



OPEN Genomic structure and life history variation of isofemale lineages of *Myzus persicae* with different levels of parasitization by *Diaeretiella rapae*

Mariane Possignolo Gomes¹, Ewerton Costa Lira¹, Idemauro Antonio Rodrigues de Lara², Rafael de Andrade Moral³, Rita Rebollo⁴, Tobias Baril⁵ & Fernando Luis Cônsoli^{1✉}

Host resistance evolution to natural enemies depends on genetic variation, symbiont associations, and trade-offs with host's fitness traits. We selected isofemale lineages of *Myzus persicae* to investigate biological traits and molecular markers associated with aphid defensive responses to the parasitic wasp *Diaeretiella rapae*. The parasitization of *M. persicae* lineages by *D. rapae* ranged from 43 to 76%. *Rickettsia* was the predominant secondary symbiont. Six lineages were selected according to *D. rapae* parasitization rate (LP = low parasitization; HP = high parasitization) and *Rickettsia* association. Life history traits differed among *M. persicae* lineages. However, the association of adaptive costs with reduced susceptibility to *D. rapae* parasitization was not consistently observed. *Rickettsia* infection did not explain the enhanced aphid defense, but it did increase aphid fecundity. Comparative small structural variants analyses of *M. persicae* lineages indicated that approximately 6% of SNPs and 49% of InDels were unique to each aphid lineage or shared among LP or HP lineages. Only 1.7% of SNPs and 0.91% of InDels influenced genes involved with aphid immune response. Further exploration of non-coding variants and additional omics-related approaches are needed to fully characterize the mechanisms behind the differential parasitization success of *M. persicae* lineages by *D. rapae*.

Keywords Biological control, Defensive symbiont, Defensive traits, Genomic small structure variants, Host-parasitoid interactions

Parasitoids use completely different life strategies when compared to true parasites as they kill their hosts upon completion of their development, putting a much stronger selection pressure for the evolution of defensive traits on their hosts^{1–5}. The successful exploitation of a wide range of hosts by parasitoids is partially due to the diverse strategies parasitoids evolved for host exploitation⁶. But host defensive strategies also lead to parasitoid avoidance and parasitization escape^{7–9}. Host populations that are maintained under strong selective pressure from parasitoids can face a bottleneck in their genetic composition due to the elimination of susceptible genotypes that are successfully parasitized from the total genetic pool available in the population,^{3,10–13} including those represented by genotypes associated with microbial symbionts^{14,15}. Host-parasitoid population densities are known to fluctuate cyclically, keeping both populations in equilibrium and rarely leading to extinction events^{16–18}. Thus, the survival of host genotypes that escape parasitization may drive selection for hosts that are resistant to parasitization^{19–21}.

Diaeretiella rapae (MacIntosh) (Hymenoptera: Braconidae), a parasitoid of more than 60 species of aphids²², including *Myzus persicae* (Sulzer) (Homoptera: Aphididae) is an example of a parasitoid exerting strong selective pressure on its hosts. *Myzus persicae* is a polyphagous pest of great economic importance, with excellent

¹Department of Entomology and Acarology, Insect Interactions Laboratory, Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba 13418-900, Brazil. ²Department of Exact Sciences, Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba 13418-900, Brazil. ³Department of Mathematics and Statistics, Maynooth University, Co. Kildare W23F2H6, Ireland. ⁴UMR203, Institut National des Sciences Appliquées de Lyon, Biologie Fonctionnelle, Insectes et Interactions, Institut National de la Recherche Agronomique, 69621 Lyon, France. ⁵Laboratory of Evolutionary Genetics, Institute of Biology, University of Neuchâtel, Neuchâtel, 2000 Neuchâtel, Switzerland. ✉email: fconsoli@usp.br

developmental capabilities on hundreds of plant species in more than 50 families²³. Its economic importance is based on the ability to cause direct damage to plants in conjunction with its feeding habits, which result in host plant dehydration, loss of shoots and retardation of vegetative growth²⁴. In addition, aphids can cause indirect damage by transmitting viruses and promoting black mold growth by excreting honeydew²⁴. Aphid infestations of agricultural crops often require the implementation of pest control measures, and a set of new management strategies has been addressed to sum up to biocontrol methods to reduce the often-used synthetic chemical insecticides and their undesired side-effects²⁵.

D. rapae is a key parasitoid in the biological control of aphids, including *M. persicae*, and exerts a strong selective pressure on aphid populations, driving the evolution of host defense mechanisms and attack strategies. Previous studies on *M. persicae*—*D. rapae* interactions have mainly focused on verifying the influence of the host plant on aphid suitability²⁶ and on parasitoid attraction²⁷, the effects of the secondary symbiont *Rickettsiella viridis* in the rate of parasitization²⁸, and the side-effects of insecticides in this host-parasitoid interaction^{29,30}. There have been also studies dedicated to compare *D. rapae* efficacy on aphid host species with different host plant ranges³¹. However, the genetic basis of the defensive mechanisms, the adaptive costs of resistance to parasitization and the role of secondary symbionts as defensive symbionts in this host-parasitoid interaction are seldom reported.

These gaps in knowledge highlight the complexity of the evolutionary arms race between aphids and their parasitoids. Co-evolution between hosts and parasitoids arises because of interactions throughout their evolutionary history, as hosts face selection pressure due to exposure to parasitoid-derived virulence factors whilst, at the same time, parasitoids face selection pressure from host defenses. As predicted by the red queen theory, each adaptation developed by one species faces a counteradaptation of the interacting species, such that the survival of both species depends on the continuous development of defense and attack strategies (arms race)^{11,32–35}. Microorganisms can modulate their host phenotype^{36,37}, and secondary symbionts have shown to modify their host response to parasitoid attack, with possible implications in the evolution of parasitoid virulence³⁸. Defensive symbionts can affect host successful parasitization by resource limitation, such as lipids³⁹, and by the production and release of metabolites toxic to natural enemies^{40,41}.

Despite advances in the understanding of defense mechanisms against natural enemies, the physiological and behavioral strategies of hosts and their interactions with secondary symbionts are still poorly understood. *Hamiltonella defensa* and its associated bacteriophage are among the best-known defensive secondary symbionts. Phage-infected *Hamiltonella defensa* can produce toxins such as Shiga-like, CdtB and YD-repeat, which increase immature parasitoid mortality and strengthen host resistance^{42–44}. *Serratia symbiotica* is another relevant secondary symbiont that affects successful parasitization by interfering with the production of plant volatiles involved in parasitoid attraction⁴⁵. In aphids, other secondary symbionts (e.g., *Rickettsia*, *Spiroplasma*, *Wolbachia*) are also present in natural environments, but their role as defensive symbionts against natural enemies is still poorly understood, and an increase in the knowledge of parasitoid-host-symbiont interactions can open new perspectives for the development of more efficient biological control programs⁴⁶.

Host-parasitoid interactions are frequency-dependent, which can be observed more strongly in laboratory than in field studies⁴⁷. The frequency-dependent interaction combined with genetic variation allows interacting species to co-evolve². Parasitoids are selected to evade defense mechanisms of common host genotypes, contributing to the selection of hosts with rare resistant genotypes⁴⁸. This process is difficult to observe in field populations since the detection of resistant individuals depends on the host encounter and on the genotype of the natural enemy. Host genotypes with a lower ability to evade natural enemies' encounters are more frequently attacked. However, parasitoid genotypes are subject to similar selection pressures, resulting in a selection process that occurs without the fixation of extreme host or parasitoid genotypes^{48,49}. In laboratory experiments, genotypes with greater and/or lesser ability to avoid parasitoid attack face similar rates of parasitoid encounter under controlled abiotic and biotic conditions and similar patch structures, allowing the study of host/parasitoid genotypes with selected responses⁴⁷.

The genetic variability that results in different host responses to parasitoid attack and parasitoid phenotypes with different levels of success in host parasitization can arise from mutation, increased gene flow, genetic drift, inbreeding depression, and selection^{50,51}. The main and fastest way to generate genetic variability is through sexual reproduction, which allows for the perpetuation of mutations in the population, as well as genetic recombination between chromosomes^{52,53}. Aphids developing in temperate regions exhibit seasonal reproductive polyphenism, alternating between asexual and sexual reproduction to produce eggs that will diapause in winter^{54–56}. However, under tropical conditions, aphid species such as *Myzus persicae* reproduce exclusively by ameiotic (apomictic) parthenogenesis, in which chromosome division occurs by mitosis. In apomixis reproduction, the resulting offspring are genetically identical to their mothers, and the sources of genetic variation to promote phenotypic expression are now limited to rare genomic events, such as mutations, chromosomal rearrangements and mitotic recombination, and/or interaction with endosymbionts^{57–59}.

Chromosomal rearrangements and interactions with symbionts have been shown to serve as sources of genetic variation leading to the manifestation of insecticide resistant, parasitoid defense and host plant adapted phenotypes^{14,60–63}. Thus, both chromosomal and extra-chromosomal variation can influence the evolution of host defense mechanisms against natural enemies, which may invariably have associated adaptive costs^{64–67}. Chromosomal rearrangements often result from the emergence of structural variants that can arise through various cellular mechanisms, including DNA replication and repair⁶⁸. These variants can be broadly categorized into five classes: deletions, duplications, insertions, inversions, and translocations⁶⁹. Structural variants introduce variability in gene copy number, position, orientation, and occasionally a combination of these effects⁷⁰. As a result, structural variants drive genotypic change and facilitate the manifestation of diverse phenotypes.

In this context, we investigated the interaction between *M. persicae* and its parasitoid *D. rapae*, considering genetic and symbiotic factors that may influence the host response to parasitization. We sought to answer

the following questions: (1) is there variation between isofemale lineages of *M. persicae* in their resistance to parasitism by *D. rapae*?; (2) does the presence of secondary symbionts influence the success of parasitism?; (3) are there biological differences between isofemale strains of *M. persicae* associated with the presence of secondary symbionts and their resistance to parasitism?; and (4) do small structural variants (SVs) in the *M. persicae* genome play a role in host adaptation and response to parasitism? Our central hypothesis is that the differential resilience to parasitism by *D. rapae* among *M. persicae* lineages is associated both with the presence of secondary symbionts and with structural variations in the genome that may modulate the host response to parasitoid attack.

Results

Secondary symbionts associated with *Myzus persicae* isofemale lineages

We evaluated the presence of secondary symbionts commonly associated with aphids in isolated infections and co-infections in 14 isofemale lineages of *M. persicae*. Diagnostic PCR analysis based on the *16S rRNA* gene of the most common symbiotic bacteria harbored by aphids detected secondary symbionts associated with 11 (nearly 79%) out of the 14 *M. persicae* isofemale lineages subjected to parasitization by *D. rapae*. *Spiroplasma* (29%) and *Rickettsia* (71%) were the only secondary symbionts detected, occurring either in single- [*Spiroplasma*—9% (one lineage); *Rickettsia*—64% (seven lineages)] or double-infected aphids (27%—three lineages) (Fig. 1A).

Response of isofemale *Myzus persicae* lineages to parasitization by *Diaeretiella rapae*

We tested the response of 14 isofemale lineages of *M. persicae* to parasitization by *D. rapae*. The observed success of parasitization of *M. persicae* isofemale lineages by *D. rapae* varied between 43 and 76%, with five lineages with successful parasitization values within the fourth quartile (above 75% percentile) and another five within the first quartile (below 25% percentile) (Fig. 1B).

Rickettsia infections (*r*+) were detected in isofemale lineages with parasitization values within both quartiles (first quartile: Iso13 and Iso14; fourth quartile: Iso2 and Iso3), whereas *Spiroplasma* infections (*s*+) were detected in an isofemale lineage with a parasitism value within the first quartile (Iso12), and co-infections were detected in isofemale lineages with a parasitism rate only in the fourth quartile (Iso1 and Iso5).

Based on the scenario of infection by secondary symbionts in the two quartiles representing the lowest (first quartile) and highest (fourth quartile) values of successful parasitization, three isofemale lineages from the first [low parasitization (LP) group—Iso10^{LP/r-}, Iso11^{LP/r-} and Iso14^{LP/r+}] and fourth [high parasitization (HP) group—Iso2^{HP/r+}, Iso3^{HP/r+} and Iso4^{HP/r-}] quartiles were selected for further comparative analysis of life history traits and fertility life table determination to verify the association of fitness costs with the defensive response of *M. persicae* to parasitization by *D. rapae* (Fig. 1B).

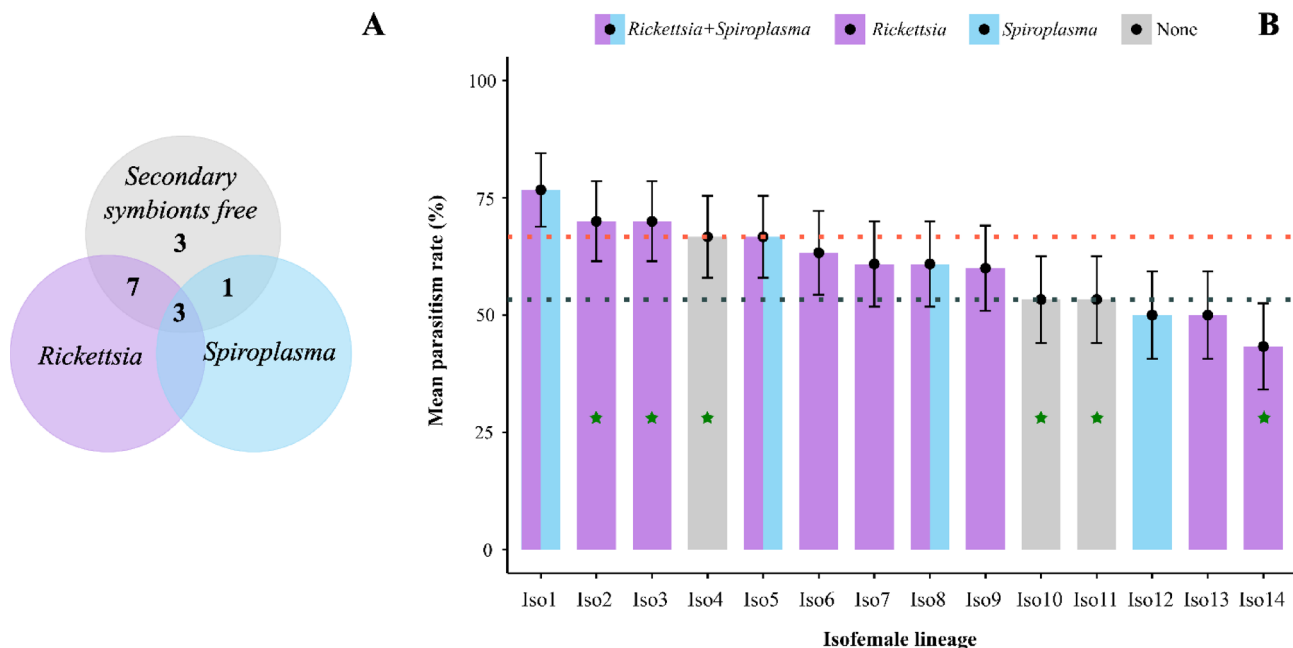


Fig. 1. Venn diagram showing the number of isofemale lineages of *Myzus persicae* with isolated infection or co-infection by the secondary symbionts *Rickettsia* and *Spiroplasma* (A) and mean parasitization (%) ($\pm$) of isofemale lineages of *Myzus persicae* by *Diaeretiella rapae* (Red dotted line = 75% percentile; black dotted line = 25% percentile) (Green stars indicate lineages selected for biological parameters and molecular markers analysis) (B).

