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A complete genome sequence enables efficient genetic mapping of adaptive traits in a phytoseiid predatory mite (*Amblyseius andersoni*)

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Abstract

Phytoseiid mites are important biological control agents in Integrated Pest Management (IPM), yet the genetic architecture underlying their adaptive traits remains largely unknown due to limited genomic and genetic resources. Here, we establish an integrated genetic framework for *Amblyseius andersoni*, generating a genome sequence and developing a high-resolution genetic mapping approach. The genome was assembled using long-read sequencing, yielding a highly contiguous assembly of approximately 202 Mb with 18,923 predicted protein-coding genes and high completeness. We then dissected resistance to two widely used insecticides, deltamethrin and spinosad, as exemplar adaptive traits. Genetic mapping via bulk segregant analysis (BSA) localized deltamethrin resistance to a major-effect QTL encompassing the voltage-gated sodium channel (VGSC). Next, F₂ linkage analysis demonstrated co-segregation of the M918L substitution in VGSC with the resistant phenotype, consistent with a target-site resistance mechanism. Spinosad resistance mapped to a region encompassing the nicotinic acetylcholine receptor (nAChR) $\alpha 6$ subunit. Altered splicing resulting in intron retention was consistent with a dysfunctional receptor in the resistant strain. Together, this framework establishes a powerful genetic tool for resolving genetic determinants of adaptive traits in predatory mites and

provides genetic markers that can support breeding programs aimed at enhancing biological control strategies within IPM systems.

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Introduction

Integrated Pest Management (IPM) aims at suppressing pest populations while reducing risks to the environment and human health. This requires minimizing pesticide applications and, when possible, using biological control as a more sustainable alternative^{1,2}. Phytoseiid predatory mites (Acari: Phytoseiidae) are biological control agents (BCAs) of major economic importance, widely applied to control phytophagous mites, thrips, whiteflies and other small arthropod pests in perennial and greenhouse crops^{3,4}. Ensuring efficient pest control with BCAs is not straightforward, as several abiotic and biotic factors can affect their performance^{5,6}. Genetic improvement programs aimed at developing strains with enhanced traits could help overcome these constraints. Although such programs are common in crops and livestock, they remain largely absent in BCAs^{5,7-9}. One of the reasons is the limited availability of genomic resources and genetic tools for non-model organisms, including most BCAs, which constrains investigation of the genetic basis of traits of interest⁷.

In recent years, interest in phytoseiids has grown, leading to the publication of few genomes for economically important species and laying the foundation for genetic and genomic studies¹⁰⁻¹³. What is still lacking, however, is an efficient method to identify genes that underlie traits of interest. Although expression studies can point to genes or gene families whose expression shifts with the phenotype, genetic mapping offers a complementary and unbiased approach by associating phenotypes with genomic regions harboring causal variants. Bulk segregant analysis (BSA) is a mapping technique which markedly reduces genotyping effort associated with traditional linkage mapping studies¹⁴. This method is particularly rewarding in small arthropods where next generation sequencing (NGS) of individual organisms is challenging, and has been widely applied in Tetranychid mites, helping to clarify the genetic basis of pesticide resistance and adaptation to challenging hosts¹⁵⁻¹⁸. BSA detects genomic regions linked to a trait of interest by assessing allele-frequency differences between selected and control pools. The resulting genomic intervals may contain causal variants, including target-site mutations, allelic differences in detoxification genes, or transcriptional regulators (acting in *cis* or *trans*)^{14,19}. In principle, this approach can be applied to any quantifiable trait, and its implementation in phytoseiid mites would allow identification of genomic regions and candidate genes underlying observed phenotypes. These candidates can then be validated through reverse-genetic approaches (e.g. RNAi or CRISPR/Cas9) which became recently available for several predatory mite species²⁰⁻²². Next, validated genetic variation can be introgressed into commercial strains via marker-assisted backcrossing, a technique which has shown to be successful for spider mites²³⁻²⁵.

Pesticide compatibility is one of the most critical aspects of IPM⁵. Phytoseiids can be severely affected by exposure, leading to lethal or sublethal effects that reduce their efficiency²⁶⁻²⁸. This is particularly problematic when they are forced to coexist with insecticides in conventional IPM programs, but also in organic orchards

and greenhouses, where only biopesticides are allowed^{29,30}. In some predatory mite populations, repeated pesticide exposure and selection has resulted in field-evolved resistance^{31–34}. Several resistance-associated mutations have been identified in predatory mite populations, although the limited sequence data does not always allow to determine whether these variants were naturally occurring or were selected³⁴. Resistant field populations provide a valuable opportunity to investigate the molecular mechanisms underlying pesticide resistance and selectivity in phytoseiid species. Such insights can guide the development of resistant strains that might become a cornerstone of well-designed IPM strategies aimed at maintaining BCA efficacy.

Apple orchards in northern Italy offer an interesting case study because both conventional and organic production systems are used. Among the most commonly applied molecules negatively affecting phytoseiids are pyrethroids, pyrethrins and spinosyns^{35–37}. Pyrethrins, and the synthetically derived Pyrethroids act as voltage-gated sodium channel (VGSC) modulators (group 3A of insecticide resistance action committee, IRAC) and are among the most widely used pesticide classes. Spinosyns are allosteric modulators of nicotinic acetylcholine receptors (nAChR) specifically targeting $\alpha 6$ subunit (IRAC group V)³⁸. The latter group includes spinosad, which is permitted in organic agriculture in the European Union. Understanding mechanisms of resistance to these pesticides in phytoseiid mites would therefore provide valuable targets for genetic improvement.

This study investigates the incidence of insecticide resistance in the generalist predatory mite *Amblyseius andersoni* (Chant), both in commercial and field-sampled populations from Italian apple orchards. We then use a comprehensive, hypothesis-free approach to identify molecular mechanisms of resistance to spinosad and deltamethrin. We first generated genomic resources through *de novo* genome sequencing, assembly and gene annotation. Using the generated contiguous genome, we subsequently implemented a high-resolution genetic mapping framework to uncover genomic regions associated with resistance. Mapped resistance loci pointed to the presence of mutations in the VGSC and mis-splicing in *nAChR- $\alpha 6$* , consistent with target-site insensitivity. Differential gene expression analysis further characterized the resistance phenotype. To support the role of VGSC mutations, we performed a phenotype to genotype F₂ linkage analysis. The application of QTL mapping in a predatory mite provides novel insights into the genetic architecture of resistance in *A. andersoni*. Beyond its relevance for identification of molecular mechanisms of resistance, our study establishes a proof-of-concept for applying genomic mapping approaches to study traits of interest in predatory mites as key beneficial arthropods.

Results

Toxicity Bioassays

To identify variation in pesticide susceptibility among *A. andersoni* strains, probit dose-response analysis was performed with deltamethrin and spinosad, two main insecticides that are frequently used in the apple orchards where *A. andersoni* field populations were collected (Supplementary Table 1). Five strains were tested for resistance to both compounds (Table 1; Supplementary Table 2), consisting of three commercially available strains (C1, C2, C3; Supplementary Table 1) and two field collected strains (FS-DAL, FS-MAR; Supplementary Table 1). Strain C1 served as the susceptible reference for both compounds. For deltamethrin, C1 showed an LC_{50} of 0.0434 mg active ingredient (a.i.) L^{-1} , whereas the other strains (C2, C3, FS-DAL, FS-MAR) exhibited LC_{50} values between 171 and 388 mg L^{-1} . Corresponding resistance ratios (RR_{50}), relative to C1, ranged from 3949 to 8936, indicating strong resistance in all tested populations. For spinosad, C1 and FS-MAR were susceptible, with nearly identical LC_{50} values (9.55 and 9.57 mg L^{-1}), while C2, C3 and FS-DAL showed high resistance (LC_{50} = 3329-7541 mg L^{-1} ; RR_{50} = 348-790).

De novo genome assembly and annotation

Because no reference genome was available for *A. andersoni*, we first generated a *de novo* genome assembly to enable dissection of the genetic basis of pesticide resistance via transcriptomic profiling and BSA-based QTL mapping. The genome of the C2 strain was assembled from Oxford Nanopore Technology (ONT) long reads (49x, read N_{50} = 23 kb), yielding a 202 Mb assembly across 143 fragments (consisting of 9 scaffolds and 134 contigs) with an N_{50} of 13.7 Mb and a maximum scaffold length of 24.2 Mb. Although we did not obtain chromosome-scale scaffolds, the total genome size is consistent with those of other phytoseiid mites, and our scaffold N_{50} is similar to the ONT-based *N. californicus* assembly, despite substantially lower sequencing coverage (Supplementary Table 3).

