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

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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 exploitation[6]. 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 aphids[22], including *Myzus persicae* (Sulzer) (Hemiptera: 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

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developmental capabilities on hundreds of plant species in more than 50 families[23]. 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 growth[24]. In addition, aphids can cause indirect damage by transmitting viruses and promoting black mold growth by excreting honeydew[24]. 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-effects[25].

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 suitability[26] and on parasitoid attraction[27], the effects of the secondary symbiont *Rickettsiella viridis* in the rate of parasitization[28], 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 ranges[31]. 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 virulence[38]. Defensive symbionts can affect host successful parasitization by resource limitation, such as lipids[39], 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 attraction[45]. 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 programs[46].

Host-parasitoid interactions are frequency-dependent, which can be observed more strongly in laboratory than in field studies[47]. The frequency-dependent interaction combined with genetic variation allows interacting species to co-evolve[2]. Parasitoids are selected to evade defense mechanisms of common host genotypes, contributing to the selection of hosts with rare resistant genotypes[48]. 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 responses[47].

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 repair[68]. These variants can be broadly categorized into five classes: deletions, duplications, insertions, inversions, and translocations[69]. Structural variants introduce variability in gene copy number, position, orientation, and occasionally a combination of these effects[70]. 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).

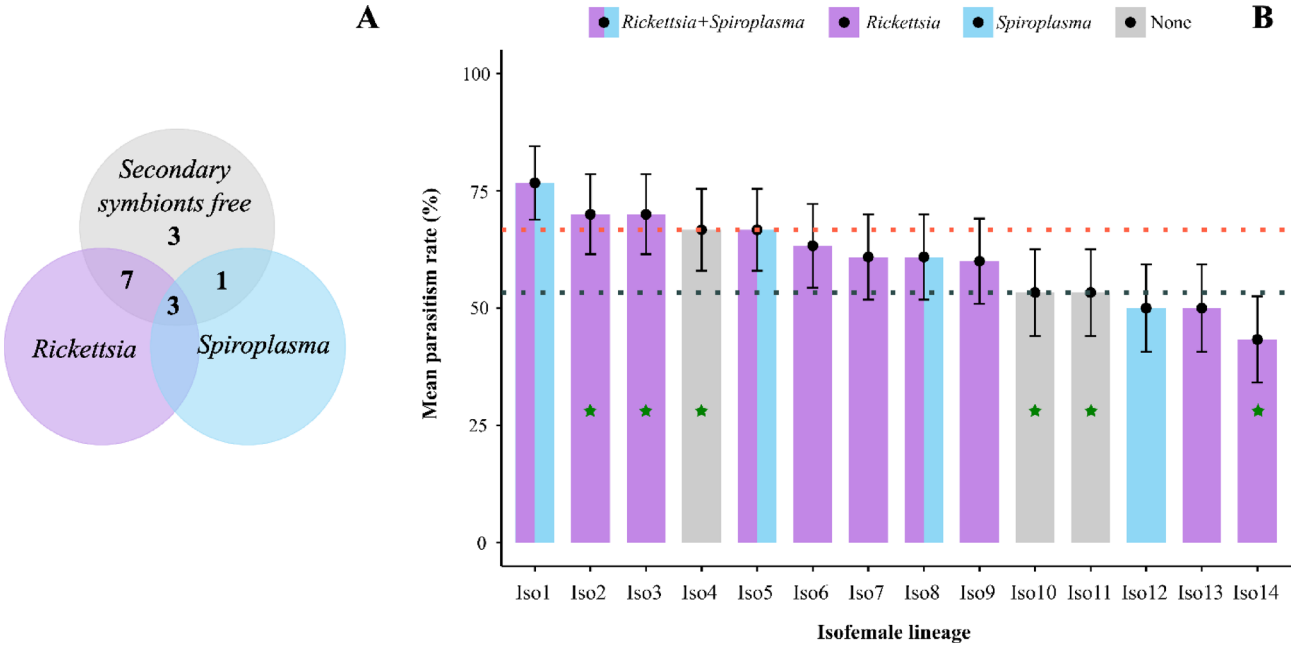

Fig. 1. Venn diagram showing the number of isofemale lineages of *Myzus persicae* with isolated infection or co-infection by the secondary symbionts *Rickettsia* and *Spiroplasma* (**A**) and mean parasitization (%) (±) of isofemale lineages of *Myzus persicae* by *Diaeretiella rapae* (Red dotted line = 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