The consequences of the differential response to parasitization by *Diaeretiella rapae* and the association with *Rickettsia* on life history traits in isofemale lineages of *Myzus persicae*

We investigated whether isofemale lineages of *M. persicae* selected for high and low parasitization by *D. rapae*, as well as infection or not by the secondary symbiont *Rickettsia* impose adaptive costs by evaluating their life history traits and fecundity table. The survival of nymphal stage differed among the selected lineages of *M. persicae* ($LR = 15.94$, $df = 5$, $p = 0.007$). The lowest probability of reaching adulthood was observed for lineage Iso10^{LP/r+} (probability of reaching adulthood = 0.776) and the highest probability of reaching adulthood was identified for lineage Iso11^{LP/r+} (probability of reaching adulthood = 0.92) (Fig. 2A, Table 1). We also detected differences in the developmental time required for nymphs to become adults ($\chi^2 = 63.10$, $df = 5$, $p < 0.001$). The development time of Iso14^{LP/r+} was the longest observed among the lineages (7.4 ± 3.5 days), differing from all other lineages except Iso4^{HP/r+}. The development time of Iso2^{HP/r+} and Iso11^{LP/r+} was intermediate and differed from the development time of Iso14^{LP/r+} and Iso4^{HP/r+}. Iso3^{HP/r+} and Iso10^{LP/r+} were among those with the shortest developmental time (5.7 ± 2.5 and 5.8 ± 3.3 days, respectively), differing from that observed for Iso4^{HP/r+} and Iso14^{LP/r+} (Fig. 2B, Table 1).

The tibia size of females, parameter used to determine the relative size of the adult aphid, also differed among the selected lineages ($F_{5,112} = 3.62$; $p = 0.0044$). The tibia of adult females from Iso2^{HP/r+} were larger than those from Iso4^{HP/r+} and Iso14^{LP/r+} which had the smallest size of the tibia. The tibia size in Iso2^{HP/r+}, Iso4^{HP/r+} and Iso14^{LP/r+} did not differ from the other lineages (Table 2). No differences in adult longevity were observed ($F_{5,174} = 0.6167$; $p = 0.6872$), while differences in female fecundity were detected only between Iso10^{LP/r+} and

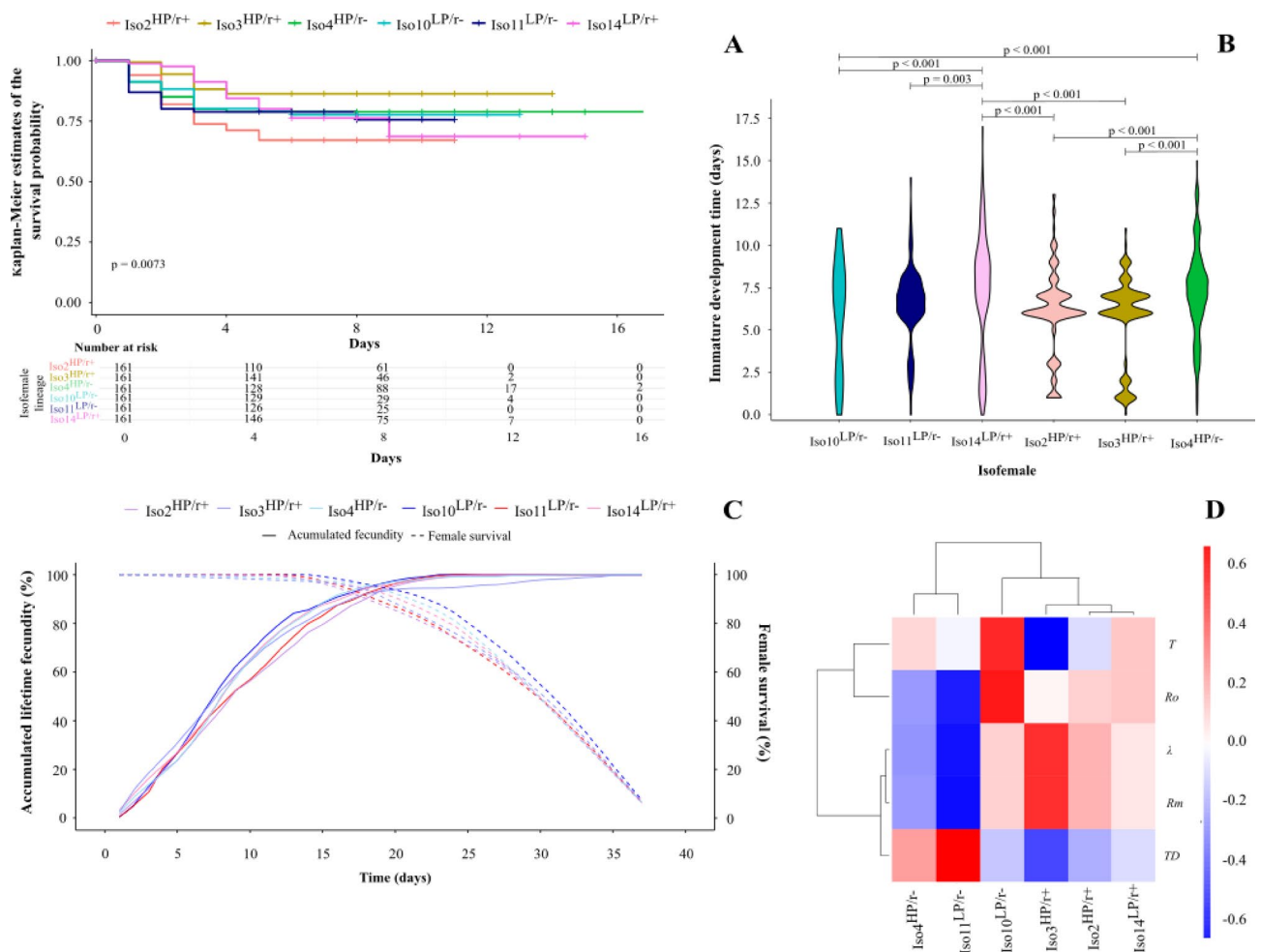


Fig. 2. Biological parameters evaluated for selected lineages of *Myzus persicae* with high (Iso2^{HP/r+}, Iso3^{HP/r+}, Iso4^{HP/r+}) and low (Iso10^{LP/r+}, Iso11^{LP/r+}, Iso14^{LP/r+}) parasitization by *Diaeretiella rapae*. **(A)** Kaplan–Meier estimates of survival curves using Cox proportional hazards models, $p < 0.05$. **(B)** Immature development time (days $\pm$ se). Treatments followed by different letters are statistically different ($p < 0.05$). Green bars = indicate the lineages of the group with lower parasitism. Blue bars = represent the isolines with higher parasitism. **(C)** Daily female survival (%) and relative accumulated observed fecundity (%). **(D)** Heatmap of reproductive life table data and associated dendrograms obtained from hierarchical clustering based on Euclidean distance using Ward's method. The color scale indicates the proximity of the mean of each isolate to the total mean of each measured parameter (white = low proximity; red = high proximity).

Isoline	Survival (%)	Development time (days)
Iso2 ^{HP/r+}	83.2 ± 6.1 ^{ab}	6.0 ± 2.5 ^a
Iso3 ^{HP/r+}	80.3 ± 4.7 ^{ab}	5.7 ± 2.5 ^a
Iso4 ^{HP/r-}	85.3 ± 11.2 ^{ab}	7.2 ± 2.6 ^{bc}
Iso10 ^{LP/r-}	77.6 ± 10.6 ^a	5.8 ± 3.3 ^a
Iso11 ^{LP/r-}	92.0 ± 6.0 ^b	6.5 ± 2.1 ^{ac}
Iso14 ^{LP/r+}	83.7 ± 5.7 ^{ab}	7.4 ± 3.5 ^b

Table 1. Mean survival (%) and mean developmental time (days) of nymphs from selected isofemale lineages of *Myzus persicae* with high (Iso2^{HP/r+}, Iso3^{HP/r+}, Iso4^{HP/r-}) and low (Iso10^{LP/r-}, Iso11^{LP/r-}, Iso14^{LP/r+}) parasitization by *Diaeretiella rapae*. Means (± s.e.) followed by the same lowercase letter are not statistically different at a 5% significance level.

Isoline	Fecundity (nymphs/female)	Tibia size (μm)	Longevity (days)
Iso2 ^{HP/r+}	32.2 ± 4.0 ^{ab}	1187.7 ± 24.5 ^a	16.3 ± 1.2 ^a
Iso3 ^{HP/r+}	30.0 ± 3.8 ^{ab}	1095.6 ± 42.2 ^{ab}	15.1 ± 1.3 ^a
Iso4 ^{HP/r-}	25.6 ± 3.0 ^{ab}	1036.7 ± 16.2 ^b	15.8 ± 1.0 ^a
Iso10 ^{LP/r-}	38.2 ± 4.5 ^a	1123.1 ± 29.3 ^{ab}	15.6 ± 1.3 ^a
Iso11 ^{LP/r-}	20.1 ± 2.4 ^b	1126.9 ± 16.1 ^{ab}	13.8 ± 1.0 ^a
Iso14 ^{LP/r+}	32.2 ± 3.2 ^{ab}	1076.4 ± 23.2 ^b	15.9 ± 1.1 ^a

Table 2. Mean fecundity (nymphs/female), tibia size (μm) and longevity (days) of adult females from selected isofemale lineages of *Myzus persicae* with high (Iso2^{HP/r+}, Iso3^{HP/r+}, Iso4^{HP/r-}) and low (Iso10^{LP/r-}, Iso11^{LP/r-}, Iso14^{LP/r+}) parasitization by *Diaeretiella rapae*. Means (± s.e.) followed by the same lower-case letter are not statistically different at a 5% significance level.

Isoline	Fertility life table parameters (± se)				
	Net reproduction rate (Ro)	Intrinsic growth rate (r_m)	Finite increase ratio (λ)	Interval between generations (T)	Doubling time (Dt)
Iso2 ^{HP/r+}	25.0 ± 14.0 ^{ab}	0.25 ± 0.05 ^{ab}	1.28 ± 0.06 ^{ab}	12.8 ± 1.8 ^{ab}	2.8 ± 0.5 ^{bc}
Iso3 ^{HP/r+}	23.4 ± 13.7 ^{abc}	0.27 ± 0.04 ^a	1.31 ± 0.05 ^a	11.6 ± 1.5 ^b	2.5 ± 0.3 ^c
Iso4 ^{HP/r-}	18.9 ± 9.0 ^{bc}	0.22 ± 0.04 ^{bc}	1.25 ± 0.05 ^{bc}	13.3 ± 1.6 ^a	3.1 ± 0.6 ^{ab}
Iso10 ^{LP/r-}	32.9 ± 18.6 ^a	0.25 ± 0.04 ^{ab}	1.28 ± 0.05 ^{ab}	14.2 ± 1.3 ^a	2.8 ± 0.4 ^{bc}
Iso11 ^{LP/r-}	13.4 ± 7.2 ^c	0.20 ± 0.04 ^c	1.22 ± 0.05 ^c	13.0 ± 1.8 ^{ab}	3.5 ± 0.7 ^a
Iso14 ^{LP/r+}	25.3 ± 11.7 ^{ab}	0.24 ± 0.03 ^{ab}	1.27 ± 0.04 ^{ab}	13.3 ± 1.9 ^a	2.9 ± 0.4 ^{bc}

Table 3. Life table parameters of selected isofemale lineages of *Myzus persicae* with high (Iso2^{HP/r+}, Iso3^{HP/r+}, Iso4^{HP/r-}) and low (Iso10^{LP/r-}, Iso11^{LP/r-}, Iso14^{LP/r+}) parasitization by *Diaeretiella rapae*. Means (± s.e.) followed by the same lower-case letter are not statistically different at a 5% significance level.

Iso11^{LP/r-} ($F_{5,174} = 2.53$; $p = 0.027$) (Table 2). All selected lineages appeared to have a similar rhythm of offspring production, with 80% of the total offspring being produced between days 13 and 15 of adulthood (Fig. 2C). Most lineages reached 80% of accumulated fecundity with almost 80% survival of females (Fig. 2C).

Analysis of fertility life table parameters of *M. persicae* lineages revealed differences in their net reproductive rate (Ro) ($F_{5,174} = 5.425$; $p < 0.001$). Lineage Iso11^{LP/r-} had the lowest Ro values, differing from Iso2^{HP/r+} ($p = 0.048$), Iso10^{LP/r-} ($p < 0.001$) and Iso14^{LP/r+} ($p = 0.038$), but not from Iso3^{HP/r+} and Iso4^{HP/r-}, which had intermediate values (Table 3). The intrinsic growth rate (r_m) ($F_{5,174} = 7.1119$; $p < 0.001$) and the finite growth rate (λ) ($F_{5,174} = 7.0172$; $p < 0.001$) were higher for lineages Iso2^{HP/r+}, Iso3^{HP/r+}, Iso10^{LP/r-}, and Iso14^{LP/r+} when compared to Iso11^{LP/r-}. r_m and λ obtained for Iso3^{HP/r+} were also higher than those for Iso4^{HP/r-} (Table 3). The time between generations (T) observed for isofemale lineage Iso3^{HP/r+} was shorter than those for Iso4^{HP/r-} ($p = 0.037$), Iso10^{LP/r-} ($p < 0.001$), and Iso14^{LP/r+} ($p = 0.025$), but like that obtained for Iso2^{HP/r+} and Iso11^{LP/r-}. The doubling time (Dt) for Iso11^{LP/r-} (3.5 d) was longer than that for all other lineages ($p < 0.001$) except Iso4^{HP/r-} (Table 3). The doubling time (Dt) for Iso4^{HP/r-} was also like that of lineages with intermediate Dt values, but different from that of Iso3^{HP/r+} (Table 3).

The results of the hierarchical clustering analysis based on Ro, r_m , T, TD and λ values revealed two major clusters, the first composed by the Iso4^{HP/r-} and Iso11^{LP/r-} isofemale lineages and the second composed by the other lineages (Iso2^{HP/r+}, Iso3^{HP/r+}, Iso10^{LP/r-} and Iso14^{LP/r+}). Iso4^{HP/r-} and Iso11^{LP/r-} grouped in a well-defined cluster. Iso2^{HP/r+} and Iso14^{LP/r+} resolved in the innermost cluster of a large cluster also containing Iso3^{HP/r+} and Iso10^{LP/r-} (Figs. 2D and S2). Iso3^{HP/r+} was the first to bifurcate from the cluster containing Iso10^{LP/r-}, Iso2^{HP/r+}, and Iso14^{LP/r+} because it had the highest values of r_m and λ , and the lowest values of Dt and T. Iso10^{LP/r-} was the

second to bifurcate out based on the highest values of R_0 and T . Iso2^{HP/r+} and Iso14^{LP/r+} resolved together as they shared similar values for all fertility life table parameters (Fig. 2D).

The life history traits and fecundity table suggest that different mechanisms may be involved in the differential response to parasitism and infection by *Rickettsia*, considering that it was not possible to detect a pattern between lineages belonging to the HP or LP group and $r+$ or $r-$.

Relation of small nucleotide variants in the genome of isofemale lineages of *Myzus persicae* selected for differential response to parasitization

We further investigated whether the difference in response to parasitization in selected isofemale lineages of *M. persicae* could be associated with small structural variants and SNPs in the genome of these isofemale lineages compared to a reference genome. Figure 3 summarizes the main results described so far (parasitism and fecundity) in relation to the detection of small structural variants (InDels) and SNPs.

DNA sequencing data

Illumina sequencing of the six selected *M. persicae* lineages resulted in a total of 140.6 million raw reads, ranging from 19.3 to 26.4 million reads per library with very high-quality scores (Table S1). The number of duplicated reads ranged from 19 to 25.5%, and only 10,806 to 23,238 bp reads per library failed the quality filters applied (Table S1). The number of paired reads ranged from 19 to 26 million/library, resulting in 138.5 million raw reads with 30% GC content (Table S1).