BUSCO analysis recovered 99.6% of conserved genes from the *arachnida_odb10* dataset, indicating a highly complete genome assembly. Repetitive elements accounted for 19.2% of the genome. Annotation predicted 18,923 protein-coding genes and 20,881 transcripts, with an average gene length of 2.7 kb. In addition, InterProScan and EggNOG analyses identified functional domains and assigned GO terms to 12,255 genes and IPR domains to 14,193 genes. Full read, assembly and annotation statistics are provided in Supplementary Table 4.

Differential expression analysis

Given that metabolic resistance often involves changes in detoxification genes expression^{16,39}, potentially regulated by transcription factors within QTL regions^{19,40}, differential expression (DE) analysis provides a useful approach to identify candidate mechanisms underlying resistance. We therefore performed DE analysis using RNA-seq data from three resistant strains (FS-MAR, FS-DAL, C2) and the susceptible strain C1. Illumina sequencing generated on average 29 million 150 bp paired-end reads per sample, of which an average of 68 % mapped uniquely against the *de novo* genome. Principal component analysis (PCA) on normalized gene

expression levels revealed distinct clustering of all strains, with PC1 and PC2 explaining 44% and 19% of the variance, respectively (Figure 1A).

To compare gene expression among strains, we identified differentially expressed genes (DEGs) for six pairwise contrasts (C2 vs C1, FS-DAL vs C1, FS-MAR vs C1, C2 vs FS-MAR, C2 vs FS-DAL, FS-DAL vs FS-MAR) using an adjusted p-value < 0.05 and $|\log_2FC| > 2$. The complete lists of DEGs are provided in Supplementary Data 1. The degree of overlap among contrasts, based on inclusive intersections, is shown in the Upset plot (Figure 1C), and revealed substantial overlap in DEGs across contrasts, with the largest intersections involving comparisons that included C2.

From these intersections, we identified core DEG sets shared among the deltamethrin-resistant strains (C2, FS-MAR, FS-DAL) and among the spinosad-resistant strains (C2, FS-DAL) when contrasted with the susceptible C1 strain. GO enrichment analyses of these core sets ($n = 127$ and 234 for deltamethrin and spinosad, respectively; Supplementary Data 1) showed a significant enrichment of oxidoreductase and monooxygenase activity, functions typically associated with cytochrome P450s, with the strongest enrichment detected in the spinosad-resistant core set and a weaker but still significant enrichment in the deltamethrin-resistant core set (Supplementary Data 2). GO enrichment analyses of the individual pairwise contrasts confirmed this pattern: P450-related GO terms (Molecular Function) were significantly enriched in all comparisons involving C1, with the most pronounced enrichment in the contrast with C2 vs C1 (Supplementary Data 3). To further examine expression patterns of these detoxification genes, we visualized the P450 genes that were differentially expressed in at least one contrast. These P450 genes were manually curated (see Methods), and official P450 nomenclature is shown in the heatmap. The corresponding Z-score heatmap (Figure 1B) shows widespread upregulation in the resistant strains, particularly in FS-DAL and C2.

Genetic mapping by Bulk Segregant Analysis

To increase the mapping resolution of our BSA approach¹⁴, we first created an inbred strain from the resistant population C2. After 20 generations of full-sib mating (Figure 2A), we confirmed that the inbred population, designated C2_i, was still resistant to both pesticides. Genome sequencing allowed to assess genome-wide heterozygosity profiles, calculated as the number of heterozygous SNPs in sliding windows along the genome, and confirmed that C2_i was successfully inbred (Figure 3A).

To identify the genomic regions underlying resistance to deltamethrin and spinosad, we created a large mapping population by crossing susceptible C1 females with resistant C2_i males (Figure 2B). After 10 generations, allowing recombination to accumulate, mites from this population were phenotyped by exposure to a diagnostic dose of either deltamethrin or spinosad. For each pesticide, three pools were generated: (i) selected pools containing individuals that survived exposure, (ii) negatively selected pools comprising dead

individuals, and (iii) unselected control pools of untreated mites. Genomic DNA was extracted from all pools as well as both parental strains, and Illumina paired-end 150 bp sequencing produced between 35.6 and 47.8 million read pairs per sample. Reads were subsequently aligned to the *A. andersoni* genome, resulting in 1,108,634 segregating SNPs prior to filtering. To identify resistance-associated genomic regions, allele frequency differences between selected and control pools were calculated for 262,685 high-quality SNPs. Parallel analyses comparing negatively selected and control pools were also performed. For deltamethrin, three biological replicates were available for all pools, allowing a permutation-based approach (adapted from Wybouw et al.¹⁸) to assess QTL significance. For spinosad, only one replicate per pool was available, and therefore no permutations were performed.

For deltamethrin, using a false discovery rate (FDR) of 0.05, a significant major peak was identified on scaffold 10 around 16.2 Mb (hereafter referred to as QTL1) (Figure 3B). Strikingly, in this QTL1 peak region, nearly complete fixation of the resistant C2_i haplotype occurred in the deltamethrin-selected populations (Supplementary Figure 1A), a pattern consistent with selection on a recessive resistance locus. Comparing the negatively selected pool (i.e. dead mites following deltamethrin treatment) with the unselected control further confirmed the location of the peak (Supplementary Figure 2) and showed selection towards the susceptible C1 haplotype in the negatively selected pool (Supplementary Figure 1A). Although still significant, the peak was less pronounced in the negatively selected pool.

Within a ~400 kb region centered on the peak (defined as the midpoint of the top window), 42 genes were present, including *VGSC*, the main target site of pyrethroids (Figure 3C). Within the *VGSC*, we identified three substitutions in the protein sequence of the resistant parent predicted from our manually curated annotation: M950L (caused by a A2848C substitution at position 16,041,229), L957V (caused by a C2869G substitution at position 16,041,250) and E1258D (caused by a A3774C substitution at position 16,044,173). Based on protein alignment with the *Musca domestica* *VGSC*, the first two correspond to the known resistance-associated mutations M918L and L925V, respectively. Both mutations were fixed in the resistant parent and absent in the susceptible parent. The resistant alleles nearly reached fixation (>90%) in the positively selected populations, but were much less frequent in the control (~25%) and negatively selected populations (~12%).

Under spinosad selection, we observed a peak on scaffold 6, designated QTL2 (Figure 3B). Allele frequencies shifted toward the resistant parent, rising to just above 0.5 (Supplementary Figure 1B) but, unlike the deltamethrin-selected populations, not approaching fixation. Comparison of the negatively selected pool with the unselected control corroborated the same locus, albeit with a less pronounced signal (Supplementary Figure 2). Surprisingly, C2_i allele frequency in the negative selected pool approached zero (i.e., the susceptible C1 genotype was fixed), meaning that heterozygous individuals did survive the discriminating dose, as also suggested by allele frequencies just above 50% in the positive selected pool (Supplementary Figure 1). Within

a 400 kb interval centered on the peak, 37 genes were annotated, including *nAChR- α 6*, which encodes the main target site of spinosyn pesticides (Figure 3D), and *nAChR- α 10*, encoding a subunit conserved within phytoseiids but divergent from other arthropods.

In *nAChR- α 10*, no coding variants nor alternative splicing events were detected in the resistant strain compared with the susceptible strain. In contrast, *nAChR- α 6* showed no amino-acid-altering mutations or large indels in the resistant parent, yet visualization of splice-junction support based on pooled RNA-seq reads from all biological replicates per strain, supports alternative splicing in the C2 strain (Figure 4). The read-coverage and junction patterns showed retention of intron 10 in C2 (red arrow in Figure 4), a pattern not observed in the susceptible C1 strain. This intron, when fully retained, introduces a premature stop codon and likely results in a nonfunctional protein, as the essential fourth transmembrane domain resides in exon 11. Intron-exon splicing junctions presented no differences between C1 and C2_i, however, three SNPs (C-to-T at position 2,163,280; G-to-C at position 2,164,531; A-to-G at position 2,164,644) were identified within intron 10 by analyzing DNA reads from the resistant parental line (C2_i) in comparison with the susceptible parental (C1). RT-PCR analysis of the region spanning the exon 10-exon 11 junction confirmed complete removal of intron 10 in C1, as indicated by a single band of the expected size after gel electrophoresis (Supplementary Figure 3, Supplementary Figure 4). In contrast, C2 and C2_i displayed multiple bands, including those corresponding to the fully spliced transcript and complete retention of intron 10, as well as two additional bands likely representing splice variants with partial intron 10 retention. These results were consistent across two distinct primer pairs and were further confirmed via Sanger sequencing.