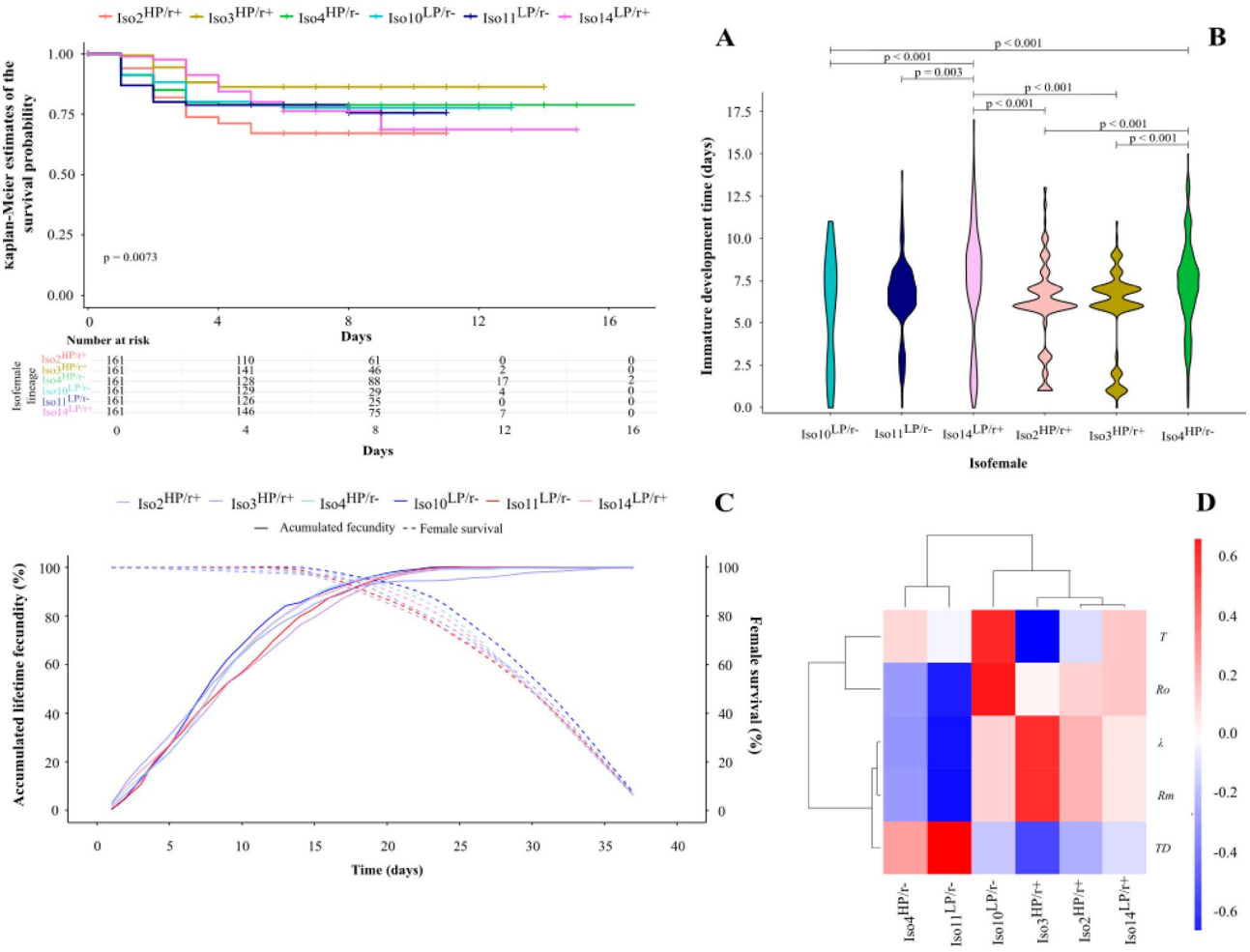

Fig. 2. Biological parameters evaluated for selected lineages of *Myzus persicae* with high ($Iso2^{HP/r+}$, $Iso3^{HP/r+}$, $Iso4^{HP/r-}$) and low ($Iso10^{LP/r-}$, $Iso11^{LP/r-}$, $Iso14^{LP/r+}$) parasitization by *Diaeretiella rapae*. (**A**) Kaplan–Meier estimates of survival curves using Cox proportional hazards models, $p < 0.05$. (**B**) Immature development time (days ± 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 isoline 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*) of $Iso4^{HP/r-}$ 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*, *Rm*, *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 *Rm* 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 *Ro* 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