SNPs and InDels variant calling

A total of 1,048,661 single nucleotide polymorphisms (SNPs) and 423,303 insertion-deletions (InDels) were detected, with 290,628 SNPs and 64,752 InDels surviving the filtering protocols used. Around 80% of all SNPs and InDels were associated with the intergenic and intron regions of the aphid genome, with only ~6% associated with coding regions (Figure S3). Most SNPs (85.1%) (Fig. 4A) and nearly half of InDels (60.8%) (Fig. 4B) were common to all lineages. The number of private SNPs detected in each lineage was quite variable, ranging from 737 in lineage Iso11^{LP/r-} to 179 in lineage Iso3^{HP/r+} (Fig. 4A). A high number of specific SNPs was associated with

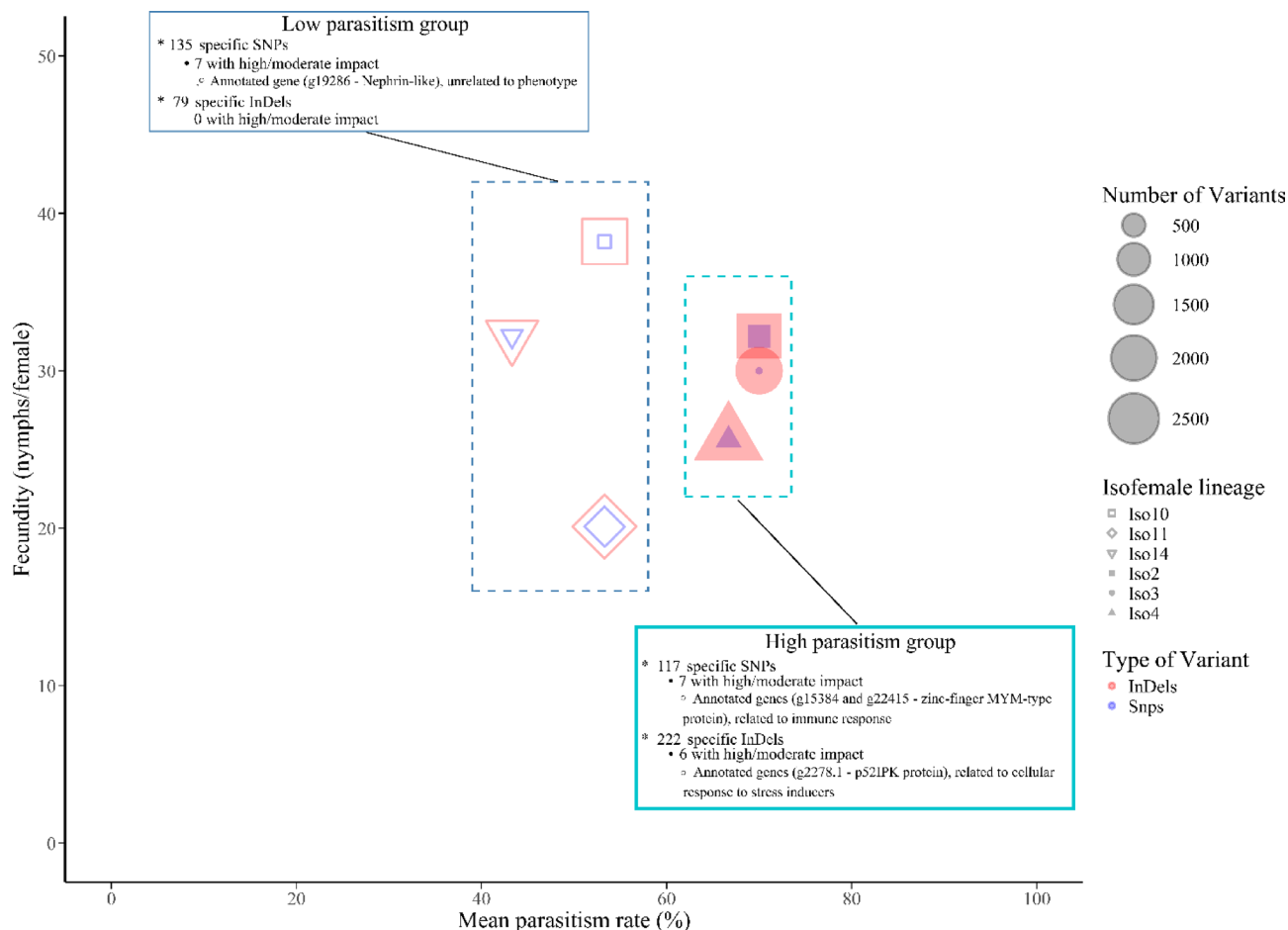


Fig. 3. Graphical summary results with parasitism, fecundity and number of variants (InDels and SNPs) in *Myzus persicae* isofemale lineages with high (Iso2^{HP/r+}, Iso3^{HP/r+}, Iso4^{HP/r+}) and low (Iso10^{LP/r-}, Iso11^{LP/r-}, Iso14^{LP/r+}) parasitization by *Diaeretiella rapae*.

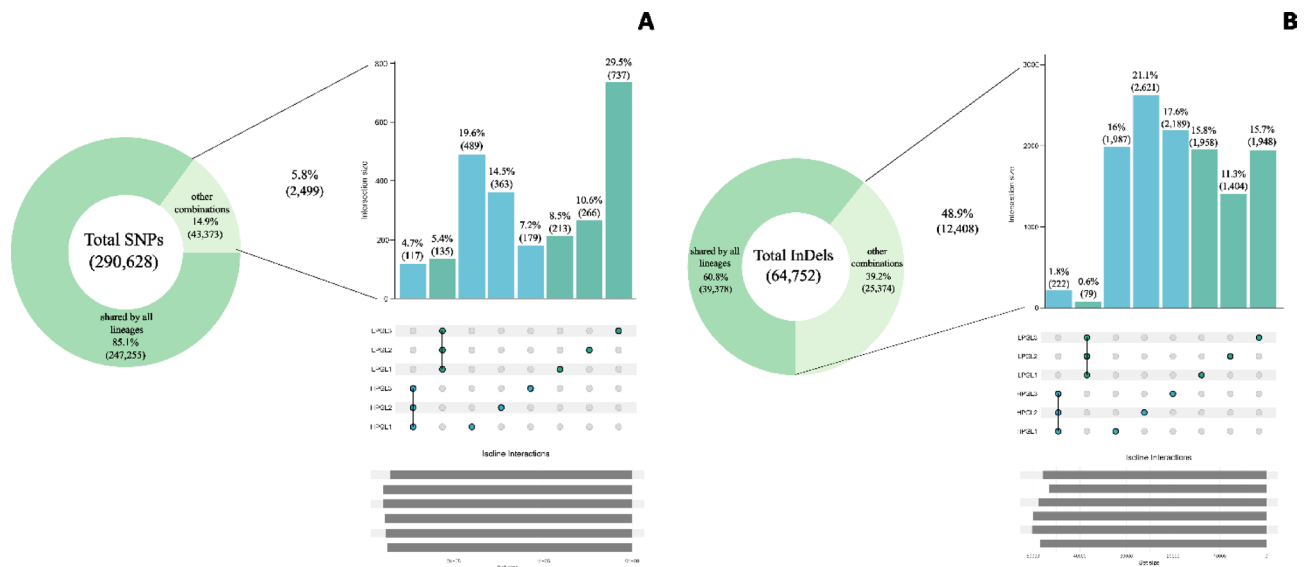


Fig. 4. Overview of the SNPs (A) and InDels (B) that survived the filtering protocols, common to all the *Myzus persicae* isofemale lineages studied (Iso2HP/r+, Iso3HP/r+, Iso4HP/r-, Iso10LP/r-, Iso11LP/r- and Iso14LP/r+), shared by two or more lineages, or unique to each group (high and low parasitism) or each lineage.

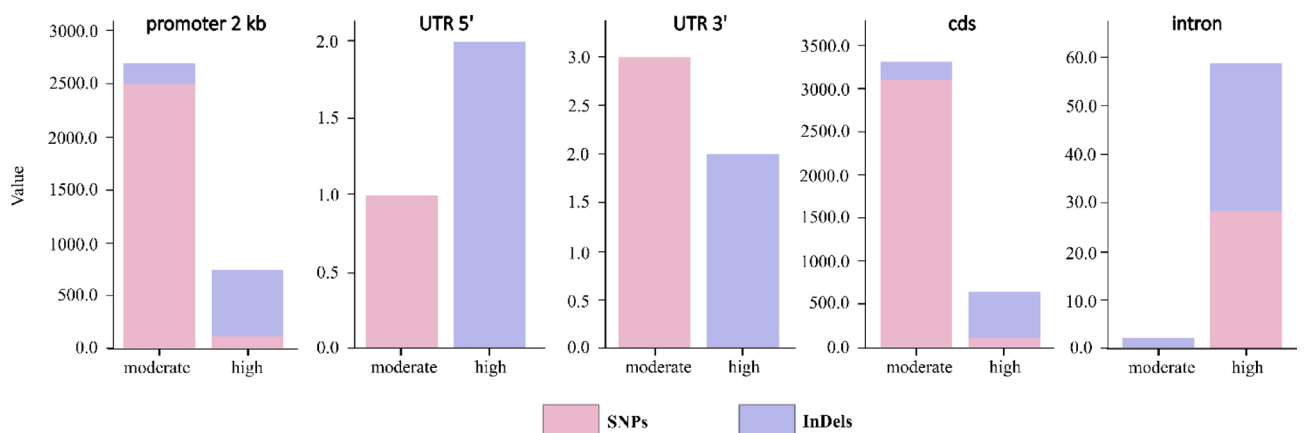


Fig. 5. Overview of the moderate and high SNPs (pink color) and Indels (purple color) by genomic regions present in the genome of *Myzus persicae* lineages with high (Iso2^{HP/r+}, Iso3^{HP/r+}, Iso4^{HP/r-}) and low (Iso10^{LP/r-}, Iso11^{LP/r-}, Iso14^{LP/r+}) parasitization by *Diaeretiella rapae*.

each group of lineages, with 135 specific SNPs in the LP group and 117 in the HP group (Fig. 4A). A very high number of InDels was specifically detected in each lineage, ranging from 1,404 in lineage Iso14^{LP/r+} to 2,621 in lineage Iso4^{HP/r-} (Fig. 4B). Lineages of the LP group shared 79 specific InDels, while those from the HP group 222 (Fig. 4B).

Nearly 1.7% SNPs and 0.91% InDels were classified as resulting in low, moderate, and high (Figure S4) according to their impact on gene function (Figures S5 and S6), and were found located with promoter 2 kb, 5'UTR, 3'UTR, cds, and intronic genomic regions, in which most SNPs and InDels are located in the promoter and codon regions (cds) (Fig. 5). Among the SNPs and InDels that were shared between lineages belonging to the LP and lineages belonging to the HP group, only seven SNPs and six InDels were classified as small structural variants with high or moderate impact in lineages of the HP group, while seven SNPs in those of the LP group (Table 4).

Functional annotation led to the identification of GO and KO terms for only 7.5% and 13.2% of the detected structural variants and SNPs with a high or moderate impact, with the majority classified into *replication, recombination and repair* (L), *carbohydrate transport and metabolism* (G), *transcription* (K) and *post-translational modification, protein turnover, chaperones* (O) COG classes (Tables S2, S3, S4, S5, Fig. 6). In the HP group, only one high InDel and two moderate SNPs were detected, the InDel promoting the deletion of an adenine nucleotide being associated with the g2278.1 gene, which codes for the p52IPK protein, a suppressor of the

Effect type	All lineages		HP group		LP group		Lineage specific		Other combination	
	SNPs	InDels	SNPs	InDels	SNPs	InDels	SNPs	InDels	SNPs	InDels
High	165	305	0	5	0	0	6	525	75	337
Moderate	4287	128	7	1	7	0	98	146	1195	92
Total	4452	433	7	6	7	0	104	671	1270	429

Table 4. Overview of high and moderate SNPs and InDels detected in selected *M. persicae* isofemale lineages with different levels of parasitization by *D. rapae* (Iso2^{HP/+}, Iso3^{HP/+}, Iso4^{HP/+}, Iso10^{LP/+}, Iso11^{LP/+} and Iso14^{LP/+}) (LP group = low parasitization group; HP group = high parasitization group).

p58IPK inhibitor of protein kinase RNA-activated (PKR), that may be associated with cellular responses to stress inducers. The SNPs resulting in the change of cytosine or guanine to thymine are related to the g15384 and g22415 genes, both of which code for a zinc-finger MYM-type protein, which may be related to immune response mechanisms. In the LP group, only one moderate SNP was found associated with the gene g19286, which encodes a nephrin-like protein, that does not appear to be associated with the phenotypes observed.

Enrichment analysis of GO terms for the high and moderate SNPs and InDels resulted in different top five terms in each category, with the most represented having catalytic or hydrolase activities (Fig. 7). KEGG pathway analysis identified InDels in 33 and SNPs in 20 pathways. Twelve genes with small structural variants specifically detected in LP lineages were associated with KEGG pathways, while no association was established for those of the HP group. KEGG pathway analysis also demonstrated that although small structural variants were associated specifically with each individual lineage or to each group (LP or HP) of lineages, all pathways identified for the LP group were also represented in at least one lineage of the HP group (Table S6).

Discussion

Isofemale lineages of *M. persicae* exhibited high phenotypic variation in response to parasitization by *D. rapae*. Phenotypic plasticity in *M. persicae* has also been demonstrated for body color and size⁷¹, pathogen vectoring capacity⁷², and susceptibility to biotic and abiotic stressors^{71,73}. However, we did not expect to observe such high variation within isofemale lineages under controlled parasitoid exposure conditions, as the offspring of parthenogenetic aphid females are clones of their mothers, once parthenogenesis occurs by apomixis⁶⁴. Previous studies with clonal lines of the aphid *Acyrtosiphon pisum* have shown contrasting variability in resistance to parasitism by *Aphidius ervi*, resulting in the selection of lines that are completely resistant and others that are highly susceptible to parasitoid attack^{74,75}, although the variability in response observed between individuals of the same line was not as high as the observed in our study.

The high variability observed in some isofemale lineages indicates the presence of factors that reduce genetic fidelity among clonal daughters. There are several examples in aphid species that demonstrate selection and evolution of clonal lines mainly in adaptation to host plants⁷¹, although several mechanisms have been reported to produce genetic variation in clonal aphids, such as DNA mutations⁷⁶, chromosomal rearrangements, mitotic recombination, and interactions with symbionts^{58,59,64}. Some of the events that generate genetic variability should not be as common in obligate asexual (anholocyclic) aphid lineages, lineages formed by asexual females that produce asexual females, as they are in holocyclic lineages, where sexual reproduction occurs during at least part of their life cycle^{71,77}. Some studies have shown that the main source of clonal variation in response to parasitization is due to interaction with the secondary symbionts *Hamiltonella defensa* and *Regiella insecticola*. The host association with these symbionts significantly increased resistance of clonal host lines to parasitoid attack^{78,79}.

In the isofemale lineages in our study, we did not detect infections with the most common defensive symbionts (*H. defensa* and *R. insecticola*), but we did examine the influence of *Rickettsia* on host fitness traits and host defense against parasitization. Our data was not conclusive on the role of *Rickettsia* as a defensive secondary symbiont, since *Rickettsia* infections were detected in aphid isofemale lineages belonging to the low and high parasitization groups. In addition, similar to a previous study with *A. pisum*, the most resistant isofemale lineages in our study exhibited levels of defense (~47–57% survival) like *A. pisum* lineages infected with defensive symbionts (~35–100%), leading us to question whether genetic variation in the strains is associated with the phenotypic variability observed^{80,81}.

Our study showed that all isofemale lineages of *M. persicae* carry specific small genomic structural variants representing specific molecular markers from its interaction with the environment, which could explain the phenotypic differences observed. Structural variants, such as SNPs and InDels, have been previously linked to phenotypic plasticity and adaptive responses in aphids and other insects^{82–85}. Despite the small number of structural variants that can induce high and moderate impact in gene functioning in LP and HP phenotypic groups of *M. persicae*, the functional annotation of one InDel and three SNPs revealed their association with stress signaling pathways and developmental processes that can be further explored to explain the observed phenotypic responses. Similar associations have been observed in studies investigating genomic adaptations in insects, where structural variants influence host resistance against parasitoids, wing plasticity and insecticide resistance^{82–85}.