Based on pooled RNA-seq reads, splice-junction patterns in the additional spinosad-resistant field strain (FS-DAL) resembled those of the susceptible strain. Still, FS-DAL contained low-frequency alternative alleles that introduced premature stop codons in exon 10 (C-to-G at position 2,165,069 and C-to-U at 2,165,334) and a two-nucleotide deletion at 2,165,422 causing a frameshift (25% for the first stop-codon variant and 10% for the other two variants).

The gene expression dataset additionally enabled us to examine whether any DEGs fell within the QTL peak regions. Using the previously defined thresholds (adjusted p-value < 0.05 and $|\log_2FC| > 2$), no differentially expressed genes were found for the resistant versus susceptible parent comparison (C2 vs C1) within the QTL1 peak. In the QTL2 region, we identified three genes that met these criteria: two without functional annotation (*AMBAN_010480*, *AMBAN_010481*) and one encoding a histone acetyltransferase (*AMBAN_010482*). For both QTLs, we inspected the same genomic interval as shown in Figure 3C–D, spanning 400 kb centered on the midpoint of the peak window.

Linking phenotype to genotype in deltamethrin resistant and susceptible strains

The presence of the M918L and L925V mutations in the VGSC was first assessed in other deltamethrin-resistant strains. PCR genotyping showed that M918L occurred in all resistant strains (C3, FS-MAR and FS-DAL), whereas L925V was fixed in C2 and C3 and nearly fixed in FS-MAR and FS-DAL. The consistent occurrence of these substitutions among resistant strains further supports their association with deltamethrin resistance in *A. andersoni*.

To formally test whether these VGSC mutations segregate with the resistance phenotype, a linkage analysis was then performed using a cross involving an independent resistant strain (Figure 5). In total, 112 F₂ individuals from reciprocal crosses between the resistant FS-DAL strain and the susceptible C1 strain were phenotyped for survival at 15 mg L⁻¹ deltamethrin and genotyped by PCR for the M918L and L925V variants. A strong genotype-phenotype relationship was observed in this data. All individuals that were either homozygous for the wild-type M918 allele (MM) or heterozygous (ML), regardless of their L925V genotype, were fully susceptible, with 0% survival (Figure 5). In contrast, all individuals homozygous for the resistant 918L (LL) allele survived, indicating a recessive mode of inheritance for the M918L mutation. The 925V mutation alone did not confer resistance: individuals carrying this mutation but lacking homozygous 918L remained fully susceptible. Pairwise statistical comparisons (Fisher exact test; Supplementary Table 5) confirmed that only homozygous 918L genotypes (LL) differed significantly in survival compared to the susceptible reference group, while all other combinations did not show significant differences.

Discussion

Improving the performance of phytoseiid predatory mites as BCAs requires insight into the genetic mechanisms that give rise to variation in key traits. Such insight depends on tools that can detect relevant loci and quantify their contribution to phenotypes. Here, building on a *de novo* long-read genome assembly, we implement genetic mapping for the first time, to our knowledge, in a phytoseiid species. Genetic mapping results were complemented by differential gene expression analysis and genotype-phenotype association in F₂ segregants to investigate deltamethrin and spinosad resistance in *A. andersoni*. This integrated approach allowed us to identify genomic regions associated with survival under pesticide exposure and to evaluate the feasibility of BSA-based resistance mapping in a BCA, while providing a framework that can be applied to other species with limited genomic resources.

The availability of a *de novo* genome assembly for *A. andersoni* represents a key step towards genetic mapping in this species. Using long-read Oxford Technology Nanopore sequencing, we obtained a contiguous and complete assembly at limited cost, illustrating how current long-read technologies have removed many of the previous barriers to generating reference genomes for non-model arthropods. This assembly was essential for accurate read mapping and variant calling, enabled downstream gene expression analyses and QTL mapping,

and provides a foundation for future functional and comparative studies in this species. The assembled genome of ~200 Mb falls within the range reported for other phytoseiid predatory mites (Supplementary Table 3). The genome of *Neoseiulus cucumeris* was estimated at ~173 Mb from an Illumina short read assembly¹². More recent long-read assemblies for *Neoseiulus californicus* and *Phytoseiulus persimilis* report genome sizes of 188 and 214 Mb, respectively^{13,41}, with ~190 Mb of the *P. persimilis* assembly anchored to four chromosomes. In contrast, the genome of the predatory mite *Galendromus occidentalis* is relatively small, with a draft assembly of ~152 Mb¹⁰.

BSA was central to our strategy for mapping resistance loci, made feasible by our ability to maintain large, recombining populations using alternative food sources such as pollen and, in particular, the bag-based *Carpoglyphus lactis* system (Figure 2). These conditions supported population sizes large enough to minimize drift and allowed recombination to accumulate over ten generations before selection, conditions that are considerably harder to sustain when using spider mites as prey. We then implemented an endpoint-selection approach, in which the recombined population was exposed once to a discriminating dose and only the survivors were pooled for sequencing. This combination of sustained population growth, multi-generation recombination, and a single strong selection step produced survivor pools of substantial size (>400 individuals per replicate for deltamethrin and >700 for spinosad), thereby maximizing the number of sampled meioses and yielding the resolution required to detect relatively narrow allele-frequency peaks (Figure 3). For deltamethrin, the allele-frequency peak was located ~150 kb from *VGSC*, whereas for spinosad, the peak was <100 kb from *nAChR-α6*. Although these distances are somewhat larger than those reported for *T. urticae*, they remain in line with expectations given our design. In *T. urticae*, Kurlovs et al.¹⁴ found that distances between BSA peaks and causal genes ranged from a few tens to several hundred kilobases, with substantial variation among replicates. Increasing biological replicates tend to reduce this variance, producing mean peak positions that fall much closer to the causal gene (often within tens of kilobases). In our study, only one replicate was available for spinosad and three for deltamethrin, in contrast to the nine or ten replicates described by Kurlovs et al.¹⁴. Additional factors likely contributed as well, including fewer generations of recombination and the use of larger sliding-window and step sizes, all of which can broaden the distance between peak positions and causal genes. Our selection regime also differed from the gradually increasing dosage schemes common in *T. urticae* BSA studies mapping pesticide resistance^{16,17,39,42}. Dose escalation is challenging to implement in predatory mites because individuals must be maintained on live prey, and both predator and prey respond to pesticide exposure. Endpoint selection, therefore, provides a practical and still informative alternative for predatory systems. The proportion of survivors in our experiment (10-15% for deltamethrin, 20-21% for spinosad) indicates that selection intensity was well-suited for detecting relevant loci. If resistance were highly

polygenic, survival after a single selection event would be expected to be much lower, and multi-generation dose escalation might be required to accumulate favorable allele combinations.

For deltamethrin, the BSA revealed a major peak (QTL1) on scaffold 10, harboring *VGSC* (Figure 3). Within this gene, we detected the M918L and L925V substitutions, which showed near-complete fixation in deltamethrin-selected populations, but occurred at much lower frequencies in control or negatively selected pools. Subsequent F_2 genotype-phenotype association in an independent resistant field strain confirmed that M918L is likely the causal mutation, conferring resistance in a recessive manner, while L925V alone had no detectable effect on survival (Figure 5). Both mutations were present in all deltamethrin-resistant field strains examined, likely reflecting the strong selective pressure acting on phytoseiids in the field. Their presence in most commercial strains is probably due to the same reason, as these mutations were likely already present in the wild individuals used to establish mass cultures. The role of M918L in resistance is well established in several insect species^{43,44}, and it has recently been reported in pyrethroid-resistant populations of several mite species^{45–48}, including phytoseiids^{49–51}. Our findings further clarify its contribution in predatory mites, where M918L alone appears sufficient to confer deltamethrin resistance. In contrast, mites carrying only L925V remained susceptible. This pattern differs from what has been reported in resistant populations of *Varroa destructor*^{52,53}. However, previous studies on *V. destructor* did not test deltamethrin specifically, raising the possibility that, in addition to species-specific differences, L925V may contribute to resistance primarily against other pyrethroids. Moreover, the frequent co-occurrence of M918L and L925V across species, including in this study, suggests that both may play a role⁴⁶. In our work, only a single discriminating dose of pyrethroids was used for selection, preventing us from drawing conclusions about whether L925V enhances pyrethroid resistance when present together with M918L. Directly assessing the contribution of L925V to pyrethroid resistance in phytoseiid mites will therefore be essential for clarifying its functional significance.