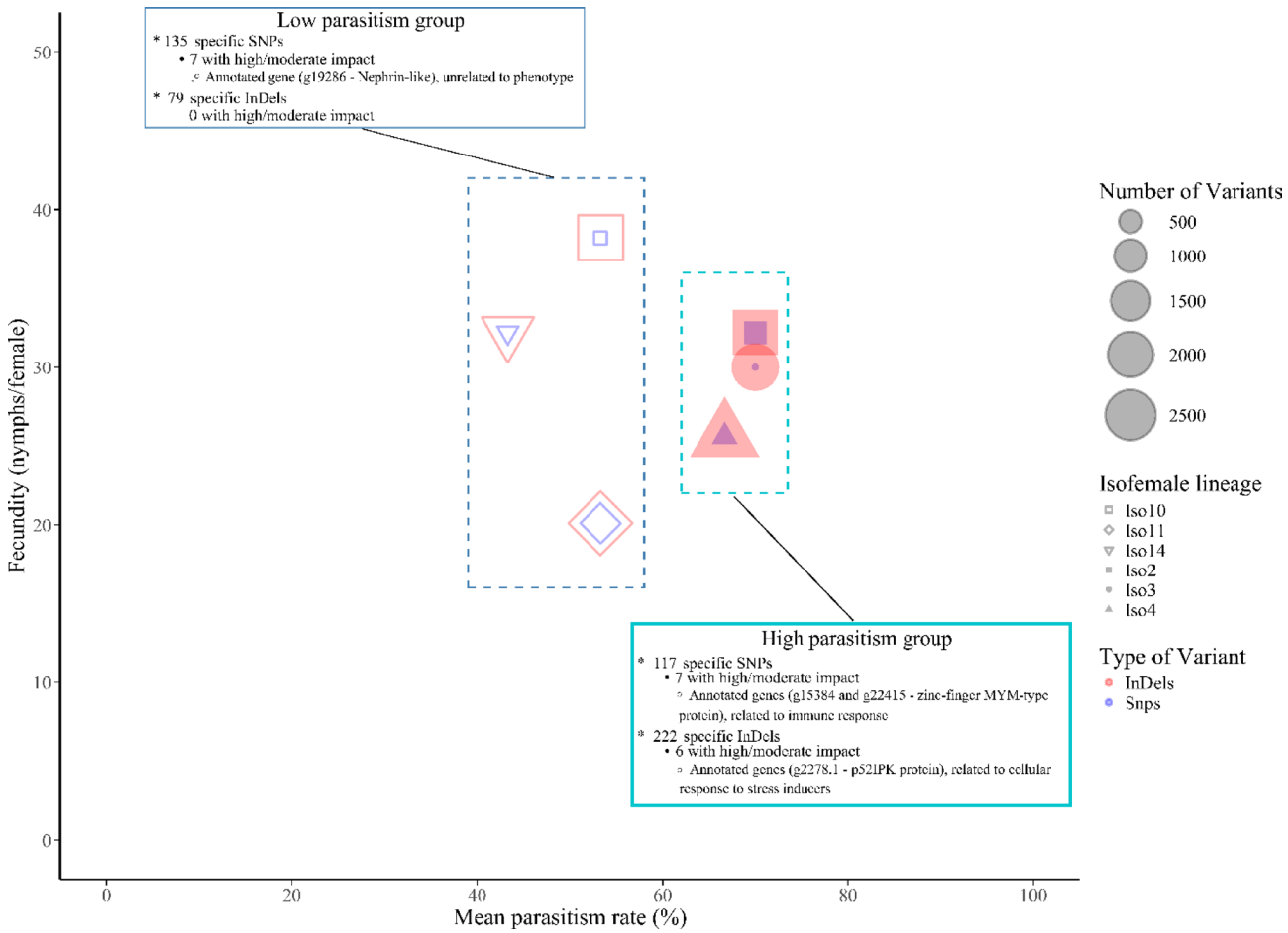

Fig. 3. Graphical summary results with parasitism, fecundity and number of variants (InDels and SNPs) in *Myzus persicae* isofemale lineages with high (Iso2$^{HP/r+}$, Iso3$^{HP/r+}$, Iso4$^{HP/r-}$) and low (Iso10$^{LP/r-}$, Iso11$^{LP/r-}$, Iso14$^{LP/r+}$) parasitization by *Diaeretiella rapae*.

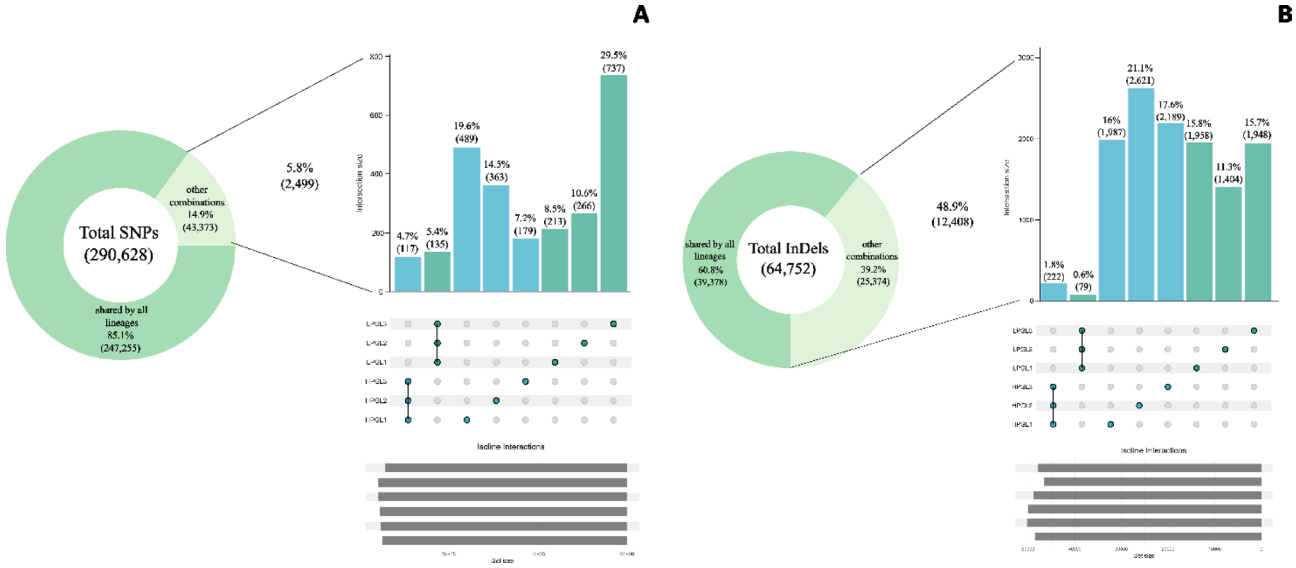

Fig. 4. Overview of the SNPs (A) and InDels (B) that survived the filtering protocols, common to all the *Myzus persicae* isofemale lineages studied (Iso2HP/ r+, Iso3HP/r+, Iso4HP/r-, Iso10LP/r-, Iso11LP/r- and Iso14LP/r+), shared by two or more lineages, or unique to each group (high and low parasitism)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

