Title: Diversity in viral resistance emerges from host genotype and infection order effects

Authors: Maija Jokinen[1], Hanna Susi[1, 2] & Anna-Liisa Laine[1]

[1] Department of Organismal and Evolutionary Biology, Faculty of Biological and Environmental Sciences, PO BOX 65 00014 University of Helsinki, Finland

[2] Department of Agricultural Sciences, Faculty of Agriculture and Forestry, PO BOX 27, 00014, University of Helsinki, Finland

Email: M.J. maija.jokinen@helsinki.fi, H.S. hanna.susi@helsinki.fi, A-L.L. anna-liisa.laine@helsinki.fi

Correspondence: Maija Jokinen, maija.jokinen@helsinki.fi and Anna-Liisa Laine, anna-liisa.laine@helsinki.fi Organismal and Evolutionary Biology Research Programme, Faculty of Biological and Environmental Sciences, PO BOX 65 00014 University of Helsinki, Finland

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Abstract

- While viruses are predicted to be the most diverse group of parasites wild plant hosts encounter, the extent and mechanisms maintaining viral resistance diversity remains poorly understood. Here, we test the hypothesis that allocation trade-offs maintain genetic variation in viral resistance and assess whether phenotypic resistance variation may may arise from altered expression under multiple viral attack.

- We inoculated clones from 24 *Plantago lanceolata* genotypes with two viruses to quantify intraspecific variation among host genotypes and test possible trade-offs in resistance to either of the viruses. Furthermore, we performed subsequent viral inoculations to investigate if prior viral infection changes host resistance phenotype.

- We found striking intraspecific variation in resistance among the 24 host genotypes against the two studied viruses, with limited evidence for trade-offs maintaining this variation. We also found that prior infection by *Plantago lanceolata enamovirus* altered the host resistance phenotype, rendering the host more vulnerable to subsequent infection.

- Jointly, our results show that intraspecific variation in resistance may have a substantial role in mitigating viral infections in wild hosts. Furthermore, our results highlight the importance of arrival order for the resistance phenotype and for shaping viral coinfections.

Introduction

The benefits of host resistance against parasites are clear, yet wild hosts are known to harbour substantial diversity in resistance across populations and among genotypes (Ericson, Burdon and Müller, 2002; Laine, 2004; Broekgaarden *et al.*, 2011; Laine *et al.*, 2011; Ekroth, Rafaluk-Mohr and King, 2019). Indeed, parasites can only colonise susceptible hosts, and hence, host resistance is expected to be an important determinant of host fitness and reproduction (Little and Ebert, 1999; Fraile and García-Arenal, 2016; Hily *et al.*, 2016; Sallinen *et al.*, 2020; Höckerstedt, Susi and Laine, 2021). Throughout their life cycle, plants are exposed to a wide range of parasites from several kingdoms of life, including fungi, bacteria, insects, and viruses. While viruses are predicted to be the most diverse group of parasites wild plant hosts encounter, much of this viral diversity still remains undiscovered (Roossinck, 2005; Maclot *et al.*, 2020; Yang *et al.*, 2022). Though many of the discovered viruses are described to be pathogenic, almost nothing is known of the intraspecific variation in plant resistance against viruses in the wild (Malmstrom, Martin and Gagnevin, 2022).

Intraspecific variation in both host resistance and parasite infectivity is predicted to be maintained through coevolution (Hamilton, 1980; Anderson and May, 1982; Gibson, 2022). Negative frequency-dependent selection favours parasites that can infect the most common host genotypes while rare host genotypes escape infection and thereby have higher fitness in the presence of parasites (Hamilton, 1980), with resistance imposing a cost on the host in terms of growth and reproduction (Leonard, 1977; Ashby and King, 2017). Indeed, experimental work on host resistance and analyses of plant resistance genes have shown resistance to be costly in some systems (Tian *et al.*, 2003; Ciota *et al.*, 2011; Auld *et al.*, 2013; Brown and Rant, 2013a; Cheatsazan *et al.*, 2013; Giolai and Laine, 2024), although there is variation in this trend across study systems (Antonovics and Thrall, 1994; Bray *et al.*, 2022).

While the cost of resistance is often studied in terms of its impact on different traits of host fitness, allocation costs against a specific parasite may constrain host resources for resistance against the myriad of other parasites the host encounters (Stearns, 1989; Bergelson and Purrington, 1996; Brown and Rant, 2013a). For example, in barley the resistance locus *mlo* conferred resistance to powdery mildew while increasing susceptibility to Ramularia leaf spot

disease (McGrann *et al.*, 2014). Conversely, limited evidence shows that a single resistance loci can have significant effects against several parasites (Ali *et al.*, 2013; Lopez-Zuniga *et al.*, 2019). Resistance may also be context-dependent, as attack by multiple parasites can alter the expression of the resistance phenotype (Brown and Rant, 2013b; Hückelhoven *et al.*, 2013; Tollenaere, Susi and Laine, 2016). Over time, resistance may also be vulnerable to resistance breakdown in the face of rapidly evolving pathogens (Bergelson *et al.*, 2001; Hillung *et al.*, 2014; González, Butković and Elena, 2019).

While coevolutionary theory considers resistance to be a fixed trait, in reality an additional layer of variation may be introduced by phenotypic plasticity whereby the expression of resistance is context dependent. Research on natural populations has revealed that multiple parasites can infect a single host simultaneously and the complex interactions between hosts and parasites play an important role in shaping these within-host parasite communities (Susi *et al.*, 2015, 2019). Within-host parasite communities are often formed through sequential coinfections, where the time and the interval of the infection events can vary (Natsopoulou *et al.*, 2015; Marchetto and Power, 2018; Karvonen, Jokela and Laine, 2019). In sequential coinfections, the initial infection can change the host resistance phenotype to be more susceptible or resistant to subsequent infection (Fukami, 2015; Debray *et al.*, 2022; Jokinen *et al.*, 2023). First infection can elevate the host immune response and thus inhibit the colonisation by subsequent parasite (Ziebell and Carr, 2010; Mauch-Mani *et al.*, 2017). On the other hand, defence against first-arriving parasite may incur costs to the host, rendering it susceptible to secondary infection (Morris, Cleary and Clarke, 2017; Wang *et al.*, 2018). Thus, the interplay between the host and its parasites may be dynamic and change during the course of infection generating phenotypic variation in host resistance that may be difficult to predict based on their genotype alone.

To address the knowledge gap of plant intraspecific variation in resistance against viral infection and the role trade-offs and phenotypic plasticity contributing to this variation, we conducted a large inoculation experiment to study intraspecific resistance variation among host genotypes during viral infection. We inoculated 24 *Plantago lanceolata* genotypes with two different *P. lanceolata* infecting viruses: *Plantago lanceolata closterovirus* and *Plantago lanceolata enamovirus*. To evaluate differences in resistance against the two viruses and to investigate possible allocation costs in defence between the studied viruses, we performed single viral inoculations with each virus species on each host genotype. Additionally, sequential viral inoculations were conducted on a subset of the genotypes to study changes in resistance

phenotypes under viral coinfection. Specifically, we ask: 1) Can we detect intraspecific variation among *P. lanceolata* genotypes in resistance against the two viruses? 2) Can we detect allocation costs in viral resistance to different viruses among host genotypes? 3) Can we identify allocation costs between resistance and fitness traits during viral infection? 4) Can we detect changes in resistance phenotype when the host is exposed to sequential infections? 5) Are there differences among host genotypes in their responses to sequential infections?

Materials and Methods

Study species

The host, *P. lanceolata*, is a perennial herb that reproduces sexually through wind-dispersed pollen and asexually via side rosettes (Sagar and Harper, 1964). *Plantago lanceolata* is distributed worldwide. In Finland, *P. lanceolata* is found in the Åland Islands, where it typically grows on dry meadows and forms a network of over 4000 populations, varying in size and connectivity (Jousimo *et al.*, 2014; Höckerstedt *et al.*, 2022). The size and location of these populations have been monitored since 1990 as part of metapopulation studies of the Glanville fritillary (*Melitaea cinxia*) butterfly (Hanski *et al.*, 1995; Ojanen *et al.*, 2013).

Viruses associated with *P. lanceolata* in the Åland Islands have been studied since 2013, and several virus families have been detected from this system with small-RNA sequencing technology (Susi *et al.*, 2019; Norberg *et al.*, 2023). Five viruses have been characterized in more detail; PCR primers have been developed for *Plantago lanceolata latent virus* (PlLV) in the genus *Capulavirus* (Susi *et al.*, 2017), *Plantago lanceolata caulimovirus* in the genus *Caulimovirus* (Susi *et al.*, 2019), *Plantago lanceolata betapartitivirus* in the genus *Betapartitivirus* (Susi *et al.*, 2019), *Plantago enamovirus* in the genus *Enamovirus* (Susi *et al.*, 2019) and *Plantago closterovirus* in the genus *Closterovirus* (Susi *et al.*, 2019). For clarity, the studied viruses are hereafter referred to by their genus. Field studies have demonstrated differences among *P. lanceolata* genotypes in the diversity of viral infections they host (Sallinen *et al.*, 2020; Jokinen *et al.*, 2023). However, whether these differences are generated by inherent differences in resistance or, e.g., differences in vector preferences have not been determined previously.