Unlike deltamethrin, the spinosad QTL offers a more ambiguous and less supported picture given a single replicate was available. The mapping peak (QTL2) contains *nAChR- α 6* and *- α 10* on scaffold 6. Subunit α 6 is the only known spinosyn target site in insects^{54,55}, and was recently confirmed as the spinosad target in the phytoseiid mite *Amblyseius swirskii*²². In contrast, subunit α 10 is a phytoseiid-specific, highly divergent subunit of unknown function and is considered unlikely to participate in spinosyn binding²². Consistent with this, no coding mutations or alternative splicing events were detected in *nAChR- α 10*, further directing attention toward *nAChR- α 6*. However, coding mutations in *nAChR- α 6* leading to disruption or amino acid substitutions were also absent. The low expression of this gene in whole-organism bulk RNA-seq limited our ability to examine isoforms or expression differences. Nonetheless, pooling RNA reads across replicates yielded sufficient coverage to assess alternative splicing. This analysis revealed the retention of intron 10 in the resistant parental strain, introducing a premature stop codon and disrupting the protein before the essential fourth transmembrane domain

encoded in exon 11. On the other hand, the susceptible C1 strain presented splicing as expected. These findings were confirmed by RT-PCR, which also revealed varying levels of intron retention in C2_i and C2, suggesting a more complex regulation of intron 10 splicing. Disruption of *nAChR-α6* due to intron retention or exon skipping is a well-established mechanism of spinosyn resistance and has been documented in several insect species⁵⁵. Moreover, co-existence of multiple splicing variants within a single population was previously described, and a linear correlation between percentage of nonsense transcripts and resistance was observed⁵⁶. Furthermore, CRISPR-mediated knockout of *nAChR-α6* in the phytoseiid *A. swirskii* recently generated highly resistant individuals²², strongly indicating that loss-of-function of nAChR-α6 confers spinosad resistance in phytoseiid mites. Nevertheless, the mutation responsible for intron retention remains unidentified: both donor and acceptor splice sites appear intact, suggesting that the causal variant may be one of the SNPs located within the intronic sequence. The region harboring α6 did not reach fixation in the positively selected pool, whereas it did in the negatively selected pool, where the C2_i resistant genotype was absent. This could be caused by dominant inheritance of the resistant trait, in which case heterozygous individuals can survive the discriminating dose of spinosad. Although loss of function of nAChRs is normally recessive in insect species^{57–59}, this might vary in chelicerates, where limited data are available about nAChRs²². In addition, the different nature of mutations that might result in loss of function might also influence the resistant phenotype.

Although genetic mapping identified *VGSC* and *nAChR-α6* alterations as major candidates for resistance, additional mechanisms contributing to the high resistance levels observed in commercial and field strains may remain undetected, as their effects might only become relevant at higher doses than those used during BSA selection. In this context, transcriptomic analysis complements the genetic mapping results by revealing consistent upregulation of numerous cytochrome P450 genes in the spinosad-resistant strains C2 and FS-DAL. In contrast, this pattern was much less pronounced in FS-MAR, which is resistant to deltamethrin but not to spinosad. Hence, we cannot exclude that P450-mediated detoxification also contributes to higher levels of spinosad resistance in *A. andersoni*, then selected for by BSA. Although both C2 and FS-DAL are resistant to spinosad, they displayed noticeable qualitative and quantitative differences in their differentially expressed P450 profiles. Together with the absence of alternative splicing and the presence of premature stop codons only at low frequency in FS-DAL, this suggests that the mechanisms underlying spinosad resistance may differ, at least partially, between the two strains. While dissecting all resistance factors shaping the resistance phenotype was beyond the scope of this study, the differential expression analysis indicates that secondary mechanisms may be involved in spinosad resistance and therefore warrant further investigation. A new genetic mapping study using FS-DAL, or selection regimes based on gradually increasing doses over multiple generations, as commonly used in *T. urticae*, may help identify additional resistance mechanisms.

One of the major challenges for the establishment of natural enemies in crop systems is their compatibility with synthetic and natural pesticides, including pyrethroid and spinosyn^{29,60}. However, the molecular mechanism of pesticide resistance in phytoseiids remain largely unknown, hindering the development of rational control programs aimed at reducing negative effects on these beneficial mites. The present study helps to clarify these mechanisms by identifying causal mutations associated with resistance, which could in the future guide the selection of resistant strains to improve phytoseiid persistence in the field when deployed in IPM programs. However, resistance is often associated with fitness costs, typically caused by alteration of the target-site itself^{24,61}. These deleterious effects, which may arise in the absence of pesticide selection pressure, could limit the practical use of resistant strains in field applications. Point mutations in VGSC have previously been linked to fitness cost of varying magnitude^{24,62–64}, whereas loss of nAChR- $\alpha 6$ has generally been associated with severe fitness disadvantage in insects^{65–67}. In this study, the occurrence of fitness cost was not assessed. However, the presence of deltamethrin and spinosad resistance mutations in commercial strains indicates that the occurrence of multiple resistance mutations does not necessarily preclude the successful mass production and biocontrol efficacy of these key BCAs. Furthermore, the observation that the same mutations are fixed or approaching fixation in field populations suggests that potential fitness costs remain negligible under field conditions. Future studies should assess potential fitness disadvantages and their impact on production and efficacy of *A. andersoni* as well as explore the introduction of additional resistance alleles into commercial strains to develop multi-resistant beneficial arthropods with enhanced value for biological control.

Overall, this study provides a proof-of-concept that QTL mapping via BSA can be successfully applied in predatory mites and, by extension, in other organisms with limited genomic resources or where experimental setup for genetic mapping are still lacking. In doing so, it also reveals key aspects of the genetic basis of deltamethrin resistance in *A. andersoni*, which is caused by target-site mutations. Moreover, although the support of QTL2 is weaker given the lack of replication, this study strongly suggests that resistance to spinosad is mainly caused by target site resistance, with possible metabolic contributions. This framework opens new opportunities to dissect the genetic architecture and causal loci underlying a wide range of agronomically relevant traits, including resistance to additional pesticides as well as environmental tolerance traits or prey preference.

Methods

Predatory mite strains and husbandry

Two field strains were collected from two commercial apple orchards in north-eastern Italy: one from a conventionally managed field (FS-MAR) and one from an organic field (FS-DAL) (Supplementary Table 1). Three

additional strains were provided by European biocontrol companies and are referred to as C1, C2 and C3 (Supplementary Table 1). For genetic mapping, we established an inbred line (C2_i) derived from the C2 strain by performing 20 consecutive generations of brother-sister mating. Predatory mite strains were reared on arenas consisting of black plastic squares placed on wet sponges, with wet cotton serving as a barrier to prevent escape. Fresh pollen from *Typha* spp. (Nutrimite, BioBest, Belgium) was provided 3 times a week. Rearing arenas were maintained in incubators at $25 \pm 1^\circ\text{C}$, $65 \pm 10\%$ RH and 16L:8D h photoperiod.

Toxicity bioassays

The pesticides used were commercial formulations of deltamethrin (25 g a.i. L⁻¹, Decis® Evo, Bayer Crop Science Italia s.r.l.) and spinosad (480 g a.i. L⁻¹, Laser®, Corteva Agriscience Italia s.r.l.). Four to five concentrations of diluted insecticides solutions were used to spray on black plastic squares (12 cm²) placed on wet cotton inside plastic boxes, with additional wet cotton along the edges. Treatments were applied using a Potter spray tower (Buckard Manufacturing) and distilled water served as the control. Each concentration was tested in five replicates, each consisting of eight mites. Once residues had completely dried (30 minutes), fresh *Typha* spp. pollen was provided to the mites. Experimental units were placed in incubators at $25 \pm 1^\circ\text{C}$, $65 \pm 10\%$ RH and 16L:8D h photoperiod. Female survival was assessed after 72h and escaped or drowned females were excluded from further analyses. Mites were considered dead if they failed to move after prodding with a fine brush. Dose-response curves (LC₅₀, RR₅₀, graphs and statistics) were generated by probit regression analysis using R (v4.2.3) and the package BioRssay (v1.1.0)⁶⁸.