One of the InDels identified in lineages of HP group is associated with the g2278.1 gene encoding for the p52IPK protein, a suppressor of the p58IPK inhibitor of protein kinase RNA-activated (PKR). PKRs are key regulators of cellular response to stress inducers by activating other pathways of stress response⁸⁶. Several of

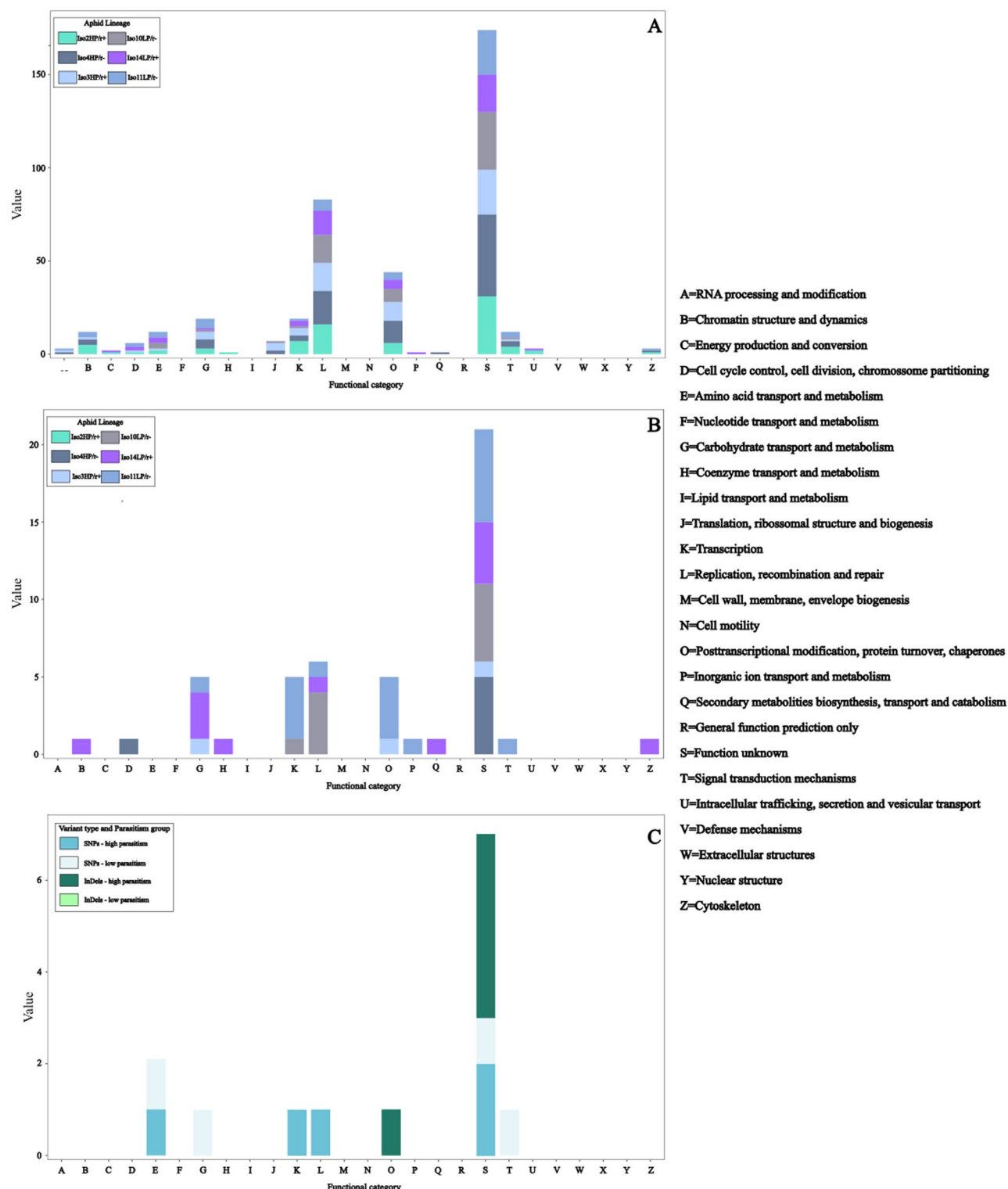


Fig. 6. The functional COG classification of genes associated with high and moderate SNPs (A) and InDels (B) detected in *Myzus persicae* lineages selected for high (Iso2HP/r+, Iso3HP/r+, Iso4HP/r-) and low (Iso10LP/r-, Iso11LP/r-, Iso14LP/r+) parasitization by *Diaeretiella rapae*, or unique to each group (high and low parasitism) (C).

the activated stress responses by the PKR pathway are involved with humoral and cellular immune responses (e.g., JNK, p38, and NFkB). p38 is a mitogen activated protein kinase (MAPK) and regulates gene expression leading to antimicrobial peptide production^{87,88}. MAPK also plays a key role in the cascade activation of the humoral and cellular immune response in insects leading to the encapsulation and melanization of wasp eggs

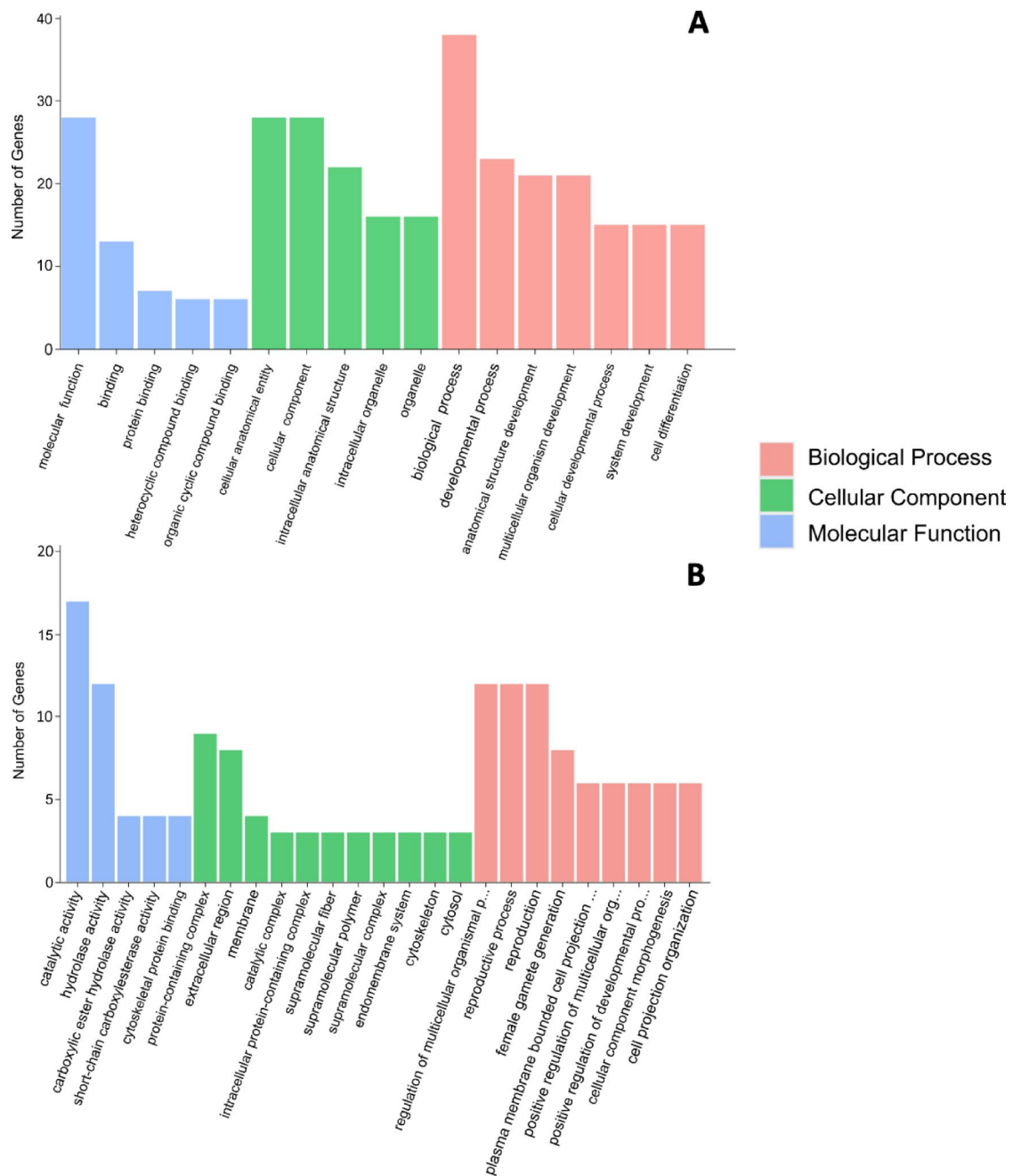


Fig. 7. Top five Gene Ontology (GO) functional analysis of genes associated with high and moderate SNPs (A) and InDels (B) unique to the high (Iso2HP/r+, Iso3HP/r+, Iso4HP/r-) or low parasitization (Iso10LP/r-, Iso11LP/r, Iso14LP/r+) group or to each individual lineage of *Myzus persicae* selected for their differential response to parasitization by *Diaeretiella rapae*.

in parasitized hosts^{89–93}. Recent data has shown the efficacy of the antimicrobial peptides in activating the Toll/Imd immune pathways to protect silkworm larvae from dipteran parasitoids successful development⁹⁴. Thus, we believe the InDel detected in the p52IPK gene could lower the capacity of the HP group of aphid lineages to build an immune response through the protein kinase cascade activation of cellular and humoral immune responses due to the reduced suppression of p58IPK, which in turn would result in a higher inhibition of PKRs by p58IPK.

SNPs detected in two genes (g15384.t1 and g22415.t1) in the HP group lineages encode zinc finger proteins of the MYM family (ZFP-MYM), which are also associated with the immune response. ZFP-MYMs are multifunction proteins with regulatory roles in SUMOylation by acting as SUMO-binding proteins, which directly interferes with the development, maturation, and activation of immune cells, precursors, and effectors^{95–98}. Thus, alterations in the protein sequence of ZFP-MYMs would affect their binding capacity to target proteins would dysregulate the development and differentiation of immune cells and impair the cellular immune response against macroinvaders.

The only small variant within a host gene represented in all lineages of the LP group was a SNP in a gene encoding for a nephrin-like protein (g19286.t1 gene). Nephrins are related to cell adhesion and signaling, regulating the structure and function of nephrocytes⁹⁹. Based on the information available, we were unable to establish a link between the alteration of this gene and the observed phenotype.

The SNPs and InDels identified in the HP lineage group offer some potential to explain the differential response of aphid lineages to the parasitization by *D. rapae*, but we believe that additional experiments on comparative differential gene expression could provide additional insights into the functional role of the large number of variants detected in intergenic/intronic regions. Intronic sequence variants can modulate genotype–phenotype relationship¹⁰⁰ and they have been demonstrated to affect insect resistance to insecticides^{101,102}, the development of human diseases¹⁰³ and the response to drugs¹⁰⁴. However, the occurrence of other mechanisms regulating gene expression in *M. persicae* should also be considered, since epigenetic changes (DNA methylation) also lead to the loss of *M. persicae* resistance to insecticides^{105,106}.

The lack of association with host-defensive secondary symbionts and the control of other factors that could interfere with the aphid response to parasitization (nutritional quality of the host plant, age of the aphid and wasp, and genetic variation of the wasp)^{107–109} indicate that the selected isofemale lineages accumulated sufficient genetic diversity to generate the observed phenotypic variability. Similar patterns of intraspecific variation in resistance to parasitism have been observed in other aphid species, such as *A. pisum*, where genetic diversity among clones contributed to differential survival against parasitoids^{74,75}. The environmental factors that produced the observed phenotypic variation require further physiological and molecular investigation, but our initial hypothesis is that structural variants associated mainly with immune genes and variants in intronic and intergenic regions in the genomes of the selected aphid lineages resulted in alterations in aphid response to parasitization.

The manifestation of defense strategies, regardless of their origin, most often results in changes in life history traits due to the energy costs involved. Changes that direct the allocation of larger amounts of energy to build a stronger immune response will often reduce the energy budget dedicated to sustaining other life history traits, such as fecundity or longevity⁶⁶. The differences in life history traits observed among the isofemale lineages tested were similar to those observed by Gwynn et al.⁶⁶ and Vorburger et al.⁶⁷. However, the relationship between fitness costs and the ability of aphids to respond to *D. rapae* was not uniform among lineages of the LP group (Iso10^{LP/r-}, Iso11^{LP/r-}, and Iso14^{LP/r+}). Only Iso11^{LP/r-} (lower fecundity, *Ro*, *Rm*, and λ , and longer *Dt*) and Iso14^{LP/r+} (delayed development) showed significant negative changes in their fitness traits. While the observation of fitness costs in fecundity is commonly reported as a trade-off between female reproduction and immunity in insects¹¹⁰, other life history traits such as development time are rarely studied¹¹¹.

The hypothesis that the reduced growth rate observed for Iso14^{LP/r+} is related to a fitness cost due to its greater ability to avoid parasitism is not supported by observations showing that the growth rate is genetically correlated positively with the larval cuticular immune response of the cotton leafworm *Spodoptera littoralis*⁸³. The observed change in life history traits of Iso14^{LP/r+} may be related to a reduced allocation of energy to food acquisition to the detriment of other physiological processes, such as immune response and xenobiotic detoxification. Similar trade-offs between fitness and activities related to defense against pathogens and natural enemies have been reported in other insects. For instance, Freitak et al.¹¹² found that immune activation in *Pieris brassicae* (L.) led to increased metabolic rates. Similarly, Kraaijeveld et al.³³ demonstrated that the adoption of defense mechanisms against parasites and parasitoids resulted in reduced size and fecundity in *Drosophila melanogaster*. SNPs and InDels in Iso14^{LP/r+} were detected in genes encoding proteins involved in insect immunity (for example, zinc finger proteins of the MYM family and protein kinase-like inhibitor) and detoxification (g12062.t1 gene—multidrug resistance-associated protein belonging to the ABC transporter family)^{113,114}. Reduced growth rates in aphids are generally associated with reduced metabolism to convert ingested food into energy¹¹⁵. Malnutrition has been reported to increase the susceptibility of insects to stress responses and to reduce their immune capacity^{116,117}. Changes in the nutritional content of aphids may also result in aphids that do not provide the correct chemical stimuli to induce female wasps to lay their eggs while assessing for host suitability^{118,119}.

The longer developmental time required for insects to complete their immature development due to malnutrition is often accompanied by changes in other life-history traits, such as fecundity. However, no reproductive changes were observed for Iso14^{LP/r+}, like other studies in which no trade-offs were detected between reproduction and resistance to parasitization^{80,134,135}. We believe that in our study this is due to its association with *Rickettsia*, given that this secondary symbiont positively affects fecundity in other host-symbiont associations^{120–122}. Thus, *Rickettsia* may mitigate the costs associated with the reduced susceptibility of Iso14^{LP/r+} to *D. rapae* parasitization. Thus, the fitness traits observed in *Rickettsia*-infected isolines suggest that *Rickettsia* does not benefit the host response to parasitization by *D. rapae*, but the fecundity of *Rickettsia*-infected isofemale lineages was higher than that of uninfected isofemale lineages. The observed dissociation between the benefits of *Rickettsia* on aphid fecundity and its lack of effect on parasitoid resistance may be explained by several factors. First, the energetic trade-offs associated with immune activation may favor reproductive investment over parasitoid resistance, as resource allocation to immunity may come at the expense of other physiological functions, such as fecundity¹²³. This trade-off has been observed in other insect systems, where increased reproductive output is often associated with a reduced ability to mount an effective immune response¹²⁴. However, it is important to note that additional research (eg., analysis of antibiotic cured *Rickettsia*-infected lines) is still required to establish a causal relationship between *Rickettsia* infection and the aphid increased fecundity.