In this study, we focused on two RNA viruses: *Closterovirus* and *Enamovirus*. *Closterovirus* belongs to the *Closteroviridae* virus family, and the members of this family are a diverse group of single-stranded RNA (ssRNA) viruses (Karasev, 2000). *Closteroviridae* typically have long, filamentous non-enveloped structure (Agranovsky *et al.*, 1995) and can colonise several economically important hosts: beet (type species: *Beet yellows virus* ;Agranovsky *et al.*, 1995), citruses (Citrus tristeza virus; Harper, 2013), carrot (Adams *et al.*, 2014) and grapevine (Al Rwahnih *et al.*, 2012). Viruses or this family are also among the most frequently detected viruses infecting *P. lanceolata* in Åland Islands (Susi *et al.*, 2019; Norberg *et al.*, 2023). *Closteroviridae* are transmitted in a semi-persistent manner, typically by aphids; however, transmission by whiteflies and mealybugs has been reported as well (Karasev, 2000). Transmission via seeds has not been reported (Fuchs *et al.*, 2020). Symptoms of *Closteroviridae* colonisation can include yellowing or reddening of the leaf tissue or vein-clearing, though symptoms can be inconspicuous and difficult to detect (Karasev, 2000; Fuchs *et al.*, 2020).

Enamovirus belongs to the family *Solemoviridae*, a group of ssRNA viruses with non-enveloped icosahedral virions (Sõmera *et al.*, 2021). Similar to *Closteroviridae*, members of the *Solemoviridae* family infect important crop species: potato (Type species of *Polerovirus*: Potato leafroll virus; Taliansky, Mayo and Barker, 2003, legumes (Southern bean mosaic virus, Pea enation mosaic virus 1; Vemulapati *et al.*, 2010; Sõmera *et al.*, 2021, rice, and papaya (Sõmera *et al.*, 2021). Most *Solemoviridae* are transmitted by aphid vectors in a persistent, circulative and non-propagative manner (Demler *et al.*, 1996). However, for some viruses belonging to the family, also mechanical transmission via wounding and abiotic transmission through soil have been described (*Sobemovirus*; Sõmera, Sarmiento and Truve, 2015). S*olemoviridae* infections can cause a variety of symptoms in their hosts with equally varying severity; the host can remain symptomless or display symptoms such as mosaic pattern, vein-clearing, necrotic lesions, yellowing, redness, rolling, and even sterility (Sõmera *et al.*, 2021).

Host and viral material for the inoculation experiment

To study intraspecific resistance variation among *P. lanceolata* genotypes during viral infection, we cloned *P. lanceolata* individuals from 24 genotypes, originating from 7 different *P. lanceolata* populations in the Åland Islands (Supplementary table 1). The maternal plants were grown from seeds collected from the Åland Islands during the autumn of 2017. The germination of the maternal plants was started at the beginning of February 2022 by placing

178 the seeds into small pots filled with potting soil and sand (3:1, respectively). The germination
179 was carried out in a growth chamber with a light-dark cycle of 16:8, and after approximately
180 three weeks, the seedlings were transferred to the greenhouse. The cloning was started five
181 weeks after sowing. The maternal plant pot was positioned on top of an 11 cm × 11 cm pot
182 filled with vermiculite and placed on a tray filled with water. The roots of the maternal plant
183 were allowed to grow through the upper pot and once they reached sufficient size, they were
184 cut and let sprout into the bottom pot. When the shoots were grown large enough, they were
185 individually planted into fresh 10 cm × 10 cm pots filled with 1:1 proportion of potting soil
186 and sand (see also Sallinen *et al.*, 2020). The cloned host individuals were grown in the
187 greenhouse until the beginning of the experiment (mid-June 2022). During the growth period
188 in the greenhouse, the plants were fertilised with NPK fertiliser (7:2:2, respectively) once a
189 week and watered when needed. Plants were regularly treated with 2% pine soap water to
190 prevent thrip damage. Before the start of the experiment, leaf samples were collected from each
191 maternal plant for RNA extraction by collecting a 3 cm^2 leaf piece and the maternal plants were
192 confirmed to be virus-free for the focal viruses by PCR (see below for a detailed description of
193 the PCR protocol).

194 The cloning success varied among the genotypes, and hence, in the experiment, the
195 genotypes were represented by 7 to 21 individuals depending on the host genotype
196 (Supplementary table 1). Furthermore, for statistical analyses, we focused on plant genotypes
197 with a successful mock inoculation (i.e., mock plants with no virus detection). Consequently,
198 we excluded two genotypes from the first inoculation treatment (Figure 1A), leaving
199 individuals from 24 genotypes for statistical analysis (n = 335). For the sequential inoculations,
200 a subset of seven genotypes were selected as they had an adequate number of clones to perform
201 both sequential and single inoculation treatments (17-21 clones, n = 129). (Figure 1B,
202 Supplementary table 1).

203 To investigate host genotypic variation against two distinct viruses, we prepared virus
204 inocula from *P. lanceolata* plants collected from wild *P. lanceolata* populations in early June
205 2022. Plants exhibiting viral symptoms were carefully uprooted from the local soil and placed
206 into 10 cm × 10 cm pots, and if needed, the pots were filled with a mixture of 1:1 soil and sand.
207 The plants were transported to the laboratory and placed into a growth chamber with a 16:8
208 light-dark cycle. To identify which viruses were present in the collected wild plants, we took 1
209 cm^2 and 3 cm^2 samples from each plant for DNA and RNA extractions, respectively, and snap-
210 froze those in liquid nitrogen. We extracted total RNA and DNA from each sample and ran

PCR reactions targeting PlLV, *Enamovirus*, *Closterovirus*, *Betapartitivirus* and *Caulimovirus* as described in Susi *et al.* (2019) and Sallinen *et al.* (2020).

The inoculation experiment was started in mid-June 2022. In the first part of the inoculation experiment (Figure 1A) each of the 24 *P. lanceolata* genotypes, represented by 3-7 individuals, depending on the genotype, received either *Closterovirus* inoculum or *Enamovirus* inoculum and the control plants received mock inoculum (phosphate buffer; Supplementary table 1). To prepare the viral inoculum, leaves from plants infected by the respective virus were collected and placed into individual plastic extraction bags (Bioreba, Switzerland) containing 5 ml of 0.02 M phosphate buffer (pH 7.4). The bags were sealed, and the leaves were crushed with a mortar. The resulting inoculum was then immediately applied to the cloned experimental plants (approx. 400-500 µl of viral inoculum per plant) by pressing the syringe tightly against the leaf. The control plants were inoculated similarly using the phosphate buffer. After inoculation, each plant was placed individually inside a mesh bag closed with a rubber band to prevent insect transmission of the viruses. Two weeks after the first inoculation, we collected samples for RNA extraction for subsequent viral detection by taking a 3 cm^2 piece of leaf tissue and snap-froze those in liquid nitrogen. In addition, we counted the number of flowers and leaves, as well as measured the length of the longest flower and the width and length of the largest leaf. We used the measurements, to calculate the plant size $n \times A$, where n is the number of leaves and $A=\pi ab$, where a is the half axis of the width of the largest leaf and b is the half axis of the length of the largest leaf.

To investigate the effects of sequential infections on plant's resistance phenotype, we carried out subsequent inoculations for seven of the genotypes included in the first inoculations (Figure 1B, Supplementary table 1). On the day following the first sampling, individuals initially inoculated with *Closterovirus* were subsequently inoculated with *Enamovirus* and vice versa, the host individuals first inoculated with *Enamovirus* were inoculated with *Closterovirus*. Additionally, to compare the effects of single and sequential infections, individuals from each genotype initially treated with phosphate buffer (mock inoculation) were now inoculated with *Closterovirus* or *Enamovirus*. In the experiment, 4-5 individuals in each treatment represented each genotype (Supplementary table 1). Lastly, one individual per genotype remained as a mock inoculated control throughout the experiment and was inoculated with phosphate buffer in the first and second inoculation steps. Sampling was repeated two weeks after the second inoculation, using the same procedure as after the first. The plants were kept in their individual mesh bags for the whole experiment.