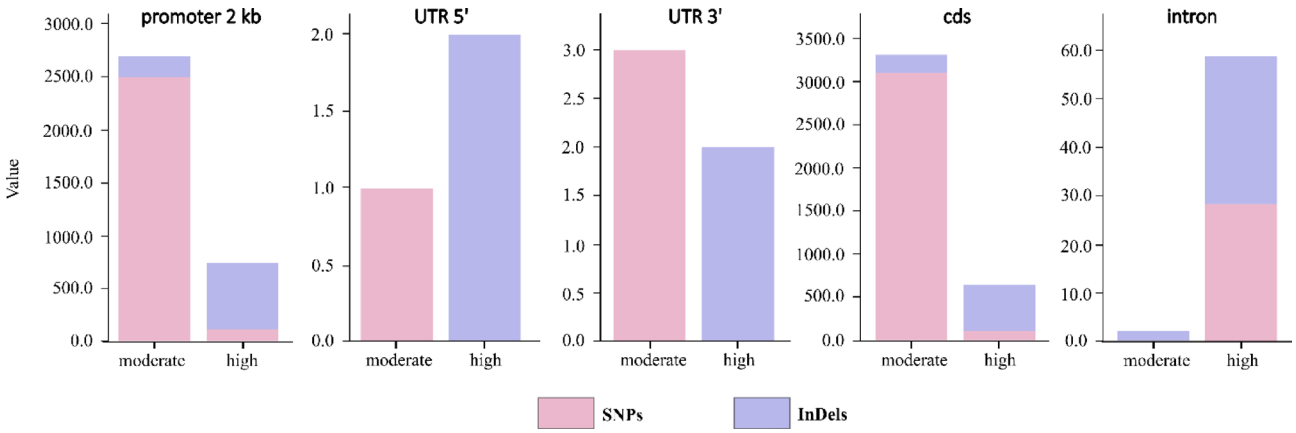

Fig. 5. Overview of the moderate and high SNPs (pink color) and Indels (purple color) by genomic regions present in the genome of *Myzus persicae* lineages with high (Iso2$^{HP/r+}$, Iso3$^{HP/r+}$, Iso4$^{HP/r-}$) and low (Iso10$^{LP/r-}$, Iso11$^{LP/r-}$, Iso14$^{LP/r+}$) parasitization by *Diaeretiella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

	All lineages		HP group		LP group		Lineage specific		Other combination	
Effect type	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$^{HP/r+}$, Iso3$^{HP/r+}$, Iso4$^{HP/r-}$, Iso10$^{LP/r-}$, Iso11$^{LP/r-}$ and Iso14$^{LP/r+}$) (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 size[71], pathogen vectoring capacity[72], 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 apomixis[64]. Previous studies with clonal lines of the aphid *Acyrthosiphon 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 plants[71], although several mechanisms have been reported to produce genetic variation in clonal aphids, such as DNA mutations[76], 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 response[86]. Several of