DNA extraction, genome assembly and annotation

About 12,000 adult mites were flash frozen in dry ice and stored at -80°C until DNA extraction. Frozen mites were crushed in a mortar filled with liquid nitrogen and the frozen powder was moved into 2 mL tubes. Homogenization was performed in each tube with 950 μL of SDS buffer (200 mM Tris-HCl, 400 mM NaCl, 10 mM EDTA, 2% SDS at pH 8.2), 18 μL Proteinase K (10 mg mL⁻¹) and 3.6 μL of RNase A (40mg mL⁻¹). Samples were incubated at 60°C with constant rotation for 1h, followed by a further incubation step of 1h at 37°C after adding additional 3.6 μL of RNase A. Subsequently, a standard phenol-chloroform extraction was executed⁶⁹. DNA concentration and quality were assessed on the Qubit Fluorometer with the Qubit dsDNA HS Assay kit (ThermoFisher) and the 4200 Tapestation System with the Genomic DNA ScreenTape assay (Agilent), respectively. Sequencing libraries were prepared following the manufacturer's recommendations for Oxford Nanopore Technologies (ONT), and run on an R10.4 flow cell (FLO-MIN114). Raw reads were first trimmed with Seqtk (v1.3-r106) to remove 50 bp from both read ends, and reads containing adapter sequences were filtered out using Seqkit (v2.0.0). To improve assembly contiguity, only reads with a minimum length of 10 kb were retained for downstream processing.

A *de novo* genome assembly was generated with Flye (v2.9.3-b1797), using an expected genome size of 200 million bp. Scaffolding was performed using Flye's built-in scaffolding functionality and was completed without the use of any additional external data sources (such as optical maps or Hi-C). The draft assembly was subsequently polished with Medaka (v2.0.1), by aligning the ONT reads back to the assembly and refining base calls to improve accuracy. Assembly completeness was evaluated with BUSCO (v5.4.6) in genome mode, using the *arachnida_odb10* database.

Genome annotation was performed using the Funannotate (v1.8.17) pipeline. Repeat elements were masked prior to gene prediction using RepeatModeler (v2.0.6) and RepeatMasker (v4.1.9). The annotation pipeline integrated multiple sources of evidence including RNA-seq data, transcript assemblies, and protein databases. Protein sequences from *G. occidentalis* (NCBI BioProject PRJNA62309) were aligned with Miniprot (v0.12-r237), and the resulting GFF3 was incorporated as additional protein evidence into Funannotate with a weight of 10. The functional annotation of predicted genes was carried out using integrated databases and tools within Funannotate, including InterProScan, PFAM, eggNOG and SignalP, to assign putative functions to gene predictions. Gene models of *VGSC* and *nAChR- $\alpha 6/\alpha 10$* were manually curated based on Miniprot/BLAST alignments of *G. occidentalis* protein sequences to the *A. andersoni* genome. CDS coordinates from the alignments were used to replace fragmented or overlapping predictions, while preserving gene and transcript identifiers from the original Funannotate annotation. nAChR subunits nomenclature follows the one proposed for phytoseiids by Mocchetti et al.²².

P450 genes were manually curated by searching each predicted sequence (identified by the InterPro domain IPR001128) using TBLASTN against the genome and by BLASTP against a curated P450 database (Arthropod P450 enciridion - arthropodp450.eu⁷⁰) to confirm gene structure. Length discrepancies or exon-structure issues were corrected when supported by the sequence evidence, and adjacent fragments were merged when they clearly represented a single gene. To detect exons or gene copies that might have been missed by the annotation, large genomic regions around problematic loci were searched using BLASTX against the P450 database. Fragments that could not be reliably reconstructed (e.g. loose exons) were excluded. The resulting curated set was used for subsequent analyses.

RNA extraction, sequencing, alignment and differential expression analysis

Total RNA was extracted from five replicates of each strain (C1, C2, FS-MAR, FS-DAL), each consisting of 100-150 adult females, using the RNeasy Plus Mini Kit (Qiagen, Germany) following the manufacturer's protocol. Quantity and quality of the extracted RNA was assessed using a DeNovix DS-11 spectrophotometer (DeNovix, USA) and by visual inspection of gel electrophoresis (1% agarose gel; 30 min; 100 V). Library preparation was performed by Novogene (Germany) and subsequently sequenced on the Illumina NovaSeqX platform to generate 150 bp paired-end reads. Reads were aligned to the *de novo* genome assembly using STAR (v2.7.9a)⁷¹

in two-pass alignment mode, with a maximum intron size of 30 kb, and using the GFF annotation from the annotation pipeline. The resulting BAM files, sorted by chromosomal coordinates, were subjected to read counting with HTSeq (v2.0.2)⁷², using the same annotation. Prior to differential expression analysis with DESeq2⁷³ (v1.40.2), genes with low expression were filtered out to improve statistical power. Specifically, genes were retained if they had an expression level greater than 1 fragment per million (FPM) in at least five samples, corresponding to the number of biological replicates per strain. Differentially expressed genes (DEGs) for each pairwise contrast were identified based on an absolute log₂ fold change > 2 and a Benjamini-Hochberg (BH) adjusted p-value < 0.05.

For assessment of potential alternative splicing, reads from all samples within each strain were pooled across the *nAChR-α6* gene region and visualized using *rmats2sashimiplo*.

GO enrichment

GO enrichment analysis was performed with the *enricher* function of the package *clusterProfiler* in R (v4.8.3), using a BH-adjusted p-value cut-off of 0.05. The background gene set consisted of the genes including in the DE analysis, thus excluding low-count genes. GO terms for Molecular Function (MF), Biological Process (BP), and Cellular Component (CC) were obtained from the output of the genome annotation pipeline.

QTL mapping using Bulk Segregant Analysis (BSA)

The initial cross of the BSA experiment was performed with C2_i and C1. Ten virgin females from the susceptible strain were crossed with three males from the resistant inbred strain on a bean leaf arena. To prevent cannibalism and contamination, the parental lines were transferred to fresh bean leaves every two days for three consecutive transfers. The resulting population was further expanded for eight generations on bean leaves supplemented twice weekly with fresh pollen and spider mites as food sources. After that, a total of 385 mites were transferred to rearing bags (Euronda Eurosteril rolls), containing 150 mL of wheat bran and *C. lactis* (provided by Biobest Group, Belgium), as food source, for two additional generations in incubators at 25 ± 1°C, 85 ± 10% RH and 16L:8D h photoperiod. This enabled exponential expansion of the segregating population, producing a larger bulk for downstream analysis.

After the 10th generation, two separate selection experiments were initiated using mites from the unselected population. In each experiment, mites were transferred onto bean leaf arenas and sprayed with a discriminating dose of either 15 mg L⁻¹ of deltamethrin or 3200 mg L⁻¹ of spinosad. These concentrations were determined based on dose-response curves and were lethal to all C1 individuals while not affecting C2. Twenty mites from each parental line were also sprayed and served as controls. Once residues dried, pollen was provided as food. After 24h (deltamethrin) or 72h (spinosad), mites were collected and pooled by phenotype (alive or dead). In the deltamethrin experiment, 1280 alive mites were recovered and divided into three

replicates, with equal numbers collected for the unselected and dead pools. In the spinosad experiment, 700 mites were pooled in the same way, but only one replicate per pool was generated.

Genomic DNA (gDNA) was extracted from the pools and the parental lines (500 mites each) by directly homogenizing them in SDS buffer with the addition of proteinase K and RNase A followed by chloroform-phenol purification as described above. DNA quantity was measured using a Denovix DS-11 spectrophotometer (DeNovix, Wilmington, DE, USA), and quality was assessed by gel electrophoresis (1% agarose gel; 30 min; 100 V). Samples were sent to Novogene (Cambridge, UK) for PCR-free library preparation, followed by sequencing on an Illumina NovaSeqX platform, which yielded 150 bp paired-end reads. All DNA reads were aligned to the *de novo* genome using BWA (0.7.17-r1188)⁷⁴ with default settings. The resulting bam files were sorted by coordinates using SAMtools (v1.6)⁷⁵, and duplicates were marked with Picard (v2.25.0) (<https://broadinstitute.github.io/picard>). Genetic variants were jointly called across all samples following GATK's (v4.2.0.0) best practices workflow⁷⁶, resulting in a final VCF file used for downstream analyses. To predict the effects of the detected variants, a custom SNPeff⁷⁷ (v5.3a) database was built based on the newly assembled reference genome and its corresponding annotation. SNPeff was then used to annotate each SNP and indel with its predicted impact on coding and non-coding regions.

BSA analysis was performed using the "RUN_BSA1.02.py" script (available at <https://github.com/rmclarklab/BSA>) with a sliding window size of 250 kb and a step size of 25 kb applied to the joint-called VCF. Prior to analysis, variants were filtered to remove low-quality calls (QD < 2.0, SOR > 3, MQ < 50.0, MQRankSum < -8, ReadPosRankSum < -8.0), and additional coverage-based filters were applied to exclude potential copy number variable regions. The script calculates allele-frequency differences between bulks across the genome and, when replicate bulks are available, estimates statistical significance through a permutation-based procedure that shifts allele-frequency profiles to distinguish signals of selection from drift. For the spinosad experiment, no replicates were available, and permutation testing was therefore omitted. For the deltamethrin experiment, 10,000 permutations were performed to determine a genome-wide significance threshold (paired-data mode) corresponding to an FDR of 0.05.