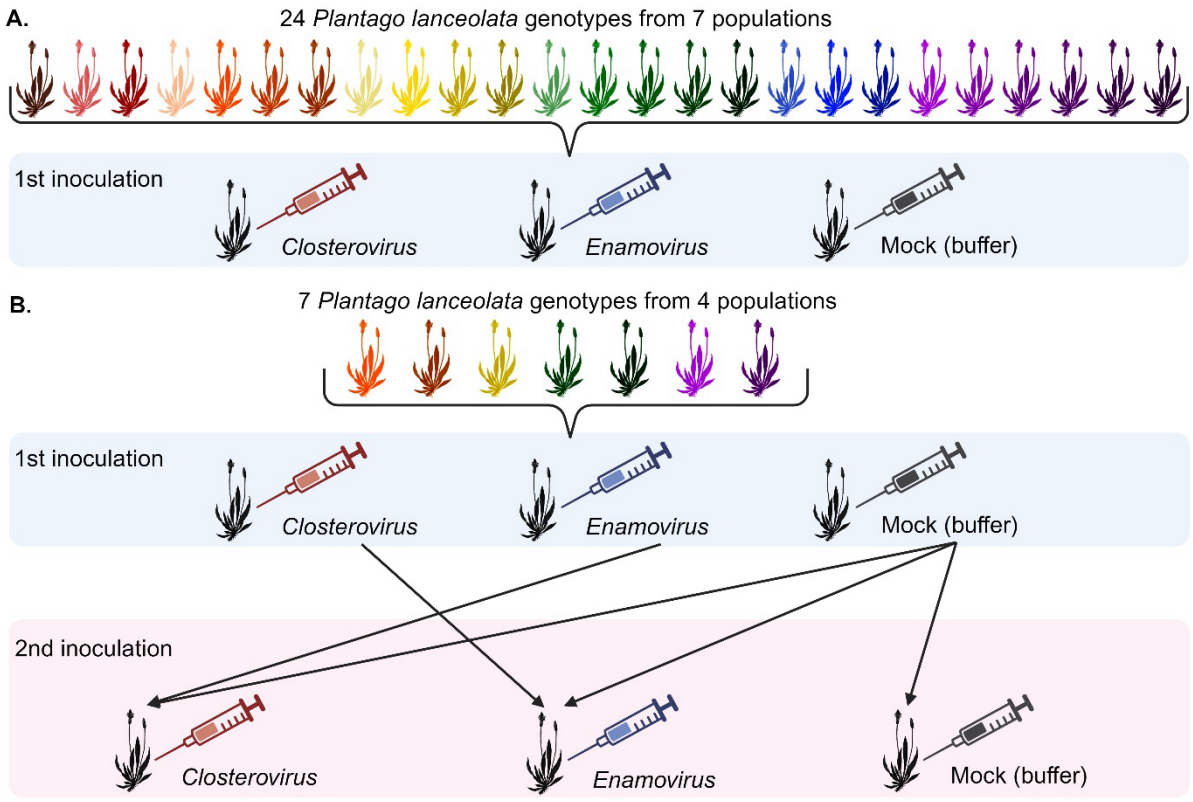

Figure 1. Experimental set-up of an inoculation experiment investigating intraspecific variation in host resistance during viral infection among *Plantago lanceolata* host genotypes (n = 24). The experiment comprised of two inoculation steps: A) first inoculations, where clones from 24 genotypes were inoculated with *Plantago lanceolata closterovirus* or *Plantago lanceolata enamovirus* or mock inoculated, and B) sequential inoculations, where seven genotypes from the first inoculation were sequentially inoculated with a different treatment than in the first inoculation. The syringe colour represents the inoculation treatment: red = *Plantago lanceolata closterovirus,* blue = *Plantago lanceolata enamovirus* and black = mock inoculation (phosphate buffer).

RNA extraction, cDNA translation and viral PCR detection from plant tissue samples

To detect *Closterovirus* and *Enamovirus* RNA from the collected samples, we extracted the total RNA using acid phenol-chloroform extraction method (Chang, Puryear and Cairney, 1993) with a few modifications. In short, first a 3 cm^2 size piece of plant tissue sample was

ground to a very fine powder using liquid nitrogen and then combined with 800 µl of warm 65°C extraction buffer (2% hexadecyltrimethylammonium bromide (Sigma-Aldrich, USA), 2% polyvinylpyrrolidone K-30 (MW 40 000, Sigma-Aldrich, USA), 100 mM Tris hydrochloride (pH 8.0; Thermo Fischer Scientific, USA), 25 mM Ethylenediaminetetraacetic acid (pH 8.9; Sigma-Aldrich, USA), 2.0 M NaCl (Sigma-Aldrich, USA) and 2% β-mercaptoethanol (Sigma-Aldrich, USA) and mixed vigorously. After, 800 µl of phenol-chloroform-isoamyl alcohol (IAA) solution (25:24:1, respectively) was added and the mixture was centrifuged at full speed (13 500 rpm) for 15 minutes. The supernatant was collected, and the acid-phenol-IAA and centrifugation steps were repeated. The supernatant was collected into a new tube and combined with 160 µl of 10 M of LiCl (Sigma-Aldrich, USA) and precipitated overnight on ice at +4 °C. The following day, the extract was centrifuged 10 000 rpm for 30 min at +4 °C. The pellet was resuspended with 500 µl of warm of SSTE buffer (1 M NaCl (Sigma-Aldrich, USA), 0.5 % Sodium dodecyl sulphate (Sigma-Aldrich, USA), 10 mM Tris hydrochloride (pH 8.0; Thermo Fischer Scientific, USA), 1mM Ethylenediaminetetraacetic acid (pH 8.9; Sigma-Aldrich, USA)) and 1 ml of Chloroform-IAA (24:1) was added and the sample was vortexed vigorously. After this, the chloroform-IAA purification step was repeated, followed by two ethanol washes (94 % and 70 %, respectively). Finally, the RNA was resuspended into 25 µl of nuclease-free water. The leaf tissue sample and the extracted RNA were stored at -80 °C.

The extracted total RNA was translated into cDNA before analysing the samples for viral presence by PCR. The concentration and purity of each RNA sample was measured with Nanodrop 2000, and 2 ng of RNA was used for each cDNA reaction. The extracted RNA was combined with 2 µl of 50 µM random hexamer primers (Promega Corporation, USA) and nuclease-free water was added to a final volume of 17.125 µl. The reaction was incubated at 70 °C for 5 min. After, the reactions were immediately placed on ice and spun down. The reverse transcription reaction was prepared as follows: 1 µl of Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT; Promega Corporation, USA), 5 µl of M-MLV RT 5x buffer (Promega Corporation, USA), 1.25 of 10 mM dNTP mix (Thermo Fischer) and 0.625 µl of RiboLock RNase inhibitor (Thermo Fischer Scientific, USA) was added. The mixture was incubated for 60 min at 37 °C and finally stored at -20 °C.

The detection of *Closterovirus* and *Enamovirus* was done by PCR (Susi *et al.*, 2017, 2019; Sallinen *et al.*, 2020). In short, for the PCR reaction, we combined 1 µl of template

cDNA, 500 nmol of each corresponding reverse and forward primer, 5 µl of GoTaq Green® 5x Mastermix (Promega Corporation, USA) and nuclease-free water to a total reaction volume of 10 µl. The PCR program consisted of initial denaturation at 95 °C for 2 min, followed by 35 cycles at 95 °C for 2 min, 53-60 °C for 40 s and 72 °C for 1 min. The final extension was done at 72 °C for 5 min. Positive control and water control were included in each run. The sizes of the PCR products were analysed on 1.5 % agarose gel, stained with GelRed (Biotium, USA) and visualised using the Bio-Rad Gel Doc XR+ imaging system (Bio-Rad Laboratories, USA).

Statistical analysis

All statistical analyses were conducted using R software (version 4.2.2., R Foundation for Statistical and Computing, Vienna, 2022). To investigate intraspecific variation among *P. lanceolata* genotypes in resistance to viral infection and to test differences in responses to *Closterovirus* and *Enamovirus*, we ran Generalized linear models (GLM) for the data of the first inoculation treatment. We included host infection status (0 = no infection, 1 = infection by *Closterovirus* or *Enamovirus*) as a binomial response variable and host genotype, viral inoculation treatment (*Closterovirus* or *Enamovirus*) along with their interaction as predictor variables. To determine the significance of the main effects we used function "Anova" in R-package "car" (Fox and Weisberg, 2019). To examine differences among host genotypes in resistance to the studied viruses and possible allocation costs in resistance, we performed pairwise comparisons of the estimated marginal means using functions "contrasts" and "emmeans" from the R-package "emmeans" (version 1.8.8, Lenth *et al.*, 2018). Specifically, we compared the infection rates of *Closterovirus* and *Enamovirus* within each host genotype, as well as between *Closterovirus* and *Enamovirus* across all genotypes.

To further assess the relationship between *Closterovirus* and *Enamovirus* infection rates among host genotypes, as well as associations between plant growth and reproductive traits (flower size and number) with each virus, we performed Pearson correlation test for each combination. For these correlations, we used the average infection rates for each virus, the average plant size, the average flower size, and the average number of flowers for each genotype.To study possible changes in resistance phenotype after the host had been exposed to sequential infection and to investigate differences in responses among genotypes to sequential infections, we analysed the data from the second inoculation for each virus. We fitted separate GLMs for individuals sequentially inoculated with *Closterovirus* or with *Enamovirus.*

Specifically, we included the host infection status (0 = no infection, 1 = infection by *Closterovirus* or *Enamovirus*) as a binomial response variable and host genotype, inoculation treatment (*Closterovirus* or mock inoculation, *Enamovirus* or mock inoculation) and their interactions as predictor variables. The significance of the main effects was determined by using function "Anova" in package "car" (Fox and Weisberg, 2019).

Results

First, we investigated whether host genotypes varied in their resistance to viral infection and whether the resistance responses among host genotypes differed against the two viruses. The GLM analysis and likelihood-ratio (LR) test showed significant effects of host genotype (Table 1; LR test $\chi^2 = 37.308$, df = 23, p = 0.00318) and inoculation treatment (Table 1; LR test $\chi^2 = 4.182$, df = 1, p = 0.04086) on host infection status after the first inoculation. Indeed, the infection rates varied across genotypes and viral treatments. Of the 24 genotypes included in the first inoculation, in 20 genotypes at least one individual became infected with either of the viruses while four genotypes were resistant to both viruses (187-1b, 187-4d, 187-6b and 853-1; Figure 2). From the 20 susceptible genotypes, 11 were susceptible to both viruses, six to *Closterovirus* only, and three to *Enamovirus* only. A total of 17 genotypes were susceptible to *Closterovirus*, and *Closterovirus* was detected in 28% of the *Closterovirus* inoculated individuals (n = 116). Infection rates (% of infected individuals) varied greatly within genotypes; for example, the infection rate for *Closterovirus* in genotype 946-4a was 75%, while for genotype 3225-2a, only 14% of the individuals were infected. Conversely, the overall infection rate for *Enamovirus* was lower at 17% (n = 115), with individuals from 14 genotypes being infected. Similar variability in infection rates within genotypes was observed for *Enamovirus*, genotype 1030-4a had the highest infection rate for *Enamovirus* (71%) and genotypes 1030-2b, 3225-2a and 946-7b harboured the lowest infection rates (14%). Furthermore, the GLM analysis showed that genotype 1030-4a was overall more likely to harbour infection, as indicated by the positive estimated coefficient and significant p-value (Supplementary table 2; estimate = 2.71, p = 0.047).