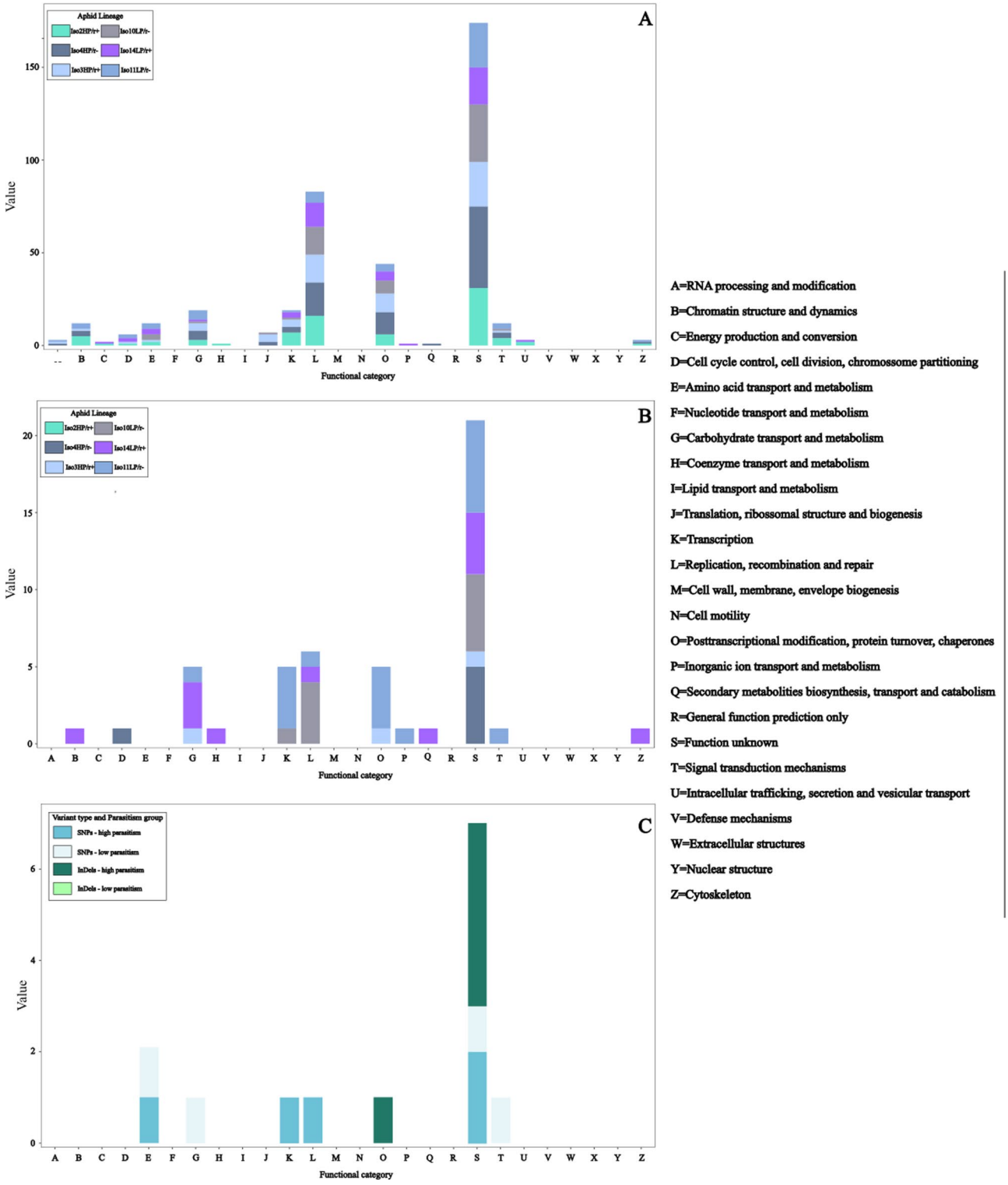

Fig. 6. The functional COG classification of genes associated with high and moderate SNPs (**A**) and InDels (**B**) detected in *Myzus persicae* lineages selected for high (Iso2HP/ r + , Iso3HP/r + , Iso4HP/r-) and low (Iso10LP/r-, Iso11LP/r, Iso14LP/r +) parasitization by *Diaeretiella rapae,* or unique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