Molecular screening of target site mutations and RT-PCR to validate intron retention

Predatory mite samples were collected from the strains described above and used for molecular screening of target-site mutations in the VGSC. Genomic DNA was extracted from 100 mites using the DNeasy Blood & Tissue Kit (Qiagen). PCRs were performed using the VGSC_fwd (5'- TCATTGTTGCACTGTCGTTACT-3') and VGSC_rvs (5'- TGTCCGTATAGTTCTTGCCGA-3') primers, using 2 µl of DNA extract as template. An initial denaturation at 95°C for 3 min was followed by 38 cycles of 94°C for 1 min, annealing at 55°C for 30 s and 1 minute at 72°C, with a final elongation at 72°C for 10 minutes. PCR products were analyzed by electrophoresis

on a 2% agarose gel for 30 minutes at 100V. PCR amplicons were Sanger sequenced at LGC genomics (Berlin, Germany). Chromatograms were visually inspected to identify segregating mutations.

To validate intron retention, RNA from C1, C2 and C2_i was extracted from 100 mites using the RNeasy mini kit as described above. Next, 2 µg of RNA were used for first strand cDNA synthesis with the Maxima First Strand cDNA synthesis kit for RT-PCR (Thermo Fisher Scientific). The region spanning intron 10 was amplified using two primer pairs. Fwd_1 (5'- CAACCTCGGCTTCATCTTCG-3') and Fwd_2 (5'- TGGTACTTCGTTCCGTGGAA-3') were designed on exon 10, while Rvs_1 (5'- CGTCGAAATTGCGGTAAATGC-3') and Rvs_2 (5'- CGAAAAGAGGCACGCGATC-3') were designed on exon 11. PCRs were performed as described above with 2 minutes 20s as elongation time, to allow amplification of the complete intron sequence when fully retained. Sanger sequencing was performed as described above.

Linking phenotype to genotype by F₂ screening

Virgin F₀ females from either C1 or FS-DAL strains were paired with males from the other population and allowed to mate for 3 days (37 FS-DAL females x 15 C1 males; 16 females C1 x 8 FS-DAL males). In the resulting F₁ population, siblings were allowed to mate for 3 days and were subsequently transferred to fresh leaves. F₂ adult females were collected and exposed to 15 mg L⁻¹ deltamethrin. After 24 hours, both surviving and dead females were collected for DNA extraction. Individual F₂ females were homogenized in 20 µl STE buffer (100 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 8) supplemented with 3 µl proteinase K (10 mg mL⁻¹). Extracts were incubated at 56 °C for 1h, followed by heating at 99 °C for 10 min. Genotyping was performed with PCR and Sanger sequencing as described above. Pairwise Fisher's exact tests were used to compare survival frequencies among F₂ genotype groups.

Statistics and reproducibility

Statistical analyses, including sample sizes and replicate numbers, are described in the corresponding Methods sections. Concentration-response curves were obtained using five replicates per concentration, each consisting of eight mites. LC₅₀-values, slopes, 95% CIs and RRs were calculated by probit analysis using R (v4.2.3) and the package BioRssay (v1.1.0)⁶⁸. For differential expression analysis, total RNA was extracted from five replicates of each strain (C1, C2, FS-MAR, FS-DAL), each consisting of 100-150 adult females. In deltamethrin BSA, 1280 alive mites were collected after selection and divided into three replicates. An equal number of mites was collected for the unselected and dead pools. In the spinosad BSA experiment, 700 mites were pooled in the same way, but only one replicate per pool was generated. In F₂ screening pairwise Fisher's exact tests were used to compare survival frequencies among F₂ genotype groups. A total of 112 mites were phenotyped.

Data availability

Raw ONT sequencing data have been deposited in NCBI under BioProject accession number PRJNA1387451, together with the associated genome assembly and annotation. The genome assembly and annotation are also available on Figshare (DOI: 10.6084/m9.figshare.32149063). WGS data used for BSA and RNA-seq data used for differential expression analysis are available in NCBI under BioProject accession numbers PRJNA1390629 and PRJNA1390710, respectively. In addition, raw RNA-seq count data and WGS-derived genotype data (VCF format) underpinning the BSA have been deposited on Figshare (DOI: 10.6084/m9.figshare.32149063). Any remaining information can be obtained from the corresponding author upon reasonable request.

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Author contributions

G.S.: Data curation, Formal analysis, Validation, Investigation, Visualization, Methodology, Writing – original draft; A.M.: Data curation, Formal analysis, Validation, Investigation, Visualization, Methodology, Writing – original draft; F.D.G.: Data curation, Formal analysis, Validation, Investigation, Visualization, Methodology, Writing – original draft; M.V.: Investigation; L.R.: Investigation; D.V.: Resources, Methodology; C.D.: Supervision, Funding acquisition; A.P.: Resources, Supervision, Funding acquisition, Project administration; T.V.L.: Conceptualization, Supervision, Funding acquisition, Writing – review and editing, Project administration

Competing interests

The authors declare no competing interests.

Table

Table 1. Lethal concentration (LC_{50}) and resistance ratio (RR_{50}) with 95% confidence intervals (CI) for deltamethrin and spinosad in different *Amblyseius andersoni* strains. RR_{50} values are calculated relative to strain C1, which served as the susceptible reference. Probit analysis parameters are specified in Supplementary Table 2.

Strain	Deltamethrin		Spinosad	
	LC_{50} (95% CI)	RR_{50} (95% CI)	LC_{50} (95% CI)	RR_{50} (95% CI)
C1	0.043 (0.023-0.007)	1	9.55 (3.74-24.35)	1
C2	210 (181-243)	4,842 (2,334-10,189)	6,969 (5,142-9,445)	729 (273-1,953)
C3	171 (151-193)	3,949 (1,951-8,095)	3,329 (2,034-5,449)	348 (121-1,004)
FS-DAL	177 (130-240)	4,075 (1,673-10,043)	7,541 (5,141-11,061)	790 (287-2,171)
FS-MAR	388 (293-500)	8,936 (3,767-20,928)	9.57 (7.85-11.68)	1 (0.39-2.61)

Figures

Figure 1. Transcriptomic analyses across *A. andersoni* strains. **a)** Principal component analysis (PCA) of normalized gene expression levels for three resistant strains (C2, FS-DAL, FS-MAR) and one susceptible strain (C1). The percentage of total variance explained by PC1 and PC2 is indicated in parentheses. **b)** Heatmap of Z-score-scaled expression levels of differentially expressed cytochrome P450 monooxygenase genes shows upregulation of multiple P450s in resistant strains compared with the susceptible strain C1. **c)** UpSet plot showing the inclusive intersections of differentially expressed genes (DEGs) across six pairwise contrasts (C2 vs C1, FS-DAL vs C1, FS-MAR vs C1, C2 vs FS-MAR, C2 vs FS-DAL, FS-DAL vs FS-MAR). DEGs were defined by an adjusted p-value < 0.05 and $|\log_2 FC| > 2$.

Figure 2. Schematic overview of inbreeding and BSA procedures. **a)** Inbreeding process started with an initial cross with a male and female from the C2 strain, from the resulting progeny a brother and sister were selected (sib-mating) and this was repeated for 20 generations. Chromosomes and inbreeding level are indicated with rectangles with red shadings. **b)** Bulk segregant analysis (BSA) is started with a founding cross from which the resulting F_1 generation is moved on bean leaves for multiple generations feeding on natural prey and pollen. Large population increase in plastic bags with factitious prey (*Carpoglyphus lactis*) precedes selection. Selection with discriminating dose of pesticides (deltamethrin or spinosad) was performed on a subset of collected mites. Unselected mites from the rearing bag, alive mites and dead mites (not pictured) were collected in three separate pools from which gDNA was extracted. Resistance alleles are indicated with black circles. Red arrows indicate the genomic position linked to resistance by BSA. Figure partially created in BioRender. VAN LEEUWEN, T. (2026) <https://BioRender.com/tyd9px7>

Figure 3. Genomic response to acaricide selections. **a)** Genome-wide heterozygosity profiles of the parental lines used for bulk segregant analysis (BSA). Upper panel: outbred susceptible C1 strain, lower panel: inbred resistant $C2_i$ line. Heterozygosity was calculated as the proportion of heterozygous SNPs in 100-kb sliding windows with a 10-kb step size. Scaffolds/contigs are arranged from longest to shortest and indicated by alternating shading. **b)** Mean allele frequency difference between deltamethrin-selected and control populations (blue) and spinosad-selected and control populations (orange), in 250 kb sliding windows with a step size of 25 kb. The blue dashed line denotes the significance threshold for the deltamethrin BSA (FDR = 0.05). No significance threshold could be calculated for the spinosad BSA as there were no replicates. Genes within selected genomic regions around QTL1 **c)** and QTL2 **d)** are colored (legend) according to their class if deemed interesting, otherwise they are shown in grey (VGSC: Voltage-Gated Sodium Channel, nAChR: nicotinic Acetylcholine Receptor). Vertical lines represent the midpoint of the peak window (blue for deltamethrin in C, orange for spinosad in D).