Post hoc analysis to evaluate differences between *Closterovirus* and *Enamovirus* resistance and possible allocation costs in resistance to the two viruses showed no significant differences within genotypes (Supplementary table 3) or among genotypes (Supplementary

table 4). Additionally, when analysing the correlation between infection rates of *Closterovirus* and *Enamovirus*, we observed a weak positive correlation. However, this correlation was not statistically significant (Figure 3; t = 1.0248, df = 22, p = 0.3166).

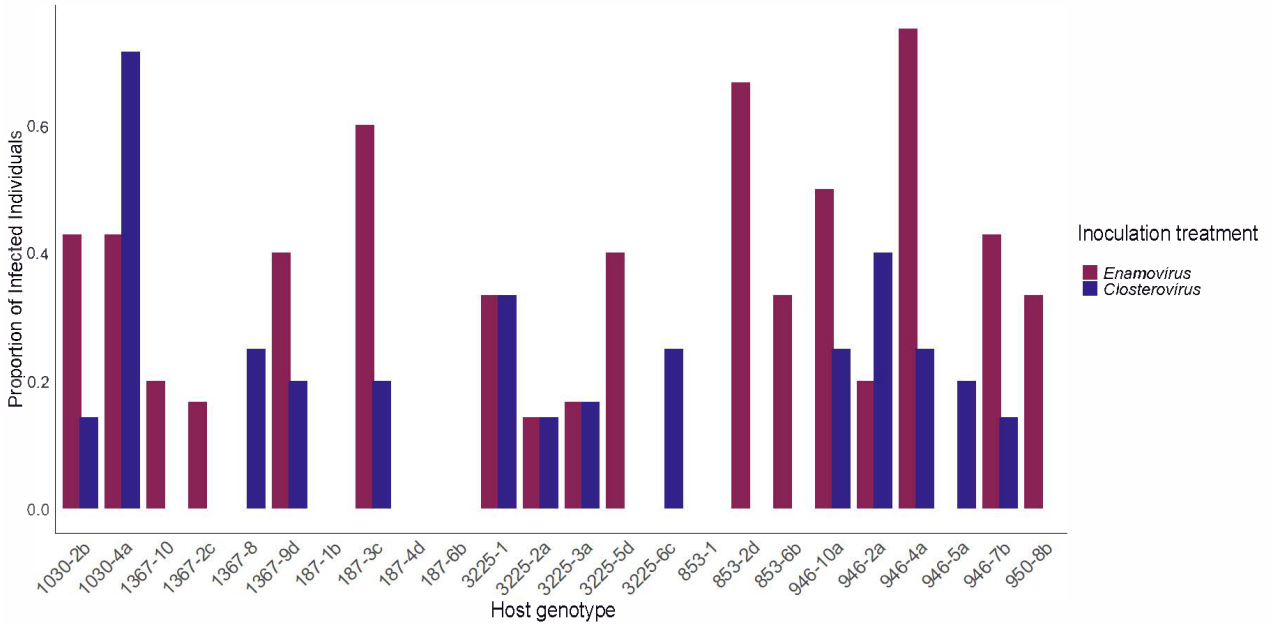

Figure 2. Infection rates of *Plantago lanceolata closterovirus* and *Plantago lanceolata enamovirus* following the first inoculation treatment grouped by host genotypes. The blue colour represent the proportion of *Enamovirus* infected individuals within the *Enamovirus* inoculation treatment and correspondingly, the red colour represents the proportion *Closterovirus* infected individuals within *Closterovirus* treatment. The absence of a bar indicates that the genotype did not harbour viral infections after the first inoculation treatment.

Table 1. Results from Generalized linear model analysis on an inoculation experiment with 24 *Plantago lanceolata* genotypes (n = 335) inoculated with *Plantago lanceolata closterovirus* or *Plantago lanceolata enamovirus* investigating the intraspecific variation among host genotypes during viral infection and the differences in host response to the studied viruses across genotypes.

Fixed effect	*LR* χ^2	*Df*	*p-value*
Genotype	**37.308**	**23**	**0.03018**
Inoculation treatment	**4.182**	**1**	**0.04086**
Genotype × Inoculation treatment	23.032	23	0.4589

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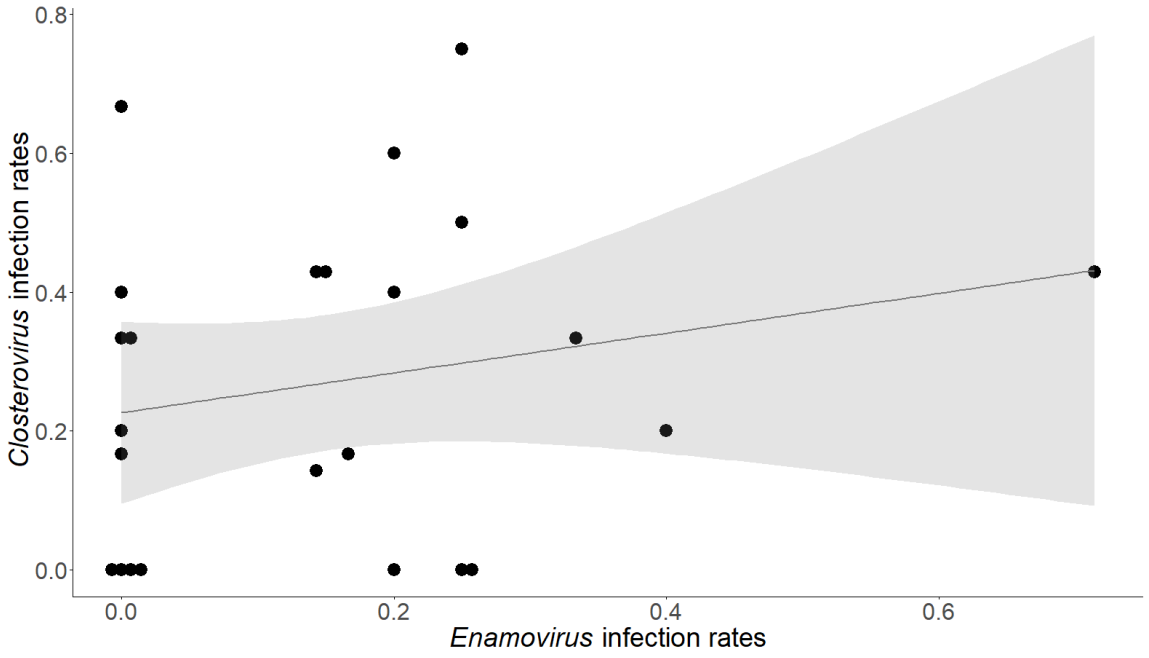

