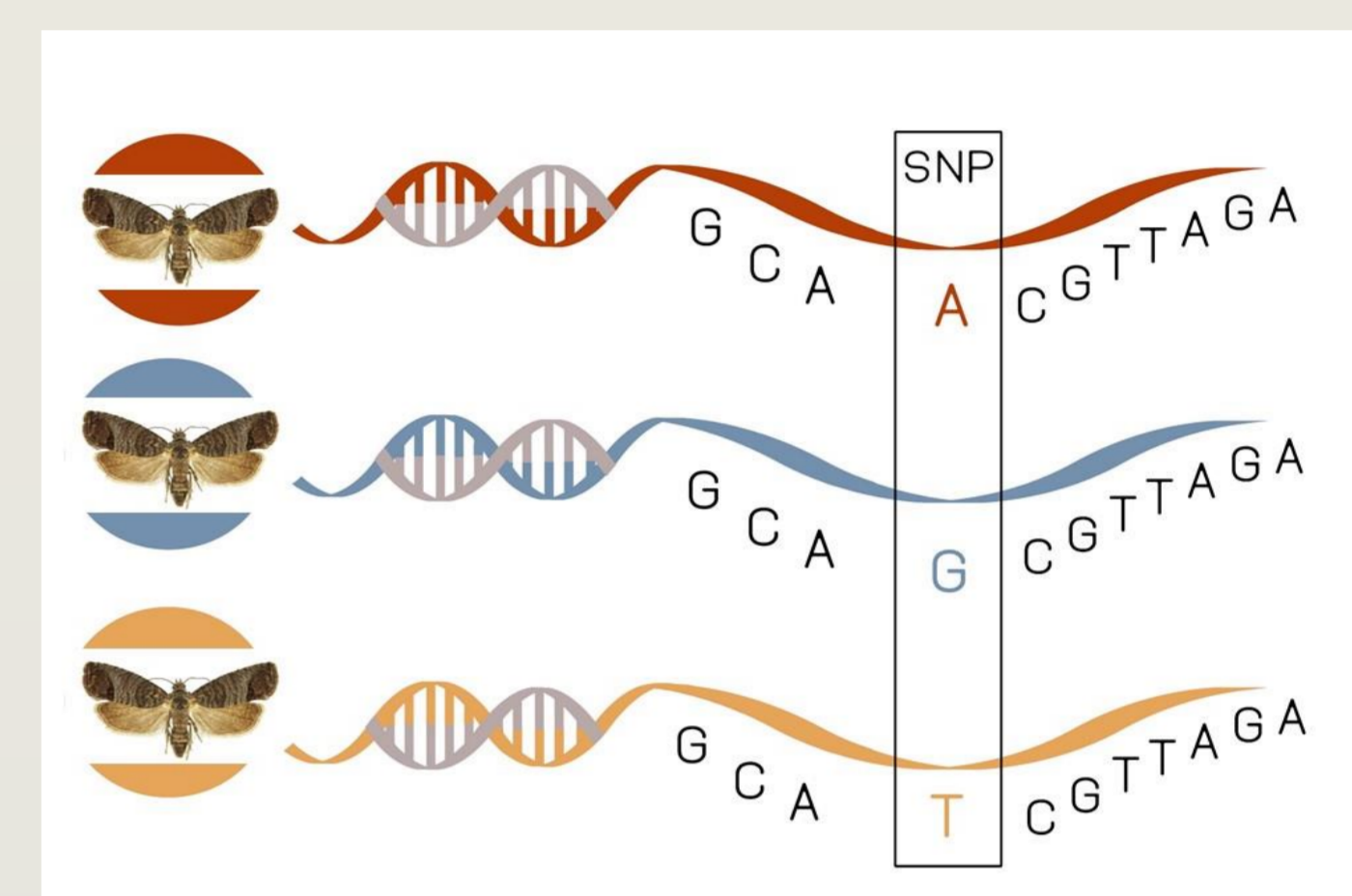
Figure 2. Example for single nucleotide polymorphisms (SNPs), single changes in the genetic code.