Figure 4. Alternative splicing of nicotinic acetylcholine receptor $\alpha 6$ (*nAChR- $\alpha 6$*) in spinosad-resistant and susceptible strains. Splice-junction support based on pooled RNA-seq reads is shown for the susceptible C1 strain, the resistant C2 strain, and the field strains FS-DAL and FS-MAR. The red arrow highlights retention of intron 10 in C2, a pattern not observed in the other strains. Numbers above the arcs indicate read counts supporting each junction. The gene model shown below the coverage and junction tracks depicts the annotated exon-intron structure of *nAChR- $\alpha 6$* . Note that the first exons display coverage but no junction arcs because the genomic distance between these exons exceeds the maximum intron length set for alignment.

Figure 5. Schematic overview of F₂ linkage analysis and results of phenotyping. **a)** Reciprocal crosses were executed between C1 and FS-DAL. Chromosomes are indicated with white and black rectangles. Sib-mating of F₁ individuals allowed segregation of resistance mutations in F₂ offspring. F₂ individuals were screened for deltamethrin resistance by applying a discriminating dose of insecticide. **b)** Genotype-to-Phenotype linkage of screened individuals is represented in the table. Green color indicates homozygosity for the susceptible allele, blue color indicates heterozygosity and red color indicates homozygosity for the resistant allele. Genotypes observed in surviving mites are highlighted in yellow. Partially created in BioRender. VAN LEEUWEN, T. (2026) <https://BioRender.com/tyd9px7>

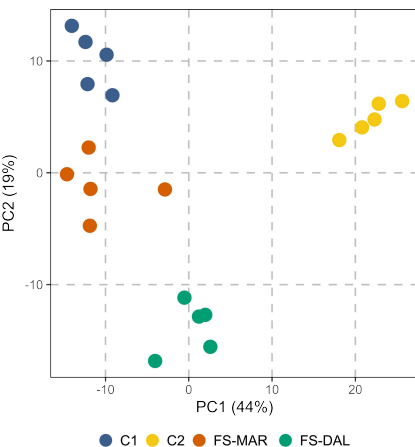
Editor's Summary

A complete genome sequence of the phytoseiid mite *Amblyseius andersoni* enables genetic mapping of causal variants underlying resistance to widely used insecticides, paving the way for future studies on adaptive traits in predatory mites.

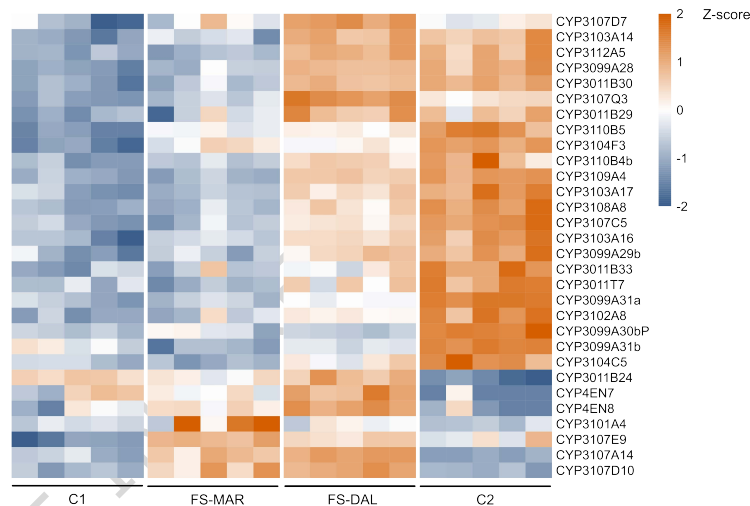
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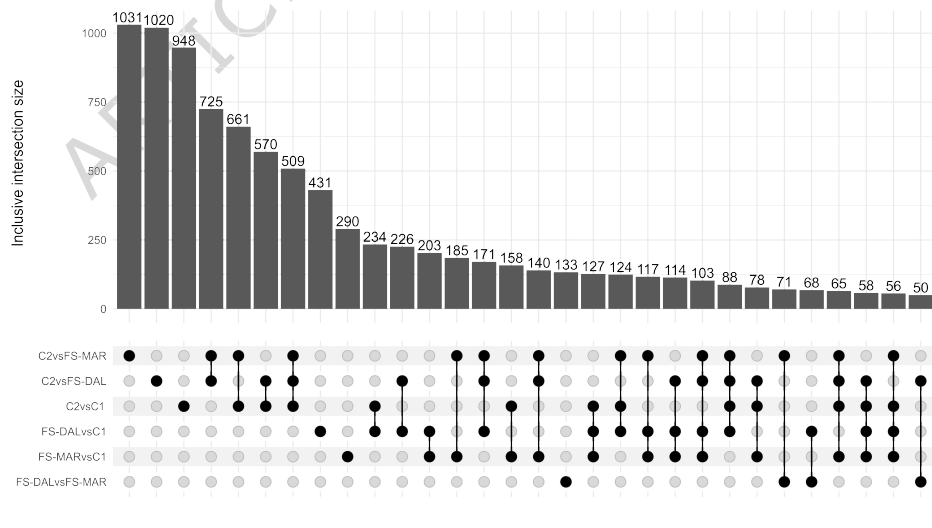
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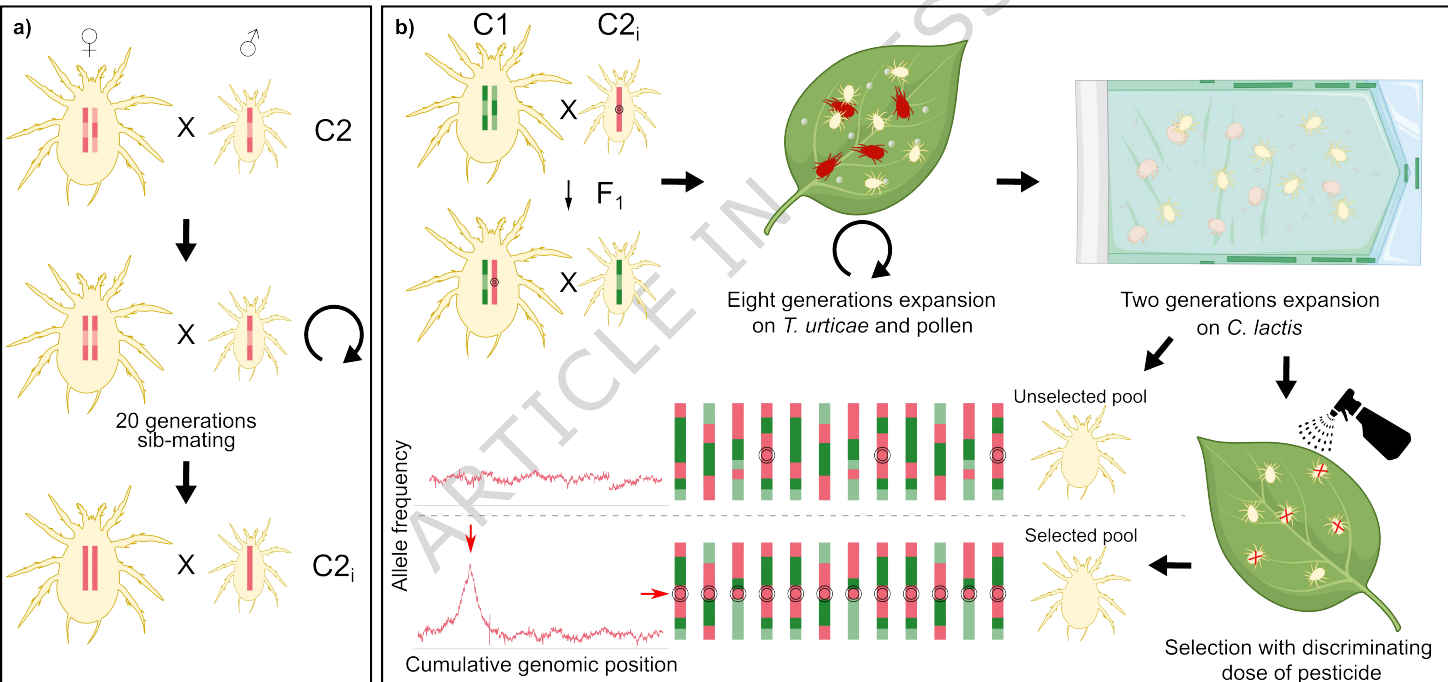