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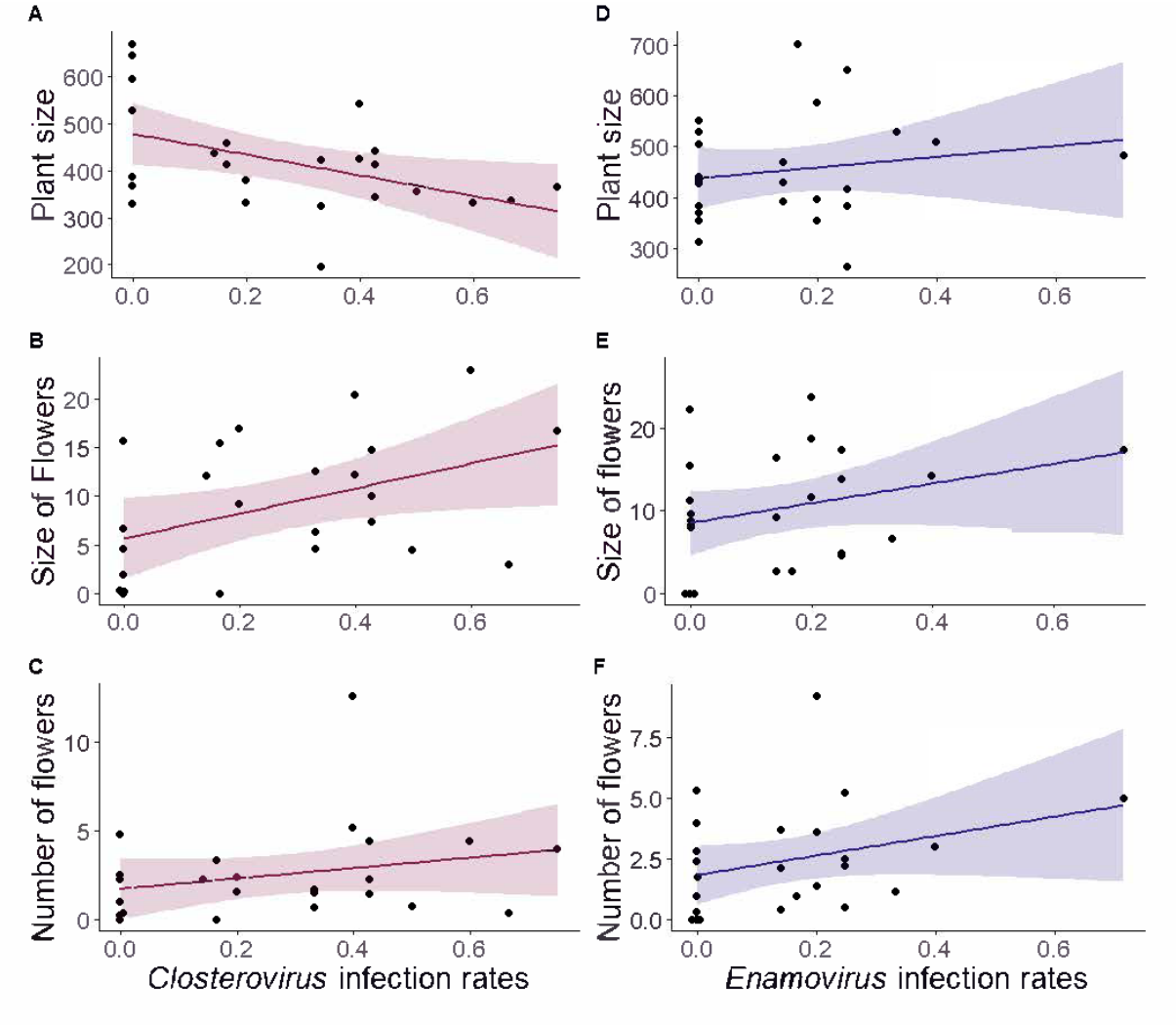
375 Figure 3. The correlation between *Plantago lanceolata closterovirus* and *Plantago lanceolata*
376 *enamovirus* infection rates among 24 *Plantago lanceolata* genotypes after the first
377 inoculation. The Pearson correlation between the infection rates of the two viruses was non-
378 significant but weakly positive (t = 1.0248, df = 22, p = 0.3166).



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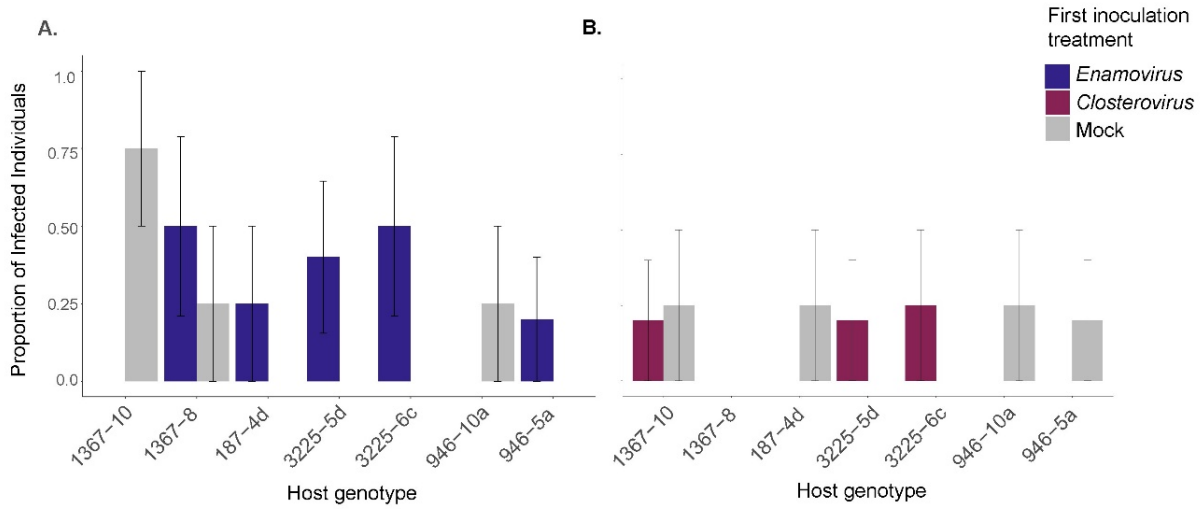
380 Figure 4. Correlations between *Plantago lanceolata closterovirus* and *Plantago lanceolata*
381 *enamovirus* infection rates and plant size (A and D), size of flowers (B and E), and umber of
382 flowers (C and F). The points represent the average infection rate for each genotype with the
383 corresponding association. The trend line indicates the linear regression and the shaded area
384 the 95% confidence interval.

385

386 Next, we investigated the associations between plant growth and reproductive traits
387 (flower size and number) and virus infection rates. We found a negative but non-significant
388 correlation (-0.459) between *Closterovirus* infection rates and host plant size (Figure 4; t =
389 2.424, df = 22, p = 0.0238). In contrast, *Closterovirus* infection rates showed moderate positive
390 correlations with flower size (0.433) and number of flowers (0.253). The correlation with
391 flower size was statistically significant (t = 2.257, df = 22, p = 0.0342), while the correlation
392 with the number of flowers was weak and statistically non-significant (t = 1.231, df = 22, p =

0.231). In comparison, the correlations between *Enamovirus* infection rates and plant growth and reproductive traits were weakly positive and did not reach statistical significance. The correlation between plant size and *Enamovirus* infection rates was weakly positive (0.175) with no significant effect (t = 0.834. df = 22, p = 0.4127). Similarly, the correlations with flower size (0.2965) and flower number (0.315) were weak and non-significant (t = 1.456, df = 22, p = 0.159 and t = 1.557, df = 22, p = 0.133, respectively).

Finally, we investigated whether the initial inoculation with *Closterovirus* or *Enamovirus* influenced the host resistance phenotype in sequential inoculation with *Enamovirus* or *Closterovirus*, respectively. After the sequential inoculations, the overall *Closterovirus* infection rate was 20%. Individuals that been previously infected with *Enamovirus* had a higher infection rate than plants that had received the mock inoculation treatment (66% vs. 33%, respectively), demonstrating how sensitive the resistance phenotype is to prior infection. Specifically, genotypes 187-4d, 3225-5d, 3225-6c and 956-5a were more susceptible to *Closterovirus* inoculation after initial inoculation with *Enamovirus* when compared to individuals that were mock inoculated during the first treatment (Figure 5A.). These observations were supported by our GLM and LR analysis, where the interaction between host genotype and initial inoculation treatment had a significant effect on *Closterovirus* resistance (Table 2A. LR χ^2 = 19.9074, df = 6, p = 0.002876). Indeed, model coefficients revealed positive estimates for interactions between genotype and first inoculation treatment with *Enamovirus* when compared to the intercept involving interaction between genotype and mock inoculation (Supplementary table 5). In contrast, we did not observe similar trends for *Enamovirus* sequential infections. The infection rates of *Enamovirus* after the sequential inoculation were generally lower than those of *Closterovirus* (Figure 5B), with only 5% of all *Enamovirus* inoculated individuals colonised by the virus. Out of the *Enamovirus* infected individuals 50% were first inoculated with *Closterovirus* and the other 50% were first mock inoculated. Our statistical analyses revealed that neither the initial *Closterovirus* inoculation nor the host genotype had a significant effect on resistance to sequential *Enamovirus* inoculation (Table 2B, Supplementary table 6).



422

423 Figure 5. Viral infection rates of plant individuals from 7 host genotypes after sequential
424 inoculation treatments. A) Infection rates of individuals after *Plantago lanceolata closterovirus*
425 sequential infection. During the first inoculation the individuals were inoculated with *Plantago*
426 *lanceolata enamovirus* (blue) or mock inoculated (grey). B) Infection rates of individuals after
427 *Plantago lanceolata enamovirus* inoculation. During the first inoculation the individuals were
428 inoculated with *Plantago lanceolata closterovirus* (red) or mock inoculated (grey).

429

430 Table 2. Likelihood Ratio Chi-Square test results for two Generalized linear model (GLM)
431 analyses of an inoculation experiment investigating the intraspecific variation and response
432 among 7 *Plantago lanceolata* genotypes during sequential viral infections. A) GLM results
433 from individuals that were first inoculated with *Plantago lanceolata enamovirus* or mock
434 inoculated and sequentially inoculated with *Plantago lanceolata closterovirus.* B) GLM results
435 from individuals that were first inoculated with *Plantago lanceolata closterovirus* or mock
436 inoculated and sequentially inoculated with *Plantago lanceolata enamovirus*.

A.

Fixed effect	*LR* χ^2	*Df*	*p-value*
Genotype	2.4445	6	0.87463
First inoculation treatment	1.317	1	0.251124
Genotype × First inoculation treatment	**19.9074**	**6**	**0.002876**

B.