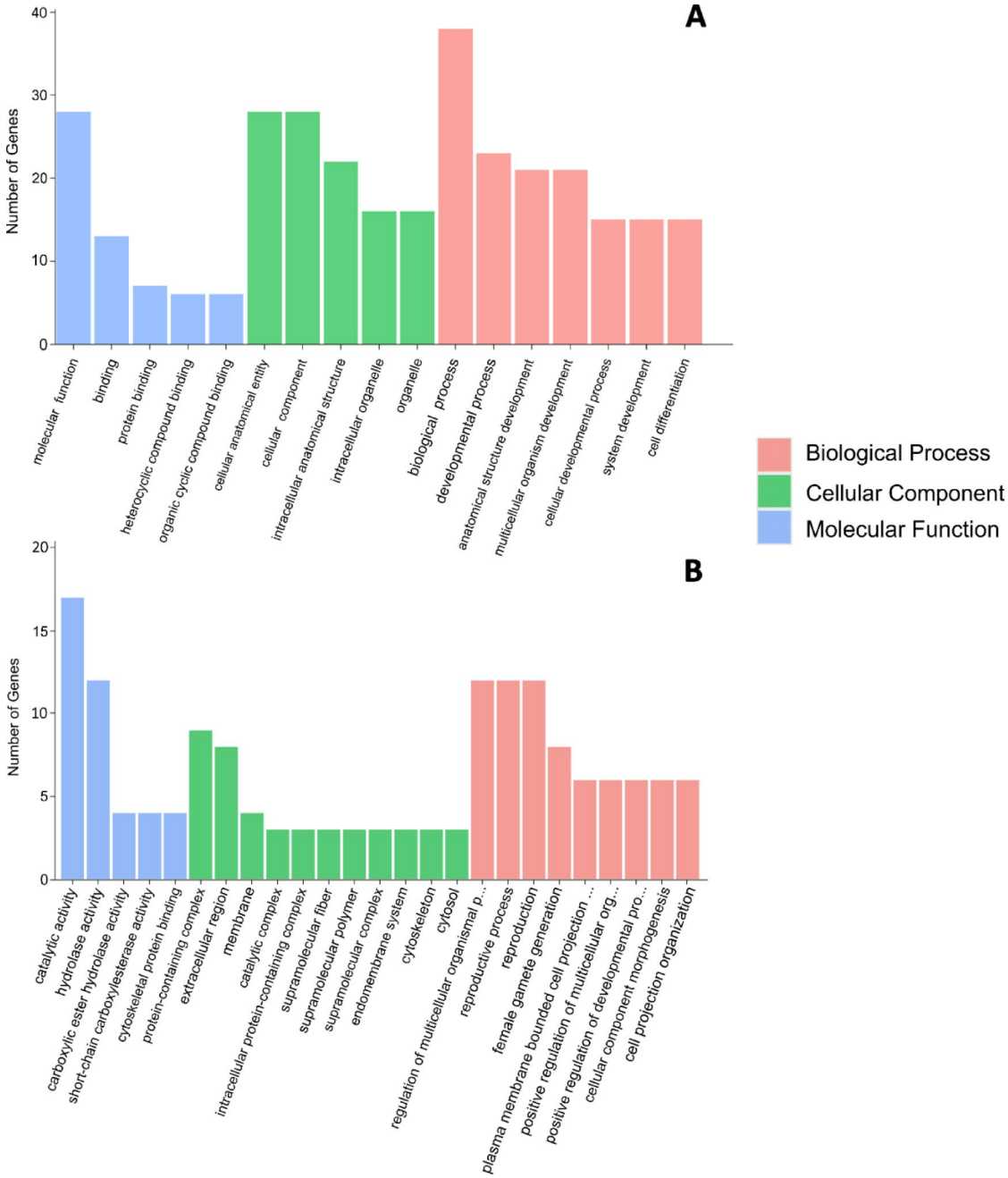

Fig. 7. Top five Gene Ontology (GO) functional analysis of genes associated with high and moderate SNPs (**A**) and InDels (**B**) unique to the high (Iso2HP/ r + , Iso3HP/r + , Iso4HP/r-) or low parasitization (Iso10LP/r-, Iso11LP/r, Iso14LP/r +) group or to each individual lineage of *Myzus persicae* 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 development[94]. 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 SUMOylating 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 nephrocytes[99]. 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 relationship[100] and they have been demonstrated to affect insect resistance to insecticides[101,102], the development of human diseases[103] and the response to drugs[104]. 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 longevity[66]. 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 insects[110], other life history traits such as development time are rarely studied[111].

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 energy[115]. 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 parasititization[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 fecundity[123]. 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 response[124]. 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 symbionts[125] 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 effects[131]. 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 conditions[132], 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 isoline 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 $ZnSO_4.H_2O$, 30 mg/L $CuSO_4.5H_2O$, and 90 mg/L FeNaEDTA) and macronutrients (140 mg/L $NH_4H_2PO_4$, 830 mg/L $MgSO_4$, 360 mg/L KNO_3, 80 mg/L NH_4NO_3, and 905 mg/L CaN_2O_6) adapted from Hoagland and Snyder[144].

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 models[145], 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 protocol[64] (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 flies[150].

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 (*Rm*), net reproduction rate (*Ro*), 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 Luiz[153], 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.0.11.8[158]. 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 software[161]. *SAMtools* v.1.21 was used to convert SAM files into sorted BAM files[162] (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.2.31.1[164], *vcf2bed* v.2.4.41[165], 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.5.2[166]. Subsequently, SNPs and InDels were specifically selected using the filtering tool provided by the *SnpSift* software v.5.2[167] 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 aligner[168], 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).

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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