Geometric morphometrics (GM)

Before DNA extraction, forewings from all individuals were removed for morphometric analysis.
We used in total 294 forewings of CM adults from populations in integrated and organic orchards and laboratory reared suseptible population. Eighteen specific points (markers) were used to assess variability in hindwing shape and size. Principal component analysis (PCA) and canonical discriminant analysis (CVA) were used.

Figure 3. Representation of the 18 morphological landmarks identified on the forewings of CM

RESULTS

Single nucleotide polymorphisms (SNPs)

Overall population estimate was applied and moderate observed heterozygosity (HO) was observed across all loci, with an estimated value of HO = 0.2535. Moderate inbreeding was observed (FIS = 0.2522). There were no significant deviations from HWE for all loci. The low overall value of the genetic structure (FST = 0.0717) estimated for the three populations suggested a lack of genetic differentiation amongst them as a whole.

Geometric morphometrics (GM)

Figure 5. Canonical Variate Analysis of the fore wing shape between different CM population

After averaging the shape variation between the different populations it is clear that morphological differences are present in the forewing shape of CM based on control practices (organic vs. integrated). We have shown a reliable pattern of differences in forewing shape related to orchard control practice type.
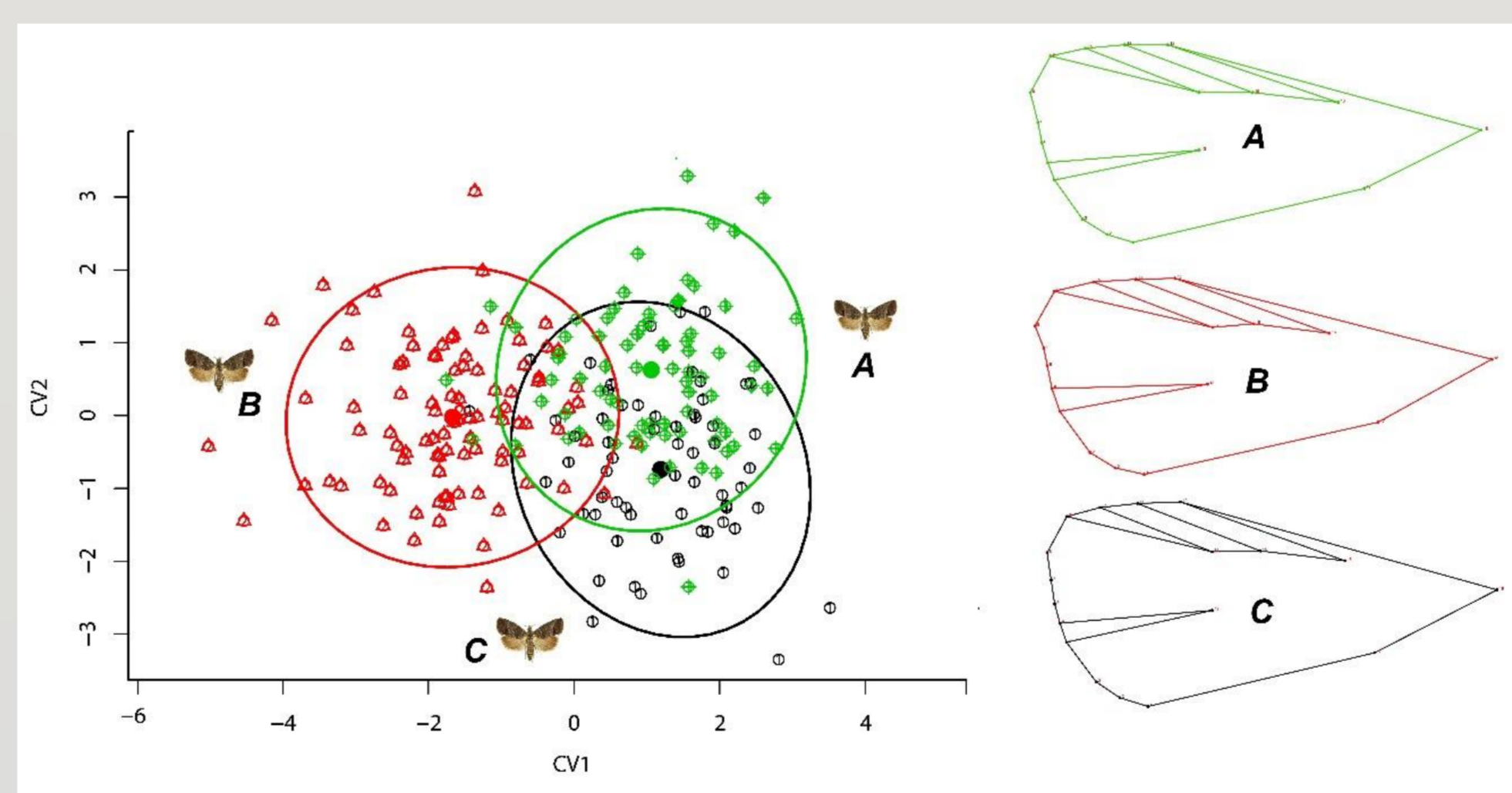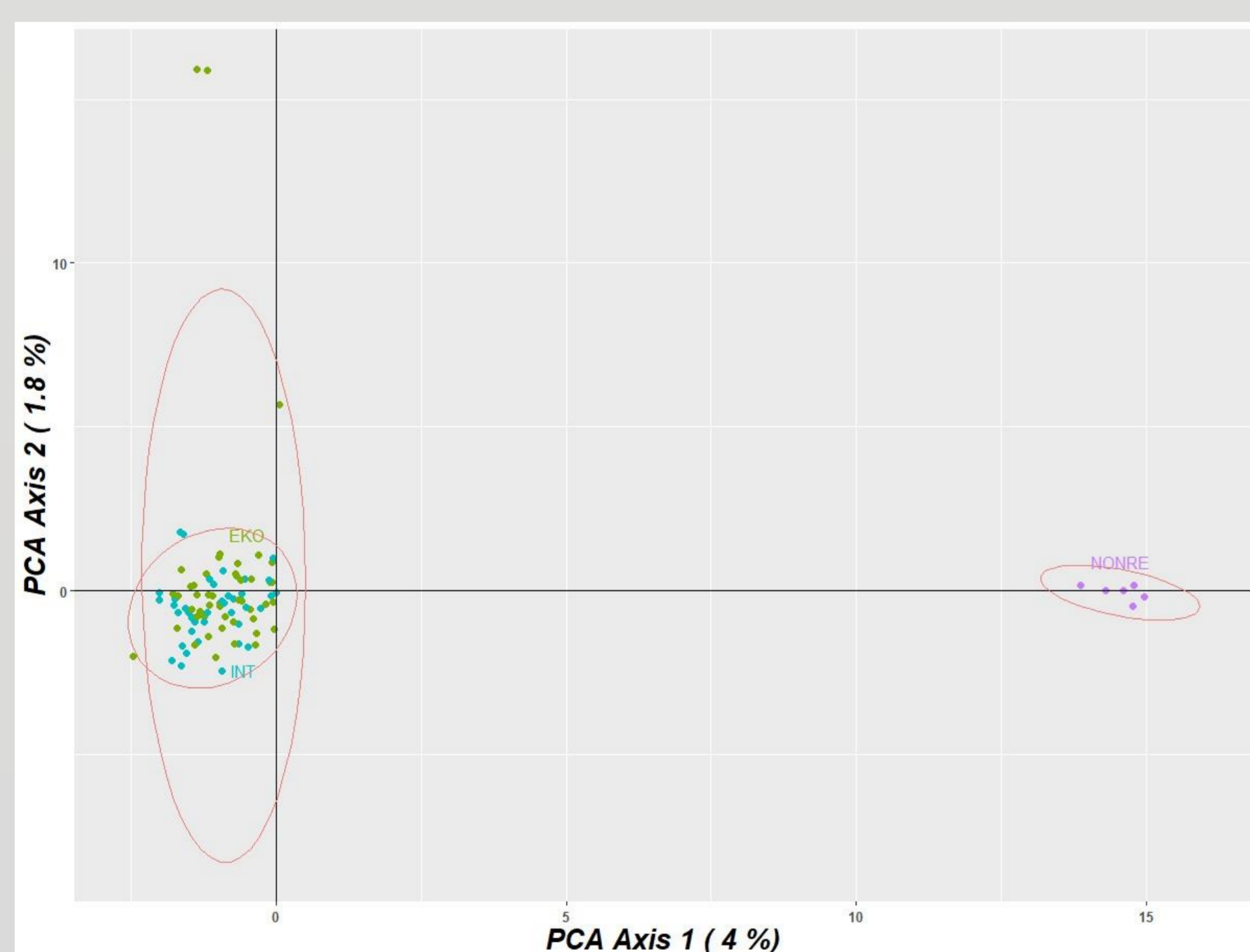
Figure 4. Principle Component Analysis of 94 CM genotypes based on DArTseq SNP markers