Fixed effect	*LR* χ^2	*Df*	*p-value*
Genotype	5.1259	6	0.5278
Inoculation treatment in timepoint A	0.0022	1	0.882
Genotype × Inoculation treatment in timepoint A	5.8739	6	0.4375

Discussion

Variation in intraspecific resistance is expected to have a key role in shaping the outcome of host-pathogen interactions (Thrall and Burdon, 2000; Laine *et al.*, 2011; Sallinen *et al.*, 2020), mitigating disease spread and preventing major outbreaks (Salvaudon, Héraudet and Shykoff, 2007; Ganz and Ebert, 2010; Jousimo *et al.*, 2014). However, much of our understanding of this derives from studies focusing on single parasite infections while in reality hosts are exposed to a wide diversity of parasites. While the true viral diversity in natural environments still remains largely undiscovered, it is well-documented that viruses are abundant and diverse across habitats, and many known viruses are pathogenic (Suttle, 2005; Roossinck, 2011; Bibby, 2013; Bass *et al.*, 2019; Koonin, Krupovic and Dolja, 2023). Yet, we know little about the intraspecific variation in resistance to viral infections in wild hosts. Here, our findings highlight the importance of host genotype as a key predictor of host viral resistance – host genotypes exhibited varying resistance profiles, ranging from resistance to susceptibility for both studied viruses. Moreover, we observed a significant change in host resistance phenotype as susceptibility to *Closterovirus* increased following prior *Enamovirus* inoculation. In addition, we observed distinct strategies in how resources were allocated between growth and resistance for the two viruses. Overall, our results highlight the role of host genotype and virus–virus interactions in mediating viral infections as well as viral community assembly.

In line with earlier studies on resistance variation in wild populations (Alexander, Antonovics and Kelly, 1993; Thrall and Burdon, 2000; Laine, 2004, 2011; Rose *et al.*, 2005; Susi and Laine, 2017), *P. lanceolata* genotypes showed high diversity in their resistance responses, showing varying levels of susceptibility and resistance to the two viruses used in this experiment. The overall susceptibility to the two viruses was relatively low and varied between the two viruses (28% for *Closterovirus* and 17% for *Enamovirus*). However, in most of the host genotypes we were able to detect viral infection in at least one individual. Infection rates varied considerably within genotypes, from over 70% of individuals being infected, to only 13% of infected individuals. We identified four genotypes that were completely resistant to viral inoculation. Three of these originate from the same host population (ID: 187), suggesting potentially spatially structured variation in selection for viral resistance (*cf.* Laine *et al.*, 2011). Genotype 1030-4a was significantly more susceptible for virus infection compared to the other genotypes, with infection rates of 71% for *Enamovirus* and 43% for *Closterovirus*. The observed variation in viral resistance among host genotypes aligns with previous research from this system, which has described ample variation in resistance to the fungal parasite *P. plantaginis* within *P. lanceolata* populations (Laine, 2004; Susi, Vale and Laine, 2015; Safdari *et al.*, 2021). Moreover, field experiments conducted during natural viral epidemics in this system revealed that viral communities varied both among *P. lanceolata* genotypes and populations (Susi *et al.*, 2019; Sallinen *et al.*, 2020; Jokinen *et al.*, 2023). Our results confirm that these differences are likely to be generated by genetic resistance variation.

In this study, we individually inoculated clones of *P. lanceolata* genotypes with *Closterovirus* or *Enamovirus* to explore possible allocation costs in resistance against the two viruses as predicted by the concept of trade-offs (Bergelson and Purrington, 1996; Webster and Woolhouse, 1999; Koskella *et al.*, 2012; Auld *et al.*, 2013). While the inoculation treatment was a significant predictor of host infections status, post hoc tests did not detect statistically significant differences in resistance against *Closterovirus* or *Enamovirus* across or within the 24 *P. lanceolata* genotypes included in the study. This lack of statistical support is likely due to the variation in infection rates within host genotypes. However, we identified several host genotypes that were resistant to one of the studied viruses while remaining susceptible to the other. For example, genotypes 853-2d, 853-6b and 950-8b were resistant to *Enamovirus*, but susceptible to *Closterovirus*, suggesting a possible trade-off in resistance. Overall, in our inoculation experiment we found a weak positive correlation between *Closterovirus* and *Enamovirus* infection rates, with several of the host genotypes being susceptible to both of the

viruses. These findings suggest that host genotype may play a key role in shaping viral co-occurrence patterns, supported by field data showing that high viral diversity tends to accumulate in certain host individuals (Susi *et al.*, 2019; Sallinen *et al.*, 2020; Jokinen *et al.*, 2023; Norberg *et al.*, 2023).

One of the most studied life-history trade-offs is the growth versus defense trade-off, which predicts the host's limited resources must be allocated between growth and defence, leading to patterns where growth is favoured over defense or vice versa (Bergelson and Purrington, 1996; Monson *et al.*, 2022; Zaret *et al.*, 2024). Indeed, we found that for both viruses higher infection rates positively correlated with larger flower size and number of flowers. These results indicate that these individuals may have allocated more resources to reproduction and, in turn, less resources to resistance against parasites. Varying resource allocation strategies create variation in wild hosts, even among host genotypes, and such trade-offs between fitness traits and defense are particularly evident in wild hosts (Giolai and Laine, 2024). Interestingly, we observed that higher infection rates with *Closterovirus* were negatively correlated with host plant size, suggesting that larger plants harbour less viral infections. In contrast, no such relationship was observed with *Enamovirus* inoculated individuals, where small size was positively correlated with viral infections. These contrasting patterns may reflect differences in the immune responses these viruses trigger or that the full extent of the trade-offs were not captured within the timeframe of the experiment (Susi and Laine, 2015; Dallas, Holtackers and Drake, 2016). A longer observation period may be necessary to observe dynamics of resource allocation and viral resistance in this system.

In addition to intraspecific variation in resistance, we observed a significant change in host resistance phenotype in several of the genotypes when the host was initially inoculated with *Enamovirus*. Specifically, host individuals first inoculated with *Enamovirus* were more susceptible to subsequent *Closterovirus* inoculation compared to those that were first mock inoculated with phosphate buffer. However, there were differences among host genotypes in their response to sequential *Closterovirus* inoculation. We observed the change in resistance phenotype in all other genotypes included in the treatment except for genotypes 1367-10 and 946-10a, indicating that there is intraspecific variation among genotypes also in their response to sequential coinfections. During coinfections, defence against the first arriving parasite can leave the host more vulnerable or resistant against subsequent parasite attack (Spoel, Johnson and Dong, 2007; Ziebell and Carr, 2010; Mauch-Mani *et al.*, 2017; Morris, Cleary and Clarke, 2017; Jokinen *et al.*, 2023). Interestingly, we did not observe similar change in resistance

phenotype when individuals were first inoculated with *Closterovirus*. There were no differences in *Enamovirus* infection rates between individuals that were first inoculated with *Closterovirus* and those that were mock inoculated. This suggest that resistance against *Closterovirus* might not impose as significant cost for the host than resistance against *Enamovirus*, which could also connect to our finding of the lack of trade-off between host plant size and resistance against *Closterovirus*. Our results also demonstrate that the assembly of viral communities is highly sensitive to the arrival order of the different viruses.

Here, we have described the importance of intraspecific variation in resistance in wild host against viral infection by using naïve *P. lanceolata* clones in an inoculation experiment. By applying both single and sequential inoculations with two wild viruses across 24 host genotypes, we were able to detect distinct differences in resistance among host genotypes against the two viruses and the sensitivity of the resistance phenotype to prior viral infection. Moreover, we detected varying strategies in resource allocation between growth and defense in response to the two viruses, reflecting a trade-off between these processes. Our findings highlight the importance of intraspecific variation in host resistance against viral infection — an important component in natural disease mitigation. Overall, our results indicate that host genotype and virus arrival sequence are key determinants of host resistance, and they may play important role in shaping disease dynamics and the assembly of within-host parasite communities in natural systems. The global trend of genetic variation in being eroded in natural populations by human actions can have far reaching consequences for disease risk (Exposito-Alonso *et al.*, 2022; Laine, 2023), which as we have shown here, is highly sensitive to host genetic variation.

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Author Contributions

Conceptualization: A.-L.L; study design: M.J. and A.-L.L.; investigation, methodology, data collection, formal analysis: M.J.; supervision: A.-L.L and H.S.; Writing: M.J., H.S. and A-L.L.

Conflict of Interest

The authors declare no conflict of interests.

Data availability statement

The data and R scripts used in this study have been submitted to GitHub (https://github.com/maijajoki/INTRA22).

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Supplementary information

Diversity in viral resistance emerges from host genotype and infection order effects

Maija Jokinen[1, 2], Hanna Susi[1] & Anna-Liisa Laine[1]

[1] Organismal and Evolutionary Biology Research Programme, Faculty of Biological and Environmental Sciences, PO BOX 65 00014 University of Helsinki, Finland

[2] Department of Evolutionary Biology and Environmental Studies, University of Zürich, 8057 Zürich, Switzerland

M.J. maija.jokinen@helsinki.fi, H.S. hanna.susi@helsinki.fi, A-L.L. anna-liisa.laine@helsinki.fi

Maija Jokinen, maija.jokinen@helsinki.fi and Anna-Liisa Laine, anna-liisa.laine@helsinki.fi
Organismal and Evolutionary Biology Research Programme, Faculty of Biological and Environmental Sciences, PO BOX 65 00014 University of Helsinki, Finland

Supplementary table 1. Data table showing all *Plantago lanceolata* genotypes included in the inoculation experiment in the first inoculations (n = 24) and the sequential inoculations (n = 7). The host genotype origin populations, first and sequential inoculation treatments, infection rates for *Enamovirus* and *Closterovirus* within each genotype and treatment, and the number individuals of each genotype within each genotype are shown.