Furthermore, interactions with other symbionts could mediate the observed effects, as previous studies have shown that *Rickettsia* can alter the density of protective symbionts¹²⁵ or reduce the number of primary aphid symbiont cells and the host fitness traits^{126,127}. However, our data does not suggest that *Rickettsia* affects *B. aphidicola* density in a way that interferes with the provision of essential amino acids and vitamins

to the host^{128–130}, because we observed higher fecundity in *Rickettsia*-infected lineages. Finally, environmental conditions and host plant quality may influence both fecundity and immune function in aphids. Studies have shown that the benefits conferred by symbionts are highly context dependent, with changes in temperature and nutrient availability altering their effects¹³¹. Aphid symbionts, including *Rickettsia*, may confer benefits under specific environmental conditions that were not assessed in this study. Future studies should aim to explore these mechanisms in more detail and determine the specific conditions under which *Rickettsia* confer fitness advantages to aphids.

The observation of fitness costs depends on the environment, and the adaptive cost varies with environmental conditions¹³², suggesting the experimental conditions used did not allow for the expression of costs associated with the different abilities of the tested lineages to respond to parasitoid attack. It is also possible that the selected lineages evolved different mechanisms to cope with parasitoid attack, as aphid defense mechanisms may rely on physiological, morphological, or behavioral strategies^{133–137}, which may or may not have associated costs. Aphid defense against parasitism may also be provided by the differential ability to metabolize and utilize host plant secondary compounds to influence the successful establishment and development of natural enemies^{138–140}.

The results of this study have important practical implications for biological control strategies of aphids using parasitoids. The phenotypic variability observed in aphid lineages suggests the existence of multiple resistance mechanisms, which may pose a challenge to biological control. However, the identification of genetic variants (SNPs and InDels) associated with immune response genes, such as p52IPK and zinc finger proteins, provides molecular targets to potentially reduce aphid defenses. Furthermore, the role of secondary symbionts, such as *Rickettsia*, which increase fecundity without affecting parasitoid resistance, suggests that symbiont manipulation may be a promising strategy. Environmental factors, such as host plant quality and temperature, also influence the effectiveness of these interactions, highlighting the need for biological control strategies adapted to specific contexts. Future research should explore these mechanisms to develop more effective and sustainable control methods.

In conclusion, we demonstrated variability among aphid isofemale lineages to parasitization and identified conserved small structure variants in all the HP lineage group that support the hypothesis of altered aphid immune function. Our comparative analysis of life history traits and fertility life table among selected lineages did not detect similar fitness costs in lineages of the LP group, suggesting they evolved different mechanisms to reduce the successful parasitization by *D. rapae*. *Rickettsia* improved aphid reproduction but did not interfere with the aphid response to the parasitoid. Since the effects of secondary symbionts on stress conditions may be dependent on the density of infection^{141–143}, further studies are required to properly investigate the contribution of *Rickettsia* to *M. persicae*. Further studies are also needed to characterize the defense mechanisms that isofemale lineages of *M. persicae* evolved to avoid the successful parasitization by *D. rapae*, particularly those involving transcriptomics and epigenomics. These findings highlight the complexity of aphid-parasitoid-symbiont interactions and provide valuable insights into biological control strategies. However, limitations remain, such as the need to further explore the role of genetic and epigenetic variants, as well as the influence of environmental factors, to develop more precise and adaptive control methods.

Methods

Insects and rearing

Aphid isofemale lineages were established from adult aphids collected from cabbage (*Brassica oleracea* var. acephala) plants in experimental plots at Esalq/USP (22° 42' 46.1" S; 47° 37' 37.8" W) and in home vegetable gardens in Piracicaba (22° 42' 38.041" S; 47° 38' 30.221" W) and Americana (22° 44' 20.216" S; 47° 18' 19.325" W), state of São Paulo, and from canola plants (*Brassica napus*) in Coxilha (28° 13' 51.809" S; 52° 24' 13.752" W), state of Rio Grande do Sul. Female aphids were isolated in plastic containers containing a leaf of cabbage var. Georgia (*Brassica oleracea* var. acephala) as a substrate for aphid feeding and reproduction. A total of 43 isofemale lineages were established. Insects were reared under controlled laboratory conditions (20 ± 2 °C; 70 ± 10% RH; 14L:10D) and cabbage leaves were replaced weekly.

The parasitoid strain was obtained from mummified aphids collected in Piracicaba, state of São Paulo (22° 42' 46.1" S; 47° 37' 37.8" W). Aphid mummies were individualized in glass tubes (8 × 1 cm) containing a droplet of honey to feed the emerging wasp. After emergence, couples were formed and allowed to mate for 24 h. After mating, females were placed in plastic cages (20 × 15 × 10 cm) containing cabbage leaves infested with aphids for parasitization for 48 h. Wasps were then removed and nymphs were kept in cages until aphid mummification. Mummies were collected and transferred to clean dishes lined with filter paper. A droplet of honey was applied to the inside of the lid as a food source for the emerging wasps. The wasps were allowed to mate, and mated females were used to parasitize new aphids. After seven generations under laboratory conditions, the isofemale lineage demonstrating the highest rate of mummification was selected for the experiments. The selected isofemale lineage was maintained under controlled conditions (20 ± 2 °C; 60 ± 10% RH; 14L:10D) and continuously reared on 2nd and 3rd instars of *M. persicae* as previously described.

Plants

Cabbage plants were obtained from seedTr (TopSeed®) sown in seedling trays of 200 cells. Seedlings with a height of 10 cm and three to four true leaves were transferred to 550 mL containers with Tropstrato HT® substrate for cultivation in a greenhouse. Plants were sprayed every two weeks with a foliar fertilizer (Home, Maxx Garden—composition: 150 g/L N, 80 g/L P, 80 g/L K, 400 mg/L B, 100 mg/L Co, 600 mg/L Cu, 500 mg/L Mn, 100 mg/L Mo, 1 g/L Zn, and 60 g/L chelating agent) to stimulate sprouting. Seedlings were also treated every other week with a hydroponic solution containing micro (20 mg/L ZnSO₄·H₂O, 30 mg/L CuSO₄·5H₂O, and 90 mg/L FeNaEDTA) and macronutrients (140 mg/L NH₄H₂PO₄, 830 mg/L MgSO₄, 360 mg/L KNO₃, 80 mg/L NH₄NO₃, and 905 mg/L CaN₂O₆) adapted from Hoagland and Snyder¹⁴⁴.

Assessing the parasitization of *Myzus persicae* isofemale lineages by *Diaeretiella rapae*

A total of 14 isofemale lineages of *M. persicae* were subjected to parasitization by *D. rapae*. Third instars *M. persicae* from each lineage were individually placed on 6 cm cabbage leaf discs placed onto a layer of 2% agar supplemented with 0.02% methyl parahydroxybenzoate (Nipagin®) in a Petri dish and allowed to settle for one hour before individual exposure to a host-fed, 24-h-old, mated *D. rapae* female for assisted observation of parasitism. Each female wasp was used to parasitize up to seven aphids randomly selected from each isofemale lineage. Nymphs were removed shortly after insertion of the parasitoid ovipositor, which was confirmed by the visualization of the oviposition hole on the cuticle surface, and transferred to a new leaf disc. The leaf discs were replaced every three days to allow the aphid full development. Aphids were maintained on the discs for 7–10 days for mummification (successful parasitization observed) or adult development (failed parasitization or successful aphid defense). Mummified aphids were collected and transferred to glass tubes for adult parasitoid emergence. The experiment was set up in a completely randomized design with 30 replicates (one replicate = one individual host aphid).

Parasitism data were statistically analyzed using Bernoulli generalized linear models¹⁴⁵, including the effects of female isoline in the linear predictor. The significance of the isofemale lineage effect was assessed by analysis of deviance. The selection of isofemale lineages for further biological and genomic analyses was based on their distribution in the first (above the 25% percentile) and fourth (below the 75% percentile) quartiles to represent isolines with low and high parasitization rates, respectively.

Assessing symbionts in aphids

Specimens of each *M. persicae* isofemale lineage used in parasitization assays with *D. rapae* were subjected to DNA extraction by a salting-out protocol⁶⁴ (Supplementary Material and Methods). Aphid gDNA was used to detect associated symbionts using diagnostic PCR primarily based on the analysis of the 16S rRNA gene of the most common symbiotic bacteria harbored by aphids (Supplementary Material and Methods; Table S7)^{146–149}. We selected representatives infected (Iso2^{HP/r+}, Iso3^{HP/r+} and Iso14^{LP/r+}) and uninfected (Iso4^{HP/r-}, Iso10^{LP/r-} and Iso11^{LP/r-}) by the *Rickettsia* among the lineages distributed in the first and fourth quartiles according to their resilience to parasitization for further biological and genomic analyses. *Rickettsia* was selected because it was the only secondary symbiont to be represented in single infection in aphid isofemale lineages represented in the first and fourth quartiles. Moreover, *Rickettsia* has also been reported to play nutritional and defensive roles in association with white flies¹⁵⁰.

Assessment of life history traits and fertility life tables of *Myzus persicae* isofemale lineages with different levels of parasitization by *Diaeretiella rapae*

The existence of adaptive costs associated with the differential response of *M. persicae* to parasitism by *D. rapae* was assessed on a selected set of isofemale lineages by evaluating life history traits at the immature and adult stages of the host. Six selected isofemale lineages were subjected to comparative biology experiments, three belonging to the high parasitism (HP) group (Iso2^{HP/r+}, Iso4^{HP/r-}, and Iso3^{HP/r+}) and three belonging to the low parasitism (LP) group (Iso10^{LP/r-}, Iso14^{LP/r+}, and Iso11^{LP/r-}). LP and HP groups contained *Rickettsia*-infected ($r+$) and uninfected ($r-$) lineages. We evaluated the immature aphid development time (days) and survival (%) by setting up 16 replicates for each selected isofemale lineage. Each replicate was represented by a pool of 10 neonates infesting a potted cabbage seedling, for a total of 160 nymphs per isofemale lineage.

Thirty newly emerged females were randomly selected for each isofemale lineage and individually placed on a cabbage plant. Females were monitored daily for survival, and daily and total fecundity (number of nymphs produced) was recorded. We fitted an inverse Gaussian generalized linear model to the longevity data (since there was no censoring), including the effects of isofemale lineage in the linear predictor. We assessed the significance of the isoline effect using F -tests. We fitted a negative binomial model to the fecundity data, including the effects of isofemale lineage in the linear predictor, and assessed the significance of the isofemale lineage effect using F -tests (since the dispersion parameter was estimated). We performed multiple comparisons by obtaining the 95% confidence intervals for the true linear predictors.

The fecundity and longevity data obtained were used to construct fertility life tables to assess the reproductive success of each selected lineage by calculating and comparing the mean interval between generations (T), finite growth rate (λ), intrinsic growth rate (R_m), net reproduction rate (R_0), and time to duplication (TD)^{151,152}. Plants and nymphs were maintained as before, and nymph mortality and adult development were checked daily.

Twenty adult females obtained from each isofemale lineage were also sampled and used to estimate aphid size by measuring the length of slide-mounted left metathoracic tibia. Measurements were performed using a stereomicroscope connected to a digital image analysis system (Motic Images Plus 2.0).

Aphid immature development time was analyzed using Cox proportional hazards models, including the effects of isofemale lineage in the linear predictor. We assessed the significance of the lineage effect using likelihood ratio (LR) tests for nested models and performed multiple comparisons by obtaining 95% confidence intervals for the true linear predictors. Survival curves were estimated using the Kaplan–Meier estimator to obtain probabilities of *M. persicae* nymphs reaching adulthood ($p < 0.05$).

Uncertainty in life table parameters was estimated by Jackknife resampling according to Maia and Luiz¹⁵³, using the SAS-based routine of Maia et al.¹⁵⁴. The obtained means were compared using the Tukey test ($p < 0.05$). Multivariate analyses were also used to evaluate the life table data using multivariate linear models, including the effects of isofemale lineage as a linear predictor. The significance of the isofemale lineage effect was assessed using Pillai's trace test, and a heatmap was created to visualize clusters of isofemale lineages based on life table parameters using hierarchical clustering based on Euclidean distances and Ward's method. A gamma generalized linear model was fitted to the tibia size data, including the effects of isofemale lineage in the linear predictor. We

assessed the significance of the isofemale lineage effect using *F*-tests and performed multiple comparisons by obtaining the 95% confidence intervals for the true linear predictors.

All analyses were performed in R (R Core Team, 2020). Package *survival*¹⁵⁵ was used to fit the Cox proportional hazards models, *ggplot2*¹⁵⁶ was used to generate the plots, and *hnp*¹⁵⁷ was used to assess the goodness-of-fit of the model.

Evaluation and identification of small structural variants (SNPs and InDels) in the selected *Myzus persicae* isofemale lineages

Genomic sequence data from the six *M. persicae* isofemale lineages selected from the parasitization assays (Iso2^{HP/r+}, Iso3^{HP/r+}, Iso4^{HP/r-}, Iso10^{LP/r-}, Iso11^{LP/r-}, and Iso14^{LP/r+}) were obtained by extracting and sequencing genomic DNA from a pool of five (5) adult aphids from each isofemale lineage. Insects were subjected to DNA extraction using the Universal AllPrep DNA/RNA/miRNA kit (Qiagen®), according to the manufacturer's recommendations. Samples with an absorbance of approximately 1.8 were sent to genomic sequencing on the Illumina Novaseq6000 platform using the paired-end strategy (150 bp) in a sequencing service provider.

The reads were subjected to quality control using the *FastQC* tool v.0.11.8¹⁵⁸. Quality trimming and adapter clipping were performed using *Trimmomatic* v.038 (parameters LEADING:15 TRAILING:15 SLIDINGWINDOW:5:15 MINLEN:50)¹⁵⁹ and sequences shorter than 50 bp were removed. Only high quality paired-reads from the libraries of each isofemale lineage were aligned against the reference genome of *M. persicae* (G006 v.3.0)¹⁶⁰ (https://bipaa.genouest.org/sp/myzus_persicae_g006/analysis/3) using *Bowtie2* v.2.5.3 aligner software¹⁶¹. *SAMtools* v.1.21 was used to convert SAM files into sorted BAM files¹⁶² (Table S8).

Nucleotide variants were detected using the *HaplotypeCaller* v.4.3.0 available in the Genome Analysis Toolkit (GATK, <https://gatk.broadinstitute.org/>)¹⁶³ with the default parameters. The *GenomicsDBImport* and *GenotypeGVFs* tools (GATK) were used to merge the gVCF files into a single VCF and to genotype the nucleotide variants. The preliminary list of identified variants was filtered using the *SelectVariants* tool (GATK), using the *-select-type-to-include* argument to obtain a subset containing only SNPs, while another subset only with InDels (insertions and deletions). The SNPs-only subset was filtered by quality metrics using the *VariantFiltration* tool (GATK) (DP < 3 || QD < 20.0 || MQ < 20.0 || FS > 10.0 || MQRankSum < -2.0 || MQRankSum > 2.0 || ReadPosRankSum < -2.0 || ReadPosRankSum > 2.0 || BaseQRankSum < -2.0 || BaseQRankSum > 2.0 || SOR > 3.0). Similarly, the subset containing only InDels was filtered by quality metrics (size = 1 to 50 bp || QD < 20.0 || FS > 30.0 || ReadPosRankSum < -2.0 || ReadPosRankSum > 2.0 || MQRankSum < 30.0 || BaseQRankSum < -2.0 || BaseQRankSum > 2.0).