PCA revealed two distinct group of CM genotypes. Suseptible individuals were separated from indivuduals from integrated and organic orchads. Suggesting that there are lots of movement among them. Which is confirmed also with low FST and moderate inbreeding
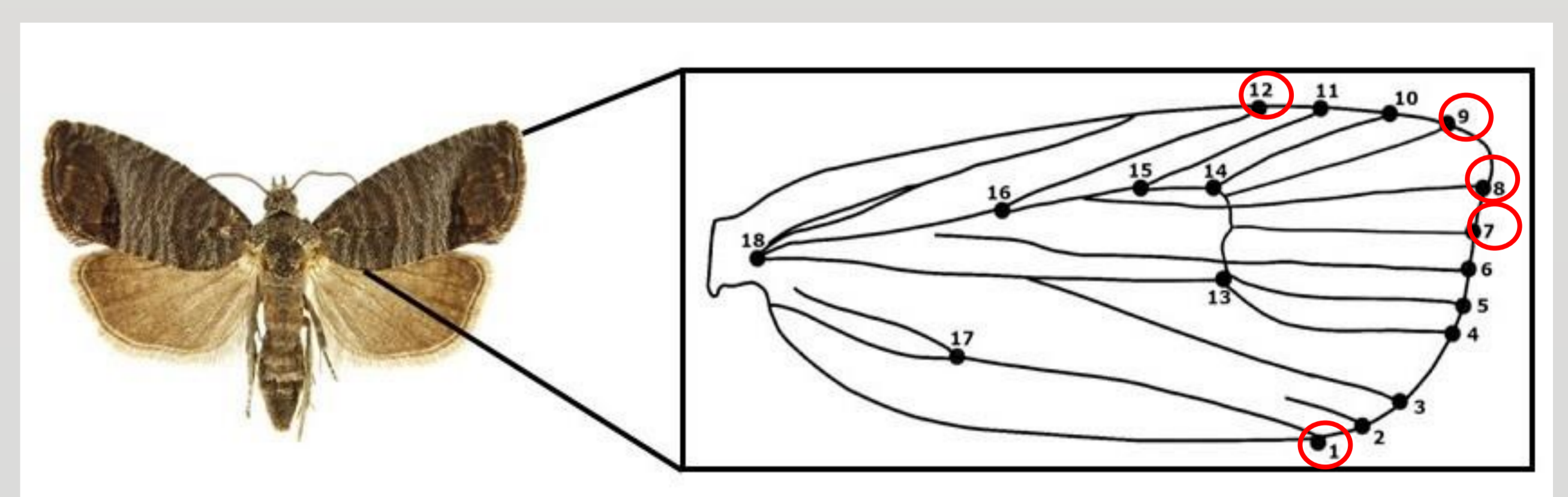
Figure 6. Eighteen specific markers on CM forewing

Populations of pests from nature (from organic and integrated farming) differ significantly in wing morphology compared to laboratory population. Variations were observed at five markers 1 7 8 9 and 12. Pest populations from integrated and organic farming had more elongated and expanded wings compared to the laboratory bred population which had an oval wing shape.

CONCLUSIONS

Results showed that there is a reliable pattern of differences related to the type of control practice. In order to prevent the spread of resistant CM populations in the field, it is crucial to detect specific biotypes on time to apply effective control measures. If the frequency of resistance alleles builds up unchecked, resistance may eventually become 'fixed' in populations. Once resistance reaches very high levels, strategies to restore susceptibility are unlikely to be effective. The detection and monitoring of resistant variants is the first step towards the implementation of anti-resistant strategies and sustainable use of pesticides in apple production worldwide when controlling CM.