Origin population	Host genotype	Timepoint	First inoculation treatment	Sequential inoculation treatement	Infection rate: Enamovirus	Infection rate: Closterovirus	Number of individuals/treatment
187	187-1b	1	*Closterovirus*		-	0.00	4
187	187-1b	1	*Enamovirus*		0.00	-	4
187	187-1b	1	Mock		0.00	0.00	2
187	187-3c	1	*Closterovirus*		-	0.60	5
187	187-3c	1	*Enamovirus*		0.20	-	5
187	187-3c	1	Mock		0.00	0.00	2
187	187-4d	1	*Closterovirus*		-	0.00	4
187	187-4d	1	*Enamovirus*		0.00	-	4
187	187-4d	1	Mock		0.00	0.00	9
853	853-1	1	*Closterovirus*		-	0.00	3
853	853-1	1	*Enamovirus*		0.00	-	3
853	853-1	1	Mock		0.00	0.00	2
853	853-2d	1	*Closterovirus*		-	0.67	3
853	853-2d	1	*Enamovirus*		0.00	-	3
853	853-2d	1	Mock		0.00	0.00	1
853	853-6b	1	*Closterovirus*		-	0.33	3
853	853-6b	1	*Enamovirus*		0.00	-	3
853	853-6b	1	Mock		0.00	0.00	2
946	946-10a	1	*Closterovirus*		-	0.50	4
946	946-10a	1	*Enamovirus*		0.25	-	4
946	946-10a	1	Mock		0.00	0.30	10
946	946-4a	1	*Closterovirus*		-	0.75	4
946	946-4a	1	*Enamovirus*		0.25	-	4
946	946-4a	1	Mock		0.00	0.00	2
946	946-5a	1	*Closterovirus*		-	0.00	5
946	946-5a	1	*Enamovirus*		0.20	-	5
946	946-5a	1	Mock		0.09	0.00	11
946	946-7b	1	*Closterovirus*		-	0.43	7
946	946-7b	1	*Enamovirus*		0.14	-	7
946	946-7b	1	Mock		0.00	0.00	1
950	950-8b	1	*Closterovirus*		-	0.33	3
950	950-8b	1	*Enamovirus*		0.00	-	3
950	950-8b	1	Mock		0.00	0.00	2
1030	1030-2b	1	*Closterovirus*			0.43	7
1030	1030-2b	1	*Enamovirus*		0.14		7
1030	1030-2b	1	Mock		0.00	0.00	1
1030	1030-4a	1	*Closterovirus*		-	0.43	7
1030	1030-4a	1	*Enamovirus*		0.71	-	7
1030	1030-4a	1	Mock		0.00	0.00	1
1367	1367-10	1	*Closterovirus*		-	0.20	5
1367	1367-10	1	*Enamovirus*		0.00	-	5
1367	1367-10	1	Mock		0.00	0.00	9
1367	1367-2c	1	*Closterovirus*		-	0.17	6
1367	1367-2c	1	*Enamovirus*		0.00	-	6
1367	1367-2c	1	Mock		0.00	0.00	1
1367	1367-8	1	*Closterovirus*		-	0.00	4
1367	1367-8	1	*Enamovirus*		0.25	-	4
1367	1367-8	1	Mock		0.11	0.00	9
1367	1367-9d	1	*Closterovirus*		-	0.40	5
1367	1367-9d	1	*Enamovirus*		0.20	-	5
1367	1367-9d	1	Mock		0.00	0.00	11
3225	3225-1	1	*Closterovirus*		-	0.33	6
3225	3225-1	1	*Enamovirus*		0.33	-	6
3225	3225-1	1	Mock		0.00	0.00	2
3225	3225-2a	1	*Closterovirus*		-	0.14	7
3225	3225-2a	1	*Enamovirus*		0.14	-	7
3225	3225-2a	1	Mock		0.00	0.00	1
3225	3225-3a	1	*Closterovirus*		-	0.17	6
3225	3225-3a	1	*Enamovirus*		0.17	-	6
3225	3225-3a	1	Mock		0.00	0.00	1
3225	3225-5d	1	*Closterovirus*			0.40	5
3225	3225-5d	1	*Enamovirus*		0.00		5
3225	3225-5d	1	Mock		0.00	0.20	10
3225	3225-6c	1	*Closterovirus*		-	0.00	4
3225	3225-6c	1	*Enamovirus*		0.25	-	4
3225	3225-6c	1	Mock		0.00	0.10	10

187	187-4d	2	*Closterovirus*	*Enamovirus*	0.00	-	4
187	187-4d	2	*Enamovirus*	*Closterovirus*	-	0.25	4
187	187-4d	2	Mock	*Closterovirus*	-	0.00	4
187	187-4d	2	Mock	*Enamovirus*	0.25	-	4
187	187-4d	2	Mock	Mock	0.00	0.00	1
946	946-10a	2	*Closterovirus*	*Enamovirus*	0.00	-	4
946	946-10a	2	*Enamovirus*	*Closterovirus*	-	0.00	4
946	946-10a	2	Mock	*Closterovirus*	-	0.25	4
946	946-10a	2	Mock	*Enamovirus*	0.25	-	4
946	946-10a	2	Mock	Mock	0.00	0.00	2
946	946-5a	2	*Closterovirus*	*Enamovirus*	0.00	-	5
946	946-5a	2	*Enamovirus*	*Closterovirus*	-	0.20	5
946	946-5a	2	Mock	*Closterovirus*	-	0.00	5
946	946-5a	2	Mock	*Enamovirus*	0.00	-	5
946	946-5a	2	Mock	Mock	-	-	1
1367	1367-10	2	*Closterovirus*	*Enamovirus*	0.20	-	5
1367	1367-10	2	*Enamovirus*	*Closterovirus*	-	0.00	5
1367	1367-10	2	Mock	*Closterovirus*	-	0.75	4
1367	1367-10	2	Mock	*Enamovirus*	0.25	-	4
1367	1367-10	2	Mock	Mock	0.00	0.00	1
1367	1367-8	2	*Closterovirus*	*Enamovirus*	0.00	-	4
1367	1367-8	2	*Enamovirus*	*Closterovirus*	-	0.50	4
1367	1367-8	2	Mock	*Closterovirus*	-	0.00	4
1367	1367-8	2	Mock	*Enamovirus*	0.00	-	4
1367	1367-8	2	Mock	Mock	0.00	0.00	1
3225	3225-5d	2	*Closterovirus*	*Enamovirus*	0.20	-	5
3225	3225-5d	2	*Enamovirus*	*Closterovirus*	-	0.40	5
3225	3225-5d	2	Mock	*Closterovirus*	-	0.00	4
3225	3225-5d	2	Mock	*Enamovirus*	0.00	-	4
3225	3225-5d	2	Mock	Mock	0.00	0.00	2
3225	3225-6c	2	*Closterovirus*	*Enamovirus*	0.25	-	4
3225	3225-6c	2	*Enamovirus*	*Closterovirus*	-	0.50	4
3225	3225-6c	2	Mock	*Closterovirus*	-	0.00	4
3225	3225-6c	2	Mock	*Enamovirus*	0.00	-	4
3225	3225-6c	2	Mock	Mock	0.00	0.00	2

Supplementary table 2. Model coefficients testing the effect of virus inoculation (*Closterovirus* or *Enamovirus*) on host infection rate among 24 different *Plantago lanceolata* genotypes. Model variables included host genotype, inoculation treatment (*Closterovirus* or *Enamovirus)* and the interaction between these two. For all variables, one level is a reference level included in the intercept.