The classification of filtered SNPs and InDels by genomic region (Figure S1) was performed using *BEDTools Intersect* v.2.31.1¹⁶⁴, *vcf2bed* v.2.4.41¹⁶⁵, and *gencode_regions* (https://github.com/saketkc/gencode_regions) tools. For these analyses, the reference genome (G006 v.3.0)¹⁶⁰ (https://bipaa.genouest.org/sp/myzus_persicae_g006/analysis/3) structural annotation file was used as the input file for the *gencode_regions* program, which allows the extraction of 3'UTR, 5'UTR, CDSs, promoters, genes, and introns from GTF/GFF files, resulting in annotation files in bed format. The bed files were then used in the *BEDTools intersect* v.2.31.1 program together with the bed file of filtered SNPs and InDels previously converted from VCF format to bed using the *vcf2bed* v.2.4.41 tool. To ensure that SNPs and InDels were counted only once, the intersection was performed in the following hierarchy: promoter 2 kb > 5'UTR > 3'UTR > cds > introns, with the bed containing all filtered SNPs and InDels being used for the first region, and the input file containing SNPs and InDels that did not match the previous region being used for subsequent regions. SNPs and InDels that did not match any of the above genomic regions were classified as belonging to intergenic regions.

Genomic variant annotation and functional effect prediction of SNPs and InDels were performed using the *SnpEff* software v.5.2¹⁶⁶. Subsequently, SNPs and InDels were specifically selected using the filtering tool provided by the *SnpSift* software v.5.2¹⁶⁷ according to the effect in genomic sequences: high—significant functional consequences; moderate—SNPs that result in missense substitutions and InDels resulting in conservative or disruptive changes; low—SNPs that did not result in amino acid changes, and InDels observed in the splice region and at initiation and termination sites; and modifier—effects predicted in intronic, intergenic, promoter and UTR regions. Gene sequences harboring high and moderate effects, present exclusively in isofemale lineages with low (LP) or high parasitism rate (HP) groups or specific isofemale lineages were queried against the NCBI non-redundant protein database (nr-NCBI) using both the *BLASTx* algorithm and the *Diamond* high-throughput sequence aligner¹⁶⁸, with an *e*-value cutoff of 10⁻³. These sequences were also subjected to functional annotation using the *EggNOG mapper* v.2.0.1 tool to obtain the gene ontology (GO) terms and the clusters of orthologous genes (COGs) classification to analyze the biological processes and pathways in which the high and moderate SNPs and InDels were involved. The mapping analysis was performed using KEGG mapper tool (<https://www.genome.jp/kegg/mapper/reconstruct.html>) against the Kyoto Encyclopedia of Genes and Genomes^{169–171}.

Data availability

The short read DNA sequencing of the six isofemale lineages of *Myzus persicae* and the molecular sequence of COI used for *Diaeretiella rapae* identification are fully available at NCBI (BioProject PRJNA1169216 and GeneBank ON243747) (Table S9). The scripts used are available at <https://github.com/MarianePossignolo/Repository-Variation-in-isolines-of-Myzus-persicae> and https://hackmd.io/@QF2W6Z2XT6qOMO2EYuxCRQ/H1S_fG31kl. Data will become publicly available upon manuscript acceptance. Data access can also be obtained by contacting the corresponding author or the first author, Mariane P. Gomes (email: mariane.gomes@usp.br).

Received: 10 January 2025; Accepted: 9 September 2025

Published online: 10 October 2025

References

1. Eggleton, P. & Gaston, K. J. "Parasitoid" species and assemblages: Convenient definitions or misleading compromises?. *Oikos* **59**, 417–421. <https://doi.org/10.2307/3545155> (1990).
2. Henter, H. J. & Via, S. The potential for coevolution in a host-parasitoid system: Genetic variation within an aphid population in susceptibility to parasitic wasp. *Evolution* **49**, 427–438. <https://doi.org/10.2307/2410267> (1995).
3. Kraaijeveld, A. R., Alphen, J. J. M. V. & Godfray, H. C. J. The coevolution of host resistance and parasitoid virulence. *Parasitology* **116**, S29–S45. <https://doi.org/10.1017/s0031182000084924> (1998).
4. Lafferty, K. D. & Kuris, A. M. Trophic strategies for animal diversity and body size. *Trends Ecol. Evol.* **17**, 507–513. [https://doi.org/10.1016/s0169-5347\(02\)02615-0](https://doi.org/10.1016/s0169-5347(02)02615-0) (2002).
5. Weinersmith, K. L. What's gotten into you? A review of recent research on parasitoid manipulation of host behavior. *Curr. Opin. Insect Sci.* **33**, 37–42. <https://doi.org/10.1016/j.cois.2018.11.011> (2019).
6. Pennacchio, F. & Strand, M. R. Evolution of developmental strategies in parasitic Hymenoptera. *Annu. Rev. Entomol.* **51**, 233–258. <https://doi.org/10.1146/annurev.ento.51.110104.151029> (2006).
7. Carton, Y. & Nappi, A. J. *Drosophila* cellular immunity against parasitoids. *Parasitol. Today* **13**, 218–227. [https://doi.org/10.1016/S0169-4758\(97\)01058-2](https://doi.org/10.1016/S0169-4758(97)01058-2) (1997).
8. Schmid-Hempel, P. Evolutionary ecology of insect immune Defenses. *Annu. Rev. Entomol.* **50**, 529–551. <https://doi.org/10.1146/annurev.ento.50.071803.130420> (2005).
9. Carton, Y., Poirié, M. & Nappi, A. J. Insect immune resistance to parasitoids. *Insect Sci.* **15**, 67–87. <https://doi.org/10.1111/j.1744-7917.2008.00188.x> (2008).
10. Godfray, H. C. J. *Parasitoids: Behavioral and Evolutionary Ecology* (Princeton University Press, 1994).
11. Kraaijeveld, A. R. & Godfray, H. C. J. Evolution of host resistance and parasitoid counter-resistance. *Adv. Parasitol.* **70**, 257–280. [https://doi.org/10.1016/S0065-308X\(09\)70010-7](https://doi.org/10.1016/S0065-308X(09)70010-7) (2009).
12. Koltz, A. M., Culler, L. E., Bowden, J. J., Post, E. & Høye, T. T. Dominant arctic predator is free of major parasitoid at northern edge of its range. *Front. Ecol. Evol.* **7**, 250. <https://doi.org/10.3389/fevo.2019.00250> (2019).
13. Moore, M. E., Hill, C. A. & Kingsolver, J. G. Differing thermal sensitivities in a host-parasitoid interaction: High, fluctuating developmental temperatures produce dead wasps and giant caterpillars. *Funct. Ecol.* **35**, 675–685. <https://doi.org/10.1111/1365-2435.13748> (2021).
14. Oliver, K. M., Campos, J., Moran, N. A. & Hunter, M. S. Population dynamics of defensive symbionts in aphids. *Proc. R. Soc. B* **275**, 293–299. <https://doi.org/10.1098/rspb.2007.1192> (2007).
15. Chaplinska, M., Gerritsma, S., Dini-Andreote, F., Falcao Salles, J. & Wertheim, B. Bacterial communities differ among *Drosophila melanogaster* populations and affect host resistance against parasitoids. *PLoS ONE* **11**, e0167726. <https://doi.org/10.1371/journal.pone.0167726> (2016).
16. Reeve, J. D. Stability, variability, and persistence in host-parasitoid systems. *Ecology* **71**, 422–426. <https://doi.org/10.2307/1940295> (1990).
17. Comins, H. N., Hassell, M. P. & May, R. M. The spatial dynamics of host-parasitoid systems. *J. Anim. Ecol.* **61**, 735–748. <https://doi.org/10.2307/5627> (1992).
18. Singh, A. Stochasticity in host-parasitoid models informs mechanisms regulating population dynamics. *Sci. Rep.* **11**, 16749. <https://doi.org/10.1038/s41598-021-96212-y> (2021).
19. Benassi, V., Frey, F. & Carton, Y. A new specific gene for wasp cellular immune resistance in *Drosophila*. *Heredity* **80**, 347–352. <https://doi.org/10.1046/j.1365-2540.1998.00303.x> (1998).
20. Kacsoh, B. Z. & Schlenke, T. A. High hemocyte load is associated with increased resistance against parasitoids in *Drosophila suzukii*, a relative of *D. melanogaster*. *PLoS ONE* **7**, e34721. <https://doi.org/10.1371/journal.pone.0034721> (2012).
21. Fors, L., Markus, R., Theopold, U. & Hambäck, P. A. Differences in cellular immune competence explain parasitoid resistance for two coleopteran species. *PLoS ONE* **9**, e108795. <https://doi.org/10.1371/journal.pone.0108795> (2014).
22. Stark, J. D. & Acheampong, S. A demographic and modeling approach to determine the suitability of two hosts, *Brevicoryne brassicae* (Linnaeus) and *Myzus persicae* (Sulzer) (Heteroptera: Aphididae) of the aphid parasitoid, *Diaeretiella rapae* (McIntosh) (Hymenoptera: Aphididae). *Pan-Pac. Entomol.* **83**, 75–79. <https://doi.org/10.3956/0031-0603-83.1.75> (2007).
23. Weber, G. Genetic variability in host plant adaptation of the green peach aphid, *Myzus persicae*. *Entomol. Exp. Appl.* **38**, 49–56. <https://doi.org/10.1111/j.1570-7458.1985.tb03497.x> (1985).
24. Dedryver, C. A., Le Ralec, A. & Fabre, F. The conflicting relationships between aphids and men: A review of aphid damage and control strategies. *C. R. Biol.* **333**, 539–553. <https://doi.org/10.1016/j.crvi.2010.03.009> (2010).
25. Javed, K. et al. Exploring innovative strategies to control aphids: Meta-analysis and a critical view on what we have and what the future is. *J. Pest Sci.* **98**, 51–57. <https://doi.org/10.1007/s10340-024-01852-4> (2025).
26. Guigo, P. L., Maingeneau, A. & Corff, J. L. Performance of an aphid *Myzus persicae* and its parasitoid *Diaeretiella rapae* on wild and cultivated Brassicaceae. *J. Plant Interac.* **7**, 326–332. <https://doi.org/10.1080/17429145.2011.628417> (2012).
27. Girling, R. D., Hassall, M., Turner, J. G. & Poppy, G. M. Behavioural responses of the aphid parasitoid *Diaeretiella rapae* to volatiles from *Arabidopsis thaliana* induced by *Myzus persicae*. *Entomol. Exp. Appl.* **120**, 1–9. <https://doi.org/10.1111/j.1570-7458.2006.00423.x> (2006).
28. Soleimannejad, S., Ross, P. A. & Hoffmann, A. A. Effect of *Rickettsiella viridis* endosymbionts introduced into *Myzus persicae* aphids on parasitism by *Diaeretiella rapae*: A combined strategy for aphid control?. *Biol. Control* **187**, 105377. <https://doi.org/10.1016/j.biocontrol.2023.105377> (2023).
29. Desneux, N. et al. *Diaeretiella rapae* limits *Myzus persicae* populations after applications of deltamethrin in oilseed rape. *J. Econ. Entomol.* **98**, 9–17. <https://doi.org/10.1093/jee/98.1.9> (2005).
30. Bacci, L. et al. Concentration-mortality responses of *Myzus persicae* and natural enemies to selected insecticides. *J. Environ. Sci. Health* **47**, 1930–1937. <https://doi.org/10.1080/03601234.2012.676494> (2012).
31. Blande, J. D., Pickett, J. A. & Poppy, G. M. Attack rate and success of the parasitoid *Diaeretiella rapae* on specialist and generalist feeding aphids. *J. Chem. Ecol.* **30**, 1781–1795. <https://doi.org/10.1023/B:JOEC.0000042401.52088.54> (2004).
32. van Valen, L. The Red Queen. *Am. Nat.* **111**, 809–810. <https://doi.org/10.1086/283213> (1977).
33. Kraaijeveld, A. R., Ferrari, J. & Godfray, H. C. J. Costs of resistance in insect-parasite and insect-parasitoid interactions. *Parasitology* **125**, S71–S82. <https://doi.org/10.1017/s0031182002001750> (2003).
34. Vienne, D. M. et al. Cospeciation vs host-shift speciation: Methods for testing, evidence from natural associations and relation to coevolution. *New Phytol.* **198**, 347–385. <https://doi.org/10.1111/nph.12150> (2013).
35. Brockhurst, M. A. et al. Running with the Red Queen: The role of biotic conflicts in evolution. *Proc. R. Soc. B* **281**, 20141383. <https://doi.org/10.1098/rspb.2014.1382> (2014).
36. Moran, N. A. Symbiosis as an adaptive process and source of phenotypic complexity. *Proc. Natl. Acad. Sci.* **104**, 8627–8633. <https://doi.org/10.1073/pnas.0611659104> (2007).
37. Feldhaar, H. Bacterial symbionts as mediators of ecologically important traits of insect hosts. *Ecol. Entomol.* **36**, 533–543. <https://doi.org/10.1111/j.1365-2311.2011.01318.x> (2011).
38. Vorburger, C. & Perlman, S. J. The role of defensive symbionts in host–parasite coevolution. *Biol. Rev.* **93**(1747–1764), 2018. <https://doi.org/10.1111/brv.12417> (2018).
39. Paredes, J. C., Herren, J. K., Schüpfer, F. & Lemaître, B. The role of lipid competition for endosymbiont-mediated protection against parasitoid wasps in *Drosophila*. *MBio* <https://doi.org/10.1128/mbio.01006-16> (2016).