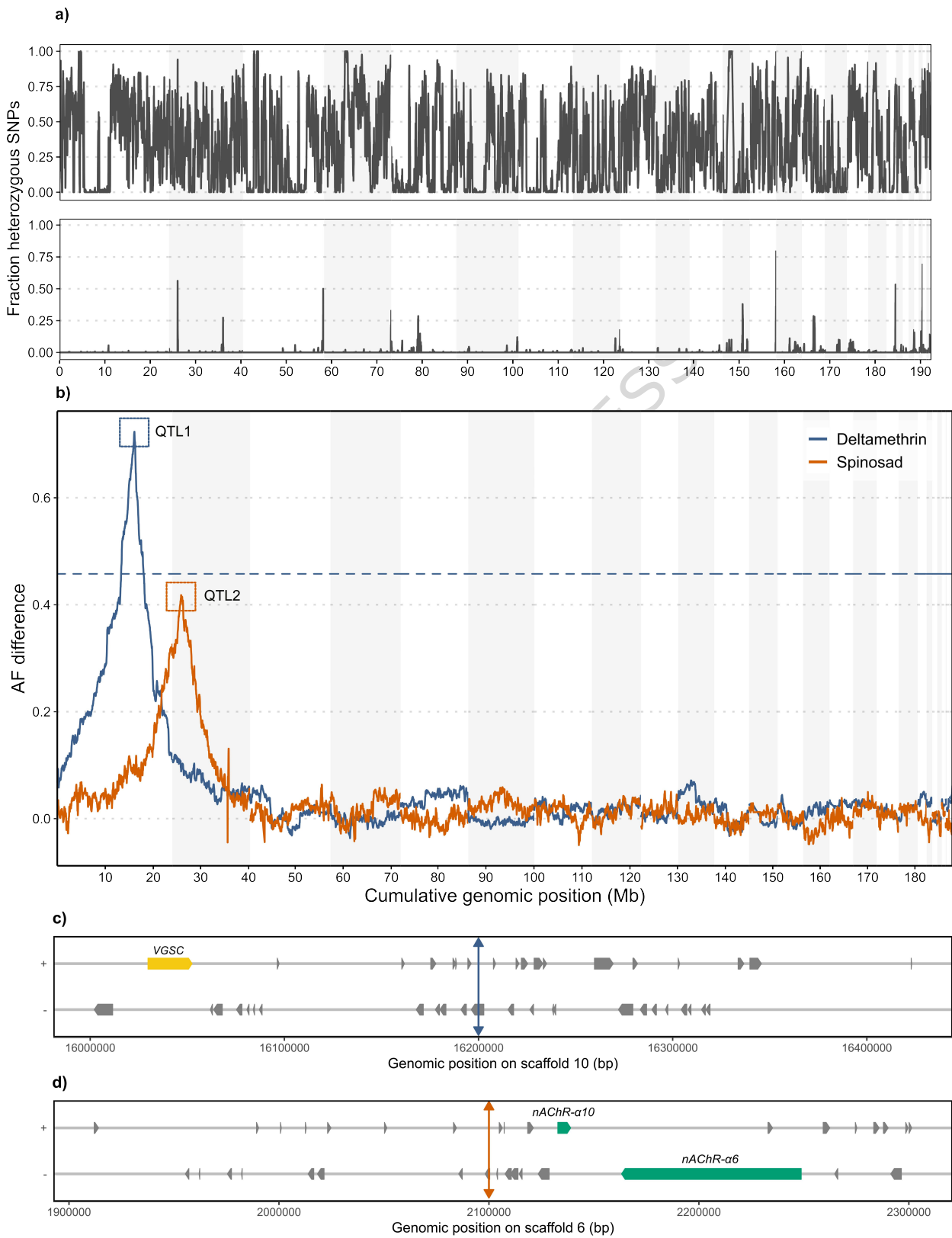
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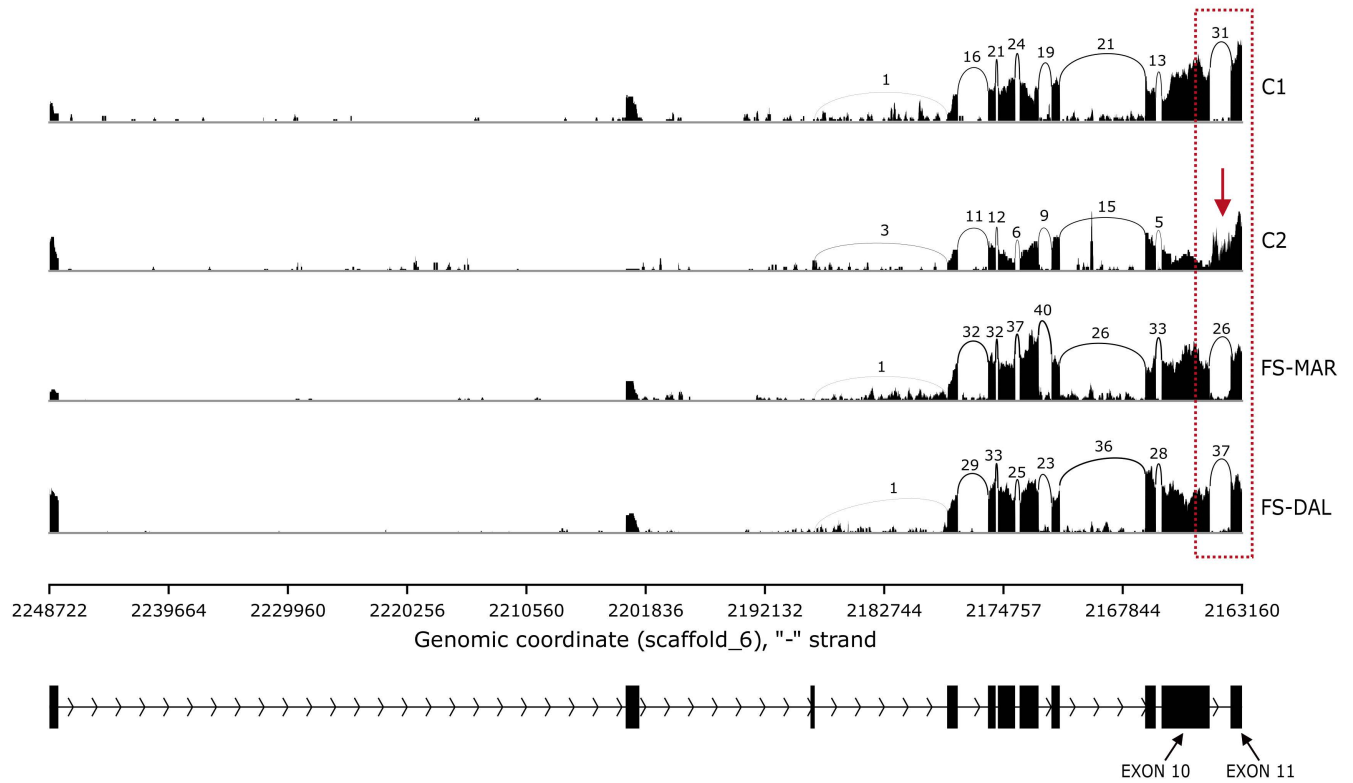


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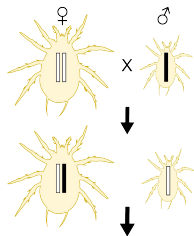
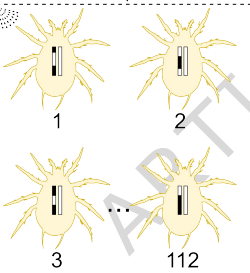








a)

F₁F₂

b)

Genotype		Phenotype		
M918L	L925V	Alive	Dead	Total
MM	LL	0	24	24
MM	LV	0	18	18
MM	VV	0	4	4
ML	LL	0	11	11
ML	LV	0	25	25
ML	VV	0	3	3
LL	LL	12	0	12
LL	LV	2	0	2
LL	VV	13	0	13
Total		27	85	112