	Estimate	Std. error	z-value	p-value
(Intercept)	-1.79	1.08	-1.66	0.097
Genotype 1030-4a	**2.71**	**1.366**	**1.98**	**0.047**
Genotype 1367-10	-17.77	4.81E+03	-3.70E-03	0.997
Genotype 1367-2c	-17.77	4.39E+03	-4.05E-03	0.997
Genotype 1367-8	0.69	1.581	0.44	0.661
Genotype 1367-9d	0.41	1.555	0.26	0.794
Genotype 187-1b	-17.77	5.38E+03	-3.31E-03	0.997
Genotype 187-3c	0.41	1.555	0.26	0.794
Genotype 187-4d	-17.77	5.38E+03	-3.31E-03	0.997
Genotype 187-6b	-17.77	5.38E+03	-3.31E-03	0.997
Genotype 3225-1	1.1	1.384	0.79	0.427
Genotype 3225-2a	1.48E-14	1.528	9.67E-15	> .999
Genotype 3225-3a	0.18	1.538	0.12	0.906
Genotype 3225-5d	-17.77	4.81E+03	-3.70E-03	0.997
Genotype 3225-6c	0.69	1.581	0.44	0.661
Genotype 853-1	-17.77	6.21E+03	-2.86E-03	0.998
Genotype 853-2d	-17.77	6.21E+03	-2.86E-03	0.998
Genotype 853-6b	-17.77	6.21E+03	-2.86E-03	0.998
Genotype 946-10a	0.69	1.581	0.44	0.661
Genotype 946-2a	1.39	1.414	0.98	0.327
Genotype 946-4a	0.69	1.581	0.44	0.661
Genotype 946-5a	0.41	1.555	0.26	0.794
Genotype 946-7b	5.94E-15	1.528	3.89E-15	> .999
Genotype 950-8b	-17.77	6.21E+03	-2.86E-03	0.998
Inoculation treatment (Enamovirus)	1.5	1.323	1.14	0.256
Genotype 1030-4a × Inoculation treatment (Enamovirus)	-2.71	1.742	-1.55	0.12
Genotype 1367-10 ×Inoculation treatment (Enamovirus)	16.68	4.81E+03	3.47E-03	0.997
Genotype 1367-2c ×Inoculation treatment (Enamovirus)	16.45	4.39E+03	3.75E-03	0.997
Genotype 1367-8 × Inoculation treatment (Enamovirus)	-19.97	5.38E+03	-3.71E-03	0.997
Genotype 1367-9d ×Inoculation treatment (Enamovirus)	-0.52	1.958	-0.27	0.789
Genotype 187-1b ×Inoculation treatment (Enamovirus)	-1.5	7.60E+03	-1.98E-04	> .999
Genotype 187-3c × Inoculation treatment (Enamovirus)	0.29	1.96E+00	0.15	0.883
Genotype 187-4d × Inoculation treatment (Enamovirus)	-1.5	7.60E+03	-1.98E-04	> .999
Genotype 187-6b × Inoculation treatment (Enamovirus)	-1.5	7.60E+03	-1.98E-04	> .999
Genotype 3225-1 × Inoculation treatment (Enamovirus)	-1.5	1.803	-0.83	0.404
Genotype 3225-2a × Inoculation treatment (Enamovirus)	-1.5	2.021	-0.74	0.457
Genotype 3225-3a × Inoculation treatment (Enamovirus)	-1.5	2.037	-0.74	0.46
Genotype 3225-5d ×Inoculation treatment (Enamovirus)	17.66	4.81E+03	3.67E-03	0.997
Genotype 3225-6c × Inoculation treatment (Enamovirus)	-19.97	5.38E+03	-3.71E-03	0.997
Genotype 853-1 × Inoculation treatment (Enamovirus)	-1.5	8.78E+03	-1.71E-04	> .999
Genotype 853-2d × Inoculation treatment (Enamovirus)	18.76	6.21E+03	3.02E-03	0.998
Genotype 853-6b × Inoculation treatment (Enamovirus)	17.37	6.21E+03	2.80E-03	0.998
Genotype 946-10a × Inoculation treatment (Enamovirus)	-0.41	2.021	-0.2	0.841
Genotype 946-2a ×Inoculation treatment (Enamovirus)	-2.48	1.958	-1.27	0.204
Genotype 946-4a ×Inoculation treatment (Enamovirus)	0.69	2.102	0.33	0.742
Genotype 946-5a × Inoculation treatment (Enamovirus)	-19.68	4.81E+03	-4.09E-03	0.997
Genotype 946-7b × Inoculation treatment (Enamovirus)	-1.29E-14	1.871	-6.92E-15	> .999
Genotype 950-8b ×Inoculation treatment (Enamovirus)	17.37	6.21E+03	2.80E-03	0.998

Supplementary table 3. Post-hoc test comparing the infection status of the host between the two inoculation treatments (*Enamovirus* and *Closterovirus*) within 24 *Plantago lanceolata* genotypes. Results of a pairwise comparison of the estimated marginal means calculated from the Generalized linear model (Table 1). Tukey adjustment was applied for multiple comparisons.

Genotype	Contrast	Estimate	Std.error	df	z-value	p-value
1030-2b	*Enamovirus - Closterovirus*	1.504	1.32	Inf	1.137	0.2555
1030-4a	*Enamovirus - Closterovirus*	-1.204	1.13	Inf	-1.063	0.2879
1367-10	*Enamovirus - Closterovirus*	18.18	4.81E+03	Inf	0.004	0.997
1367-2c	*Enamovirus - Closterovirus*	17.957	4.39E+03	Inf	0.004	0.9967
1367-8	*Enamovirus - Closterovirus*	-18.467	5.38E+03	Inf	-0.003	0.9973
1367-9d	*Enamovirus - Closterovirus*	0.981	1.44	Inf	0.68	0.4968
187-1b	*Enamovirus - Closterovirus*	0	7.60E+03	Inf	0	1
187-3c	*Enamovirus - Closterovirus*	1.792	1.44	Inf	1.241	0.2145
187-4d	*Enamovirus - Closterovirus*	0	7.60E+03	Inf	0	1
187-6b	*Enamovirus - Closterovirus*	0	7.60E+03	Inf	0	1
3225-1	*Enamovirus - Closterovirus*	0	1.23	Inf	0	1
3225-2a	*Enamovirus - Closterovirus*	0	1.53	Inf	0	1
3225-3a	*Enamovirus - Closterovirus*	0	1.55	Inf	0	1
3225-5d	*Enamovirus - Closterovirus*	19.161	4.81E+03	Inf	0.004	0.9968
3225-6c	*Enamovirus - Closterovirus*	-18.467	5.38E+03	Inf	-0.003	0.9973
853-1	*Enamovirus - Closterovirus*	0	8.78E+03	Inf	0	1
853-2d	*Enamovirus - Closterovirus*	20.259	20.259	Inf	0.003	0.9974
853-6b	*Enamovirus - Closterovirus*	18.873	6.21E+03	Inf	0.003	0.9976
946-10a	*Enamovirus - Closterovirus*	1.099	1.53	Inf	0.719	0.472
946-2a	*Enamovirus - Closterovirus*	-0.981	1.44	Inf	-0.68	0.4968
946-4a	*Enamovirus - Closterovirus*	2.197	1.63	Inf	1.346	0.1785
946-5a	*Enamovirus - Closterovirus*	-18.18	4.81E+03	Inf	-0.004	0.997
946-7b	*Enamovirus - Closterovirus*	1.504	1.32	Inf	1.137	0.2555
950-8b	*Enamovirus - Closterovirus*	18.873	6.21E+03	Inf	0.003	0.9976

Supplementary table 4. Post-hoc test comparing the inoculation treatments (*Enamovirus* and *Closterovirus*) across all *Plantago lanceolata* genotypes. Results of a pairwise comparison of the estimated marginal means calculated from the Generalized linear model (Table 1). Tukey adjustment was applied for multiple comparisons.

Contrast	*Estimate*	*SE*	*Df*	*Z-value*	*p-value*
Inoculation treatment (*Enamovirus - Closterovirus*)	2.71	923	Inf	0.003	0.997

Supplementary table 5. Model coefficients testing the effect of first inoculation (*Enamovirus* or mock) on sequential inoculation success of *Closterovirus* among 7 different *Plantago lanceolata* genotypes. Model variables included host genotype, first inoculation treatment (*Enamovirus* or mock) and the interaction between these two. For all variables, one level is a reference level included in the intercept.

	Estimate	Std. error	z-value	p-value
(Intercept)	1.1	1.155	0.951	0.341
Genotype 1367-8	-20.66	5.38E+03	-4.00E-03	0.997
Genotype 187-4d	-20.66	5.38E+03	-4.00E-03	0.997
Genotype 3225-5d	-20.66	5.38E+03	-4.00E-03	0.997
Genotype 3225-6c	-20.66	5.38E+03	-4.00E-03	0.997
Genotype 946-10a	-2.2	1.633	-1.346	0.178
Genotype 946-5a	-20.66	4.81E+03	-4.00E-03	0.997
First inoculation treatment (Enamovirus)	-20.66	4.81E+03	-4.00E-03	0.997
Genotype 1367-8 × First inoculation treatment (Enamovirus)	40.23	7.21E+03	6.00E-03	0.996
Genotype 187-4d × First inoculation treatment (Enamovirus)	39.13	7.21E+03	5.00E-03	0.996
Genotype 3225-5d ×First inoculation treatment (Enamovirus)	39.83	7.21E+03	6.00E-03	0.996
Genotype 3225-6c × First inoculation treatment (Enamovirus)	40.23	7.21E+03	6.00E-03	0.996
Genotype 946-10a × First inoculation treatment (Enamovirus)	2.2	7.21E+03	0	1
Genotype 946-5a × First inoculation treatment (Enamovirus)	38.84	6.80E+03	6.00E-03	0.995

Supplementary table 6. Model coefficients testing the effect of first inoculation (*Closterovirus* or mock) on sequential inoculation success of *Enamovirus* among 7 different *Plantago lanceolata* genotypes. Model variables included host genotype, first inoculation treatment (*Closterovirus* or mock) and the interaction between these two. For all variables, one level is a reference level included in the intercept.

	Estimate	Std. error	z-value	p-value
(Intercept)	-1.1	1.155	-0.951	0.341
Genotype 1367-8	-19.47	8.87E+03	-2.00E-03	0.998
Genotype 187-4d	1.29E-14	1.63E+00	0	1
Genotype 3225-5d	-19.47	8.87E+03	-2.00E-03	0.998
Genotype 3225-6c	-19.47	8.87E+03	-2.00E-03	0.998
Genotype 946-10a	-1.17E-15	1.63E+00	0	1
Genotype 946-5a	-19.47	7.93E+03	-2.00E-03	0.998
First inoculation treatment (Closterovirus)	-0.29	1.61E+00	-0.179	0.858
Genotype 1367-8 × First inoculation treatment treatment (Closterovirus)	0.29	1.25E+04	0	1
Genotype 187-4d × First inoculation treatment (Closterovirus)	-19.18	8.87E+03	-2.00E-03	0.998
Genotype 3225-5d × First inoculation treatment (Closterovirus)	19.47	8.87E+03	2.00E-03	0.998
Genotype 3225-6c × First inoculation treatment (Closterovirus)	19.76	8.87E+03	2.00E-03	0.998
Genotype 946-10a × First inoculation treatment (Closterovirus)	19.18	8.87E+03	-2.00E-03	0.998
Genotype 946-5a × First inoculation treatment (Closterovirus)	0.29	1.12E+04	0	1