40. Ballinger, M. J., Gawryluk, R. M. R. & Perlman, S. J. Toxin and genome evolution in a *Drosophila* defensive symbiosis. *Genome Biol. Evol.* **11**, 253–262. <https://doi.org/10.1093/gbe/evy272> (2019).
41. Oliver, K. M. & Perlman, S. J. Toxin-mediated protection against natural enemies by insect defensive symbionts. *Adv. Insect Physiol.* **58**, 277–316. <https://doi.org/10.1016/bs.aip.2020.03.005> (2020).
42. Oliver, K. M., Russel, J. A., Moran, N. A. & Hunter, M. S. Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proc. Natl. Acad. Sci.* **100**, 1803–1807. <https://doi.org/10.1073/pnas.0335320100> (2003).
43. Oliver, K. M., Degnan, P. H., Hunter, M. S. & Moran, N. A. Bacteriophages encode factors required for protection in a symbiotic mutualism. *Science* **325**, 992–994. <https://doi.org/10.1126/science.1174463> (2009).
44. Rouil, J., Jousselin, E., Coeurdacier, A., Cruaud, C. & Manzano-Marín, A. The protector within: comparative genomics of APSE phages across aphids reveals rampant recombination and diverse toxin arsenals. *Genome Biol. Evol.* **12**, 878–889. <https://doi.org/10.1093/gbe/evaa089> (2020).
45. Frago, E., Mala, M. & Weldegergis, B. T. Symbionts protect aphids from parasitic wasps by attenuating herbivore-induced plant volatiles. *Nat. Commun.* **8**, 1860. <https://doi.org/10.1038/s41467-017-01935-0> (2017).
46. Sentis, A., Hemptinne, J. L., Magro, A. & Outreman, Y. Biological control needs evolutionary perspectives of ecological interactions. *Evol. Appl.* **15**, 1537–1554. <https://doi.org/10.1111/eva.13457> (2022).
47. Stiling, P. D. The frequency of density dependence in insect host-parasitoid systems. *Ecology* **68**, 844–856. <https://doi.org/10.2307/1938356> (1987).
48. Carius, H. J., Little, T. J. & Ebert, D. Genetic variation in a host-parasite association: Potential for coevolution and frequency-dependent selection. *Evolution* **55**, 1136–1145. <https://doi.org/10.1111/j.0014-3820.2001.tb00633.x> (2001).
49. Barrett, J. A. Frequency dependent selection in plant fungal interactions. *Philos. Trans. R. Soc. B* **319**, 473–483. <https://doi.org/10.1098/rstb.1988.0060> (1988).
50. Amos, W. Factors affecting levels of genetic diversity in natural populations. *Philos. Trans. R. Soc. B* **353**, 177–186. <https://doi.org/10.1098/rstb.1998.0200> (1998).
51. Vellend, M. & Geber, M. A. Connections between species diversity and genetic diversity. *Ecol. Lett.* **8**, 767–781. <https://doi.org/10.1111/j.1461-0248.2005.00775.x> (2005).
52. Crow, J. F. Advantages of sexual reproduction. *Dev. Genet.* **15**, 205–213. <https://doi.org/10.1002/dvg.1020150303> (1994).
53. Gerber, N. & Kokko, H. Sexual conflict and the evolution of asexuality at low population densities. *Proc. R. Soc. B* **283**, 20161280. <https://doi.org/10.1098/rspb.2016.1280> (2016).
54. Moran, N. A. The evolution of aphid life cycles. *Annu. Rev. Entomol.* **37**, 321–348. <https://doi.org/10.1146/annurev.en.37.010192.001541> (1992).
55. Vorburger, C., Lancaster, M. & Sunnucks, P. Environmentally related patterns of reproductive modes in the aphid *Myzus persicae* and the predominance of two “superclones” in Victoria, Australia. *Mol. Ecol.* **12**, 3493–3504. <https://doi.org/10.1046/j.1365-294X.2003.01998.x> (2003).
56. Shah, M. A. et al. Population ecology of aphid pests infesting potato. *Sustain. Agric. Rev.* **28**, 153–181. https://doi.org/10.1007/978-3-319-90309-5_5 (2018).
57. Sunnucks, P., England, P. R., Taylor, A. C. & Hales, D. F. Microsatellite and chromosome evolution of parthenogenetic *Sitobion* aphids in Australia. *Genetics* **144**, 747–756. <https://doi.org/10.1093/genetics/144.2.747> (1996).
58. Oliver, K. M., Moran, N. A. & Hunter, M. S. Costs and benefits of a superinfection of facultative symbionts in aphids. *Proc. R. Soc. B* **273**, 1273–1280. <https://doi.org/10.1098/rspb.2005.3436> (2006).
59. Guerrero, R., Margulis, L. & Berlanga, M. Symbiogenesis: The holobiont as a unit of evolution. *Int. J. Microbiol.* **16**, 133–143. <https://doi.org/10.2436/20.1501.01.188> (2013).
60. Brown, P. A. & Blackman, R. L. Karyotype variation in the corn leaf aphid, *Rhopalosiphum maidis* (Fitch), species complex (Hemiptera: Aphididae) in relation to host-plant and morphology. *Bull. Entomol. Res.* **78**, 351–363. <https://doi.org/10.1017/S0007485300013110> (1988).
61. Blackman, L., Spence, J. M., Field, M. & Devonshire, A. L. Chromosomal location of the amplified esterase genes conferring resistance to insecticides in *Myzus persicae* (Homoptera: Aphididae). *Heredity* **75**, 297–302. <https://doi.org/10.1038/hdy.1995.138> (1995).
62. Russell, J. A. & Moran, N. A. Costs and benefits of symbiont infection in aphids: Variation among symbionts and across temperatures. *Proc. R. Soc. B* **273**, 603–610. <https://doi.org/10.1098/rspb.2005.3348> (2006).
63. Kikuchi, Y. et al. Symbiont-mediated insecticide resistance. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 8618–8622. <https://doi.org/10.1073/pnas.1200231109> (2012).
64. Sunnucks, P. & Hales, D. F. Numerous transposed sequences of mitochondrial cytochrome oxidases I–II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Mol. Biol. Evol.* **13**, 510–524. <https://doi.org/10.1093/oxfordjournals.molbev.a025612> (1996).
65. Rigby, M. C., Hechinger, R. F. & Stevens, L. Why should parasite resistance be costly? *Trends Parasitol.* **18**, 116–120. [https://doi.org/10.1016/S1471-4922\(01\)02203-6](https://doi.org/10.1016/S1471-4922(01)02203-6) (2002).
66. Gwynn, D. M., Callaghan, A., Gorham, J., Walters, K. F. A. & Fellowes, M. D. E. Resistance is costly: Trade-offs between immunity, fecundity, and survival in the pea aphid. *Proc. R. Soc. B* **272**, 1803–1808. <https://doi.org/10.1098/rspb.2005.3089> (2005).
67. Vorburger, C., Gouskov, A. & Von Burg, S. Genetic covariation between effectiveness and cost of defense in aphids. *Biol. Lett.* **4**, 674–676. <https://doi.org/10.1098/rsbl.2008.0382> (2008).
68. Hastings, P. J., Lupski, J. R., Rosenberg, S. M. & Ira, G. Mechanisms of change in gene copy number. *Nat. Rev. Genet.* **10**, 551–564. <https://doi.org/10.1038/nrg2593> (2009).
69. Hastings, P. J., Ira, G. & Lupski, J. R. A microhomology-mediated break-induced replication model for the origin of human copy number variation. *PLoS Genet.* **5**, e1000327. <https://doi.org/10.1371/journal.pgen.1000327> (2009).
70. Freeman, J. L. et al. Copy number variation: New insights in genome diversity. *Genome Res.* **16**, 949–961. <https://doi.org/10.1101/gr.3677206> (2006).
71. Loxdale, H. D. The nature and reality of the aphid clone: Genetic variation, adaptation, and evolution. *Agr. Forest. Entomol.* **10**, 81–90. <https://doi.org/10.1111/j.1461-9563.2008.00364.x> (2008).
72. Terradot, L. et al. Molecular characterization of clones of the *Myzus persicae* complex (Hemiptera: Aphididae) differing in their ability to transmit the potato *Leafroll luteovirus* (PLRV). *Bull. Entomol. Res.* **89**, 355–363. <https://doi.org/10.1017/S0007485399000498> (1999).
73. Losey, J., Harmon, J., Ballantyne, F. & Brown, C. A polymorphism maintained by opposite patterns of parasitism and predation. *Nature* **388**, 269–272. <https://doi.org/10.1038/40849> (1997).
74. Henter, H. J. & Via, S. The potential for coevolution in a host-parasitoid system I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. *Evolution* **49**, 427–438. <https://doi.org/10.1111/j.1558-5646.1995.tb02275.x> (1995).
75. Ferrari, J., Müller, C. B., Kraaijeveld, A. R. & Godfray, H. C. J. Clonal variation and covariation in aphid resistance to parasitoids and a pathogen. *Evolution* **55**, 1805. [https://doi.org/10.1554/0014-3820\(2001\)055\[1805:cvacia\]2.0.co](https://doi.org/10.1554/0014-3820(2001)055[1805:cvacia]2.0.co) (2001).
76. Loxdale, H. D. Rapid genetic changes in natural insect populations. *Ecol. Entomol.* **35**, 155–164. <https://doi.org/10.1111/j.1365-2311.2009.01141.x> (2010).
77. Loxdale, H. D. & Lushai, G. Maintenance of aphid clonal lineages: Images of immortality? *Infect. Genet. Evol.* **3**, 259–269. [https://doi.org/10.1016/S1567-1348\(03\)00091-1](https://doi.org/10.1016/S1567-1348(03)00091-1) (2003).
78. Oliver, K. M., Moran, N. A. & Hunter, M. S. Variation in resistance to parasitism in aphids is due to symbionts not host genotype. *Proc. Natl. Acad. Sci.* **102**, 12795–12800. <https://doi.org/10.1073/pnas.0506131102> (2005).

79. Vorburger, C., Gehrer, L. & Rodriguez, P. A strain of the bacterial symbiont *Regiella insecticola* protects aphids against parasitoids. *Biol. Lett.* **6**, 109–111. <https://doi.org/10.1098/rsbl.2009.0642> (2010).
80. Martinez, A. J., Weldon, S. R. & Oliver, K. M. Effects of parasitism on aphid nutritional and protective symbioses. *Molec. Ecol.* **23**, 1594–1607. <https://doi.org/10.1111/mec.12550> (2013).
81. Martinez, A. J. et al. Aphid-encoded variability in susceptibility to a parasitoid. *BMC Evol. Biol.* **14**, 127. <https://doi.org/10.1186/1471-2148-14-127> (2014).
82. Hita, M. T. et al. Genetic localization of a *Drosophila melanogaster* resistance gene to a parasitoid wasp and physical mapping of the region. *Genome Res.* **9**, 471–481 (1999).
83. Fellowes, M. & Godfray, H. The evolutionary ecology of resistance to parasitoids by *Drosophila*. *Heredity* **84**, 1–8. <https://doi.org/10.1046/j.1365-2540.2000.00685.x> (2000).
84. Singh, K. S. et al. Global patterns in genomic diversity underpinning the evolution of insecticide resistance in the aphid crop pest *Myzus persicae*. *Commun. Biol.* **4**, 847. <https://doi.org/10.1038/s42003-021-02373-x> (2021).
85. Gregory, L. E., Driscoll, R. M. H., Parker, B. J. & Brisson, J. A. Impacts of body colour, symbionts and genomic regions on the pea aphid wing plasticity variation. *Molec. Ecol.* **34**, e17660. <https://doi.org/10.1111/mec.17660> (2025).
86. Gale, M. et al. P52^{IPK} regulates the molecular cochaperone P58^{IPK} to mediate control of the RNA-dependent protein kinase in response to cytoplasmic stress. *Biochemistry* **41**, 11878–11887. <https://doi.org/10.1021/bi020397e> (2002).
87. Han, Z. S. et al. A conserved p38 mitogen-activated protein kinase pathway regulates *Drosophila* immunity gene expression. *Mol. Cell. Biol.* **18**, 3527–3539. <https://doi.org/10.1128/MCB.18.6.3527> (1998).
88. Martin-Blanco, E. p38 MAPK signaling cascades: Ancient roles and new functions. *BioEssays* **22**, 637–645. [https://doi.org/10.1002/1521-1878\(200007\)22:7%3C637::AID-BIES6%3e3.0.CO;2-E](https://doi.org/10.1002/1521-1878(200007)22:7%3C637::AID-BIES6%3e3.0.CO;2-E) (2000).
89. Cytirynska, M. Studies on the role of protein kinase A in humoral immune response of *Galleria mellonella* larvae. *J. Insect Physiol.* **52**, 744–753. <https://doi.org/10.1016/j.jinsphys.2006.04.002> (2006).
90. Tsakas, S. & Marmaras, V. J. Insect immunity and its signalling: An overview. *Invertebr. Surviv. J.* **7**, 228–238 (2010).
91. Eleftherianos, I. et al. Haemocyte-mediated immunity in insects: Cells, processes and associated components in the fight against pathogens and parasites. *Immunology* **164**, 401–432. <https://doi.org/10.1111/imm.13390> (2021).
92. Trainor, J. E. & Mortimer, N. T. Immune cell production is targeted by parasitoid wasp virulence in a *Drosophila*–parasitoid wasp interaction. *Pathogens* **10**, 49. <https://doi.org/10.3390/pathogens10010049> (2021).
93. Mahanta, D. K. et al. Insect-pathogen crosstalk and the cellular-molecular mechanisms of insect immunity: Uncovering the underlying signaling pathways and immune regulatory function of non-coding RNAs. *Front Immunol.* **14**, 1169152. <https://doi.org/10.3389/fimmu.2023.1169152> (2023).
94. Yang, J. et al. The Toll/IMD pathways mediate host protection against dipteran parasitoids. *J. Insect Physiol.* **153**, 104614. <https://doi.org/10.1016/j.jinsphys.2024.104614> (2024).
95. Guzzo, C. M. et al. SUMO-binding proteins as effectors and regulators of SUMO modification. *Cancer Res.* **70**, 2184–2184. <https://doi.org/10.1158/0008-5472.FBCR09-B69> (2009).
96. Paddibhatla, I., Lee, M. J., Kalamaz, M. E., Ferrarese, R. & Govind, S. Role for sumoylation in systemic inflammation and immune homeostasis in *Drosophila* larvae. *PLoS Pathog.* **6**, e1001234. <https://doi.org/10.1371/journal.ppat.1001234> (2010).
97. Hegde, S., Soory, X. A., Kaduskar, B. & Ratnaparkhi, G. SUMO conjugation regulates immune signaling. *Fly* **14**, 62–79. <https://doi.org/10.1080/19336934.2020.1808402> (2020).
98. Sajeev, T. K. et al. SUMO and SUMOylation pathway at the forefront of host immune response. *Front. Cell Dev. Biol.* **9**, 681057. <https://doi.org/10.3389/fcell.2021.681057> (2021).
99. Zhuang, S. et al. Sns and Kirre, the *Drosophila* orthologs of Nephrin and Nephrin, direct adhesion, fusion and formation of a slit diaphragm-like structure in insect nephrocytes. *Development* **136**, 2335–2344. <https://doi.org/10.1242/dev.031609> (2009).
100. Cooper, D. N. Functional intronic polymorphisms: Buried treasure awaiting discovery within our genes. *Hum. Genom.* **4**, 284–288. <https://doi.org/10.1186/1479-7364-4-5-284> (2010).
101. Baxter, S. W. et al. Mis-spliced transcripts of nicotinic acetylcholine receptor $\alpha 6$ are associated with field evolved spinosad resistance in *Plutella xylostella* (L.). *PLoS Genet.* **6**, e1000802. <https://doi.org/10.1371/journal.pgen.1000802> (2010).
102. Vaulin, O. V., Karagodin, D. A., Novgorodova, T. A. & Glupov, V. V. Analysis of *Anopheles messeae* sl intron gene polymorphism associated with imidacloprid resistance. *J. Vector Ecol.* **45**, 220–232. <https://doi.org/10.1111/jvec.12393> (2020).
103. Salih, M. H., Al-Azzawie, A. F. & Al-Assie, A. H. A. Intronic SNPs and genetic diseases: A review. *IJRASB* **8**, 267–274. <https://doi.org/10.31033/ijrasb.8.2.36> (2021).
104. Wang, D., Guo, Y., Wrighton, S. A., Cooke, G. E. & Sadee, W. Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. *Pharmacogenom. J.* **11**, 274–286. <https://doi.org/10.1038/tpj.2010.28> (2011).
105. Field, L. M., Devonshire, A. L., Ffrench-Constant, R. H. & Forde, B. G. Changes in DNA methylation are associated with loss of insecticide resistance in the peach-potato aphid *Myzus persicae* (Sulz.). *FEBS Lett.* **243**, 323–327. [https://doi.org/10.1016/0014-5793\(89\)80154-1](https://doi.org/10.1016/0014-5793(89)80154-1) (1989).
106. Field, L. M. Methylation and expression of amplified esterase genes in the aphid *Myzus persicae* (Sulzer). *Biochem. J.* **349**, 863–868. <https://doi.org/10.1042/bj3490863> (2000).
107. Hufbauer, R. A. Pea aphid-parasitoid interactions: Have parasitoids adapted to differential resistance? *Ecology* **82**, 717–725. <https://doi.org/10.2307/2680191> (2001).
108. Wang, S. Y., Chi, H. & Liu, T. X. Demography and parasitic effectiveness of *Aphelinus asychis* reared from *Sitobion avenae* as a biological control agent of *Myzus persicae* reared on chili pepper and cabbage. *Biol. Control* **92**, 111–119. <https://doi.org/10.1016/j.biocontrol.2015.10.010> (2016).
109. Kumar, S., Kashyap, S. & Soni, S. The foraging behavior of *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae) and *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) on *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). *Phytoparasitica* **47**, 351–360. <https://doi.org/10.1007/s12600-019-00735-0> (2019).
110. Schwenke, R. A., Lazzaro, B. P. & Wolfner, M. F. Reproduction–immunity trade-offs in insects. *Annu. Rev. Entomol.* **61**, 239–256. <https://doi.org/10.1146/annurev-ento-010715-023924> (2016).
111. Cotter, S. C., Kruuk, L. E. B. & Wilson, K. Costs of resistance: Genetic correlations and potential trade-offs in an insect immune system. *J. Evol. Biol.* **17**, 421–429. <https://doi.org/10.1046/j.1420-9101.2003.00655.x> (2004).
112. Freitag, D., Ots, I., Vanatoo, A. & Hörak, P. Immune response is energetically costly in white cabbage butterfly pupae. *Proc. R. Soc. Lond.* **270**, S220–S222. <https://doi.org/10.1098/rsbl.2003.0069> (2003).
113. Kang, X. L., Zhang, M., Wang, K., Qiao, X. F. & Chen, M. H. Molecular cloning, expression pattern of multidrug resistance associated protein 1 (mrp1, abcc1) gene, and the synergistic effects of verapamil on toxicity of two insecticides in the bird cherry-oat aphid. *Arch. Insect Biochem. Physiol.* **92**, 65–84. <https://doi.org/10.1002/arch.21334> (2016).
114. Huang, X., Liu, D., Zhang, R. & Shi, X. Transcriptional responses in defense-related genes of *Sitobion avenae* (Hemiptera: Aphididae) feeding on wheat and barley. *Mol. Entomol.* **112**, 382–395. <https://doi.org/10.1093/jee/toy329> (2019).
115. Calow, P. Ecology, evolution and energetics: A study in metabolic adaptation. *Adv. Ecol. Res.* **10**, 1–62. [https://doi.org/10.1016/S0065-2504\(08\)60233-0](https://doi.org/10.1016/S0065-2504(08)60233-0) (1977).
116. Ojala, K., Julkunen-Tiitto, R., Lindström, L. & Mappes, J. Diet affects the immune defence and life-history traits of an Arctiid moth *Parasemia plantaginis*. *Evol. Ecol. Res.* **7**, 1153–1170 (2005).
117. Singer, M. S., Mason, P. A. & Smilanich, A. M. Ecological immunology mediated by diet in herbivorous insects. *Integr. Comp. Biol.* **54**, 913–921. <https://doi.org/10.1093/icb/ucu089> (2014).

118. Vinson, S. B. & Iwantsch, G. F. Host regulation by insect parasitoids. *Q. Rev. Biol.* **55**, 143–165. <https://doi.org/10.1086/411731> (1980).
119. Sequeira, R. & Mackauer, M. Covariance of adult size and development time in the parasitoid wasp *Aphidius ervi* in relation to the size of its host, *Acyrtosiphon pisum*. *Evol. Ecol.* **6**, 34–44. <https://doi.org/10.1007/BF02285332> (1992).
120. Himler, A. G. et al. Rapid spread of a bacterial symbiont in an invasive whitefly is driven by fitness benefits and female bias. *Science* **332**, 254–256. <https://doi.org/10.1126/science.1199410> (2011).
121. Cass, B. N. et al. Conditional fitness benefits of the *Rickettsia* bacterial symbiont in an insect pest. *Oecologia* **180**, 169–179. <https://doi.org/10.1007/s00442-015-3436-x> (2015).
122. Klot, A., Kontsedalov, S., Lebedev, G., Czosnek, H. & Ghanim, M. Combined infection with *Tomato yellow leaf curl virus* and *Rickettsia* influences fecundity, attraction to infected plants and expression of immunity-related genes in the whitefly *Bemisia tabaci*. *J. Gen. Virol.* **100**, 721–731. <https://doi.org/10.1099/jgv.0.001233> (2019).
123. Bashir-Tanoli, S. & Tinsley, M. C. Immune response costs are associated with changes in resource acquisition and not resource reallocation. *Funct. Ecol.* **28**, 1011–1019. <https://doi.org/10.1111/1365-2435.12236> (2014).
124. Nystrand, M. & Dowling, D. K. Transgenerational interactions involving parental age and immune status affect female reproductive success in *Drosophila melanogaster*. *Proc. R. Soc. B* **281**, 20141242. <https://doi.org/10.1098/rspb.2014.1242> (2014).
125. Leclair, M. et al. Consequences of coinfection with protective symbionts on the host phenotype and symbiont titres in the pea aphid system. *Insect Sci.* **24**, 798–808. <https://doi.org/10.1111/1744-7917.12380> (2017).
126. Chen, D. Q., Montllor, C. B. & Purcell, A. H. Fitness effects of two facultative endosymbiotic bacteria on the pea aphid, *Acyrtosiphon pisum*, and the blue alfalfa aphid. *A. Kondoi. Entomol. Exp. Appl.* **95**, 315–323. <https://doi.org/10.1046/j.1570-7458.2000.00670.x> (2000).
127. Sakurai, M., Koga, R., Tsuchida, T., Meng, X.-Y. & Fukatsu, T. *Rickettsia* symbiont in the pea aphid *Acyrtosiphon pisum*: Novel cellular tropism, effect on host fitness, and interaction with the essential symbiont *Buchnera*. *Appl. Environ. Microbiol.* **71**, 4069–4075. <https://doi.org/10.1128/AEM.71.7.4069-4075.2005> (2005).
128. Baumann, P., Moran, N. A. & Baumann, L. The evolution and genetics of aphids endosymbionts. *Bioscience* **47**, 12–20. <https://doi.org/10.2307/1313002> (1997).
129. Silva, F. J., Ham, R. C. H. J., Sabater, B. & Latorre, A. Structure and evolution of the leucine plasmids carried by the endosymbiont (*Buchnera aphidicola*) from aphids of the family Aphididae. *Microbiol. Lett.* **168**, 43–49. <https://doi.org/10.1111/j.1574-6968.1998.tb13253.x> (1998).
130. Viñuelas, J. et al. Conservation of the links between gene transcription and chromosomal organization in the highly reduced genome of *Buchnera aphidicola*. *BMC Genom.* **8**, 143. <https://doi.org/10.1186/1471-2164-8-143> (2007).
131. Corbin, C. et al. Heritable symbionts in a world of varying temperature. *Heredity* **118**, 10–20. <https://doi.org/10.1038/hdy.2016.71> (2017).
132. Hunt, J., Bussière, L. F., Jennions, M. D. & Brooks, R. What is genetic quality?. *Trends Ecol. Evol.* **19**, 329–333. <https://doi.org/10.1016/j.tree.2004.03.035> (2004).
133. Minchella, D. J. Host life-history variation in response to parasitism. *Parasitology* **90**, 205. <https://doi.org/10.1017/S0031182000049143> (1985).
134. Ferrari, J., Muller, C. B., Kraaijeveld, A. R. & Godfray, H. C. J. Clonal variation and covariation in aphid resistance to parasitoids and a pathogen. *Evolution* **55**, 1805–1814. [https://doi.org/10.1554/0014-3820\(2001\)055\[1805:CVACIA\]2.0.CO;2](https://doi.org/10.1554/0014-3820(2001)055[1805:CVACIA]2.0.CO;2) (2001).
135. von Burg, S., Ferrari, J., Müller, C. B. & Vorburger, C. Genetic variation and covariation of susceptibility to parasitoids in the aphid *Myzus persicae*—No evidence for trade-offs. *Proc. R. Soc. B* **275**, 1089–1094. <https://doi.org/10.1098/rspb.2008.0018> (2008).
136. Sandrock, C., Gouskov, A. & Vorburger, C. Ample genetic variation but no evidence for genotype specificity in an all-parthenogenetic host-parasitoid interaction. *J. Evol. Biol.* **23**, 578–585. <https://doi.org/10.1111/j.1420-9101.2009.01925.x> (2010).
137. Vilcinskis, A. The role of epigenetics in host–parasite coevolution: Lessons from the model host insects *Galleria mellonella* and *Tribolium castaneum*. *Zoology* **119**, 273–280. <https://doi.org/10.1016/j.zool.2016.05.004> (2016).
138. Turlings, T. C. J. & Benrey, B. Effects of plant metabolites on the behavior and development of parasitic wasps. *Écoscience* **5**, 321–333. <https://doi.org/10.1080/11956860.1998.11682472> (1998).
139. Desneux, N., Barta, R. J., Hoelmer, K. A., Hopper, K. R. & Heimpel, G. E. Multifaceted determinants of host specificity in an aphid parasitoid. *Oecologia* **160**, 387–398. <https://doi.org/10.1007/s00442-009-1289-x> (2009).
140. Harvey, J. A. & Gols, R. Effects of plant-mediated differences in host quality on the development of two related endoparasitoids with different host-utilization strategies. *J. Insect Physiol.* **107**, 110–115. <https://doi.org/10.1016/j.jinsphys.2018.03.006> (2018).
141. Brown, B. L., Creed, R. P., Skelton, J., Rollins, M. A. & Farrell, K. J. The fine line between mutualism and parasitism: Complex effects in a cleaning symbiosis demonstrated by multiple fields experiments. *Oecologia* **170**, 199–207. <https://doi.org/10.1007/s00442-012-2280-5> (2012).
142. Martinez, J. et al. Should symbionts be nice or selfish? Antiviral effects of *Wolbachia* are costly but reproductive parasitism is not. *PLoS Pathog.* **11**, e1005021. <https://doi.org/10.1371/journal.ppat.1005021> (2015).
143. Hopkins, S. R., Wojdak, J. M. & Belden, L. K. Defensive symbionts mediate host–parasite interactions at multiple scales. *Trends Parasitol.* **33**, 53–64. <https://doi.org/10.1016/j.pt.2016.10.003> (2017).
144. Hoagland, D. R. & Snyder, W. C. Nutrition of strawberry plant under controlled conditions: (a) effects of deficiencies of boron and certain other elements: (b) susceptibility to injury from sodium salts. *PASHS* **30**, 288–294 (1934).
145. McCullagh, P. & Nelder, J. A. *Generalized Linear Models* 2nd edn. (Chapman & Hall, 1989).
146. Haine, E. R. Symbiont-mediated protection. *Proc. R. Soc.* **275**, 353–361. <https://doi.org/10.1098/rspb.2007.1211> (2008).
147. Moran, N. A., McCutcheon, J. P. & Nakabachi, A. Genomics and evolution of heritable bacterial symbionts. *Annu. Rev. Genet.* **42**, 165–190. <https://doi.org/10.1146/annurev.genet.41.110306.130119> (2008).
148. Oliver, K. M., Degnan, P. H., Burke, G. R. & Moran, N. A. Facultative symbionts of aphids and the horizontal transfer of ecologically important traits. *Annu. Rev. Entomol.* **55**, 247–266. <https://doi.org/10.1146/annurev-ento-112408-085305> (2010).
149. Vorburger, C. Symbiont-conferred resistance to parasitoids in aphids—Challenges for biological control. *Biol. Control* **116**, 17–26. <https://doi.org/10.1016/j.biocontrol.2017.02.004> (2018).
150. Fan, Z. Y. et al. *Rickettsia* infection benefits its whitefly hosts by manipulating their nutrition and defense. *Insects* **13**, 1161. <https://doi.org/10.3390/insects13121161> (2022).
151. Krips, O. E., Witul, A., Willems, P. E. L. & Dicke, M. Intrinsic rate of population increase of the spider mite *Tetranychus urticae* on the ornamental crop gerbera: Intraspecific variation in host plant and herbivore. *Entomol. Exp. Appl.* **89**, 159–168. <https://doi.org/10.1046/j.1570-7458.1998.00395.x> (1998).
152. Ricklefs, R. E. *A economia da natureza* 5th edn. (Guanabara Koogan, 2003).
153. Maia, A. H. N. & Luiz, A. J. B. Programa SAS para análise de tabelas de vida e fertilidade de artrópodes: o método jackknife. (Comunicado Técnico, 33), Embrapa: Jaguariúna, p. 11 (2006).
154. Maia, A. H. N., Luiz, A. J. B. & Campanhola, C. Statistical inference on associated fertility life table parameters using Jackknife technique: Computational aspects. *J. Econ. Entomol.* **93**, 511–518. <https://doi.org/10.1603/0022-0493-93.2.511> (2000).
155. Therneau, T. A package for survival analysis in R. R package version 3.2-7. Available online at: <https://CRAN.R-project.org/package=survival> (2020).
156. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis* (Springer, 2016).
157. Moral, R. A., Hinde, J. & Demétrio, C. G. B. Half-normal plots and overdispersed models in R: The hnp Package. *J. Stat. Softw.* **81**, 1–23. <https://doi.org/10.18637/jss.v081.i10> (2017).

158. Andrews, S. FastQC: A quality control tool for high throughput sequence data [Online]. Available online at: <http://www.bioinformatics.babraham.ac.uk/projects/fastqc> (2010).
159. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170> (2014).
160. Singh, K. S. et al. Global patterns in genomic diversity underpinning the evolution of insecticide resistance in the aphid crop pest *Myzus persicae*. *Commun. Biol.* **4**, 847. <https://doi.org/10.1038/s42003-021-02373-x> (2021).
161. Langmead, B., Trapnell, C., Pop, M. & Salzberg, S. L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* **10**, R25. <https://doi.org/10.1186/gb-2009-10-3-r25> (2009).
162. Li, H. et al. 1000 Genome project data processing subgroup, the sequence alignment/map format and SAMtools. *Bioinformatics* **25**(16), 2078–2079. <https://doi.org/10.1093/bioinformatics/btp352> (2009).
163. Poplin, R. et al. Scaling accurate genetic variant discovery to tens of thousands of samples. *bioRxiv* 201178. <https://doi.org/10.1101/201178> (2017).
164. Quinlan, A. R. & Hall, I. M. BEDTools: A flexible suite of utilities for comparing genomic features. *Bioinformatics* **26**, 841–842. <https://doi.org/10.1093/bioinformatics/btq033> (2010).
165. Neph, S. et al. BEDOPS: High-performance genomic feature operations. *Bioinformatics* **28**, 1919–1920. <https://doi.org/10.1093/bioinformatics/bts277> (2012).
166. Cingolani, P. et al. A program for annotating and predicting the effects of single nucleotide polymorphisms. *SnpEff. Fly* **6**, 80–92. <https://doi.org/10.4161/fly.19695> (2012).
167. Cingolani, P. et al. Using *Drosophila melanogaster* as a model for genotoxic chemical mutational studies with a new program. *SnpSift. Front. Genet.* **15**, 3–35. <https://doi.org/10.3389/fgene.2012.00035> (2012).
168. Buchfink, B., Xie, C. & Huson, D. Fast and sensitive protein alignment using DIAMOND. *Nat. Methods* **12**, 59–60. <https://doi.org/10.1038/nmeth.3176> (2015).
169. Kanehisa, M. & Goto, S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **28**, 27–30. <https://doi.org/10.1093/nar/28.1.27> (2000).
170. Kanehisa, M. Toward understanding the origin and evolution of cellular organisms. *Protein Sci.* **28**, 1947–1951. <https://doi.org/10.1002/pro.3715> (2019).
171. Kanehisa, M., Furumichi, M., Sato, Y., Matsuura, Y. & Ishiguro-Watanabe, M. KEGG: Biological systems database as a model of the real world. *Nucleic Acids Res.* **53**, D672–D677. <https://doi.org/10.1093/nar/gkac909> (2025).

Acknowledgements

We thank Dr. Silvana Savaris, Dr. Eduardo Shimbori (ESALQ/USP) and Dr. Ana Paula Wengrat for the identification of the insect species used in this work. To Dr. Roberto A. Zucchi (ESALQ/USP) for allowing the use of his laboratories for the development of part of the experiments reported. We also thank the São Paulo Research Foundation (FAPESP) for providing the grant to the first author (Grant: 2019/06876-1, 2020/07896-3), Brazilian National Research Council (CNPq) (grant: 2022/164221) and the Coordination of Superior Level Staff Improvement (CAPES) (Finance Code 001) for additional funds.

Author contributions

Conceptualization and methodology: MPG and FLC. Investigation and data curation: MPG. Formal analysis: MPG, RAM, IARL, TB, RR and ECL. Supervision and funding acquisition: FLC. Writing—original draft: MPG. Writing—editing: MPG and FLC. Review: All authors.

Funding

Funds were provided by the São Paulo Research Foundation (FAPESP) (grant: 2019/06876-1, 2020/07896-3), the Brazilian National Research Council (CNPq) (grant: 2022/164221) and the Coordination of Superior Level Staff Improvement (CAPES) (Finance Code 001).

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-025-19494-6>.

Correspondence and requests for materials should be addressed to F.L.C.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2025