Is within-host viral community assembly shaped by local adaptation?

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Abstract

Host-parasite coevolution describes the continuous reciprocal selection driving host defense and parasite infectivity, with direct consequences for disease dynamics. While abundant evidence exists for coevolution shaping host-parasite dynamics within the 'one host-one parasite' framework, hosts are typically infected by multiple parasites and the extent to which coevolutionary processes shape within-host parasite communities remains poorly understood. Investigating these interactions is essential for understanding how coevolution drives parasite diversity, competition, and coexistence within hosts. Here, we conducted a local adaptation experiment to investigate the effects of coevolution on within-host viral community assembly in *Plantago lanceolata*. Greenhouse-grown individuals were reciprocally transplanted into wild populations during natural viral epidemics. We combined small-RNA sequencing to identify the viral communities and joint species distribution modelling to quantify the effects of local adaptation, population and host characteristics on viral community assembly. Our results show that host populations vary in the extent to which local adaptation influences within-host viral diversity. Across all populations, host maternal line and origin population were the main determinants of viral community composition and infection status. The effects varied across virus families, suggesting virus-specific assembly processes and variation in the potential for coevolution to shape these interactions.

Introduction

Coevolutionary theory predicts reciprocal selection to drive key interaction traits in hosts and parasites – resistance and infectivity, respectively (1,2). Coevolution is fundamental for understanding host-parasite interactions and disease dynamics in nature, as the presence of parasites depends on the availability of susceptible hosts. Indeed, host-parasite interactions provide some of the most compelling evidence for the theory of coevolution, often demonstrated through local adaptation experiments (3–6). However, much of this work has focused on the one-host-one-parasite framework, although in nature hosts are rarely infected by a single parasite and often support complex parasite communities (7–10). Despite the growing interest in within-host parasite communities in natural environments, there remains a gap in our understanding of how coevolution can shape these complex communities (11,12).

Genetic variation and genotype-genotype specificity in the interaction are prerequisites for coevolution. Indeed, the ability to infect or resist infection can be genotype-dependent: some parasite genotypes can infect only certain host genotypes, while some host genotypes exhibit resistance to specific parasite genotypes (13–15). This variation is maintained by evolutionary mechanisms, such as parasite-imposed negative frequency-dependent selection and arms-race dynamics, which can favour different host genotypes in different populations, contributing to local adaptation (3,15,16). Notably, the outcome of host genotype × parasite genotype interactions may be altered under multiple parasite attack (17,18), with co-occurring parasites influencing community assembly either directly through parasite-parasite interactions (19,20) or indirectly through host-mediated responses (14,21). If host colonization ability – a trait expected to be shaped by coevolution – is sensitive to co-occurring parasites, then we may expect community assembly to be shaped by both ecological and evolutionary dynamics (22). The community monopolization hypothesis – evoked to explain evolutionary priority effects – predicts that locally adapted resident species can have a competitive advantage over later

arriving individuals, potentially influencing parasite community dynamics (23,24). It has been demonstrated that adaptation can reduce competitive dominance with direct consequences for community assembly (24), and that locally adapted parasites can influence the composition of the entire community (25,26).

Viruses, similar to other parasites, can form highly diverse communities (20,27–32). As obligate parasites, viral reproduction relies on the virus' ability to infect and hijack host cell machinery (33), making host-virus interactions a key factor in shaping viral communities (14,34,35). Here, to investigate how viral community assembly is influenced by coevolution, we conducted a reciprocal transplant experiment, by placing naïve *Plantago lanceolata* individuals as sentinels in sympatric and allopatric populations during naturally occurring viral epidemics. We sampled the plant individuals at the end of the growth season for small-RNA sequencing to characterize viral communities and used joint species distribution modelling (36) to tease apart the effects of local adaptation, population and host characteristics on viral community assembly. Specifically, we ask: i) Can we detect viral local adaptation? ii) What is the importance of local adaptation in determining viral community assembly? iii) What is the role of population and host characteristics in viral community assembly?

Materials and Methods

Study species

The host, *Plantago lanceolata,* is a perennial herb reproducing clonally with side rosettes or sexually with wind-dispersed pollen (37). *Plantago lanceolata* occurs worldwide, and in Finland, *P. lanceolata* can be found mainly in SW Finland. In the Åland Islands (an area spanning 50 × 70 km), *P. lanceolata* forms a large network consisting of over 4000 small fragmented populations (38).

The *P. lanceolata* host populations in the Åland Islands harbour complex viral communities (19,20). Five novel *P. lanceolata* infecting viruses have been characterised from this system, and PCR primers have been developed for their detection (14,39,40). Viral symptoms in wild hosts are challenging to identify but can include yellowing or redness of the leaf, curliness and necrotic lesions (40–42). *Plantago lanceolata latent virus* (PILV) infection has been linked to yellowing of the leaf (40,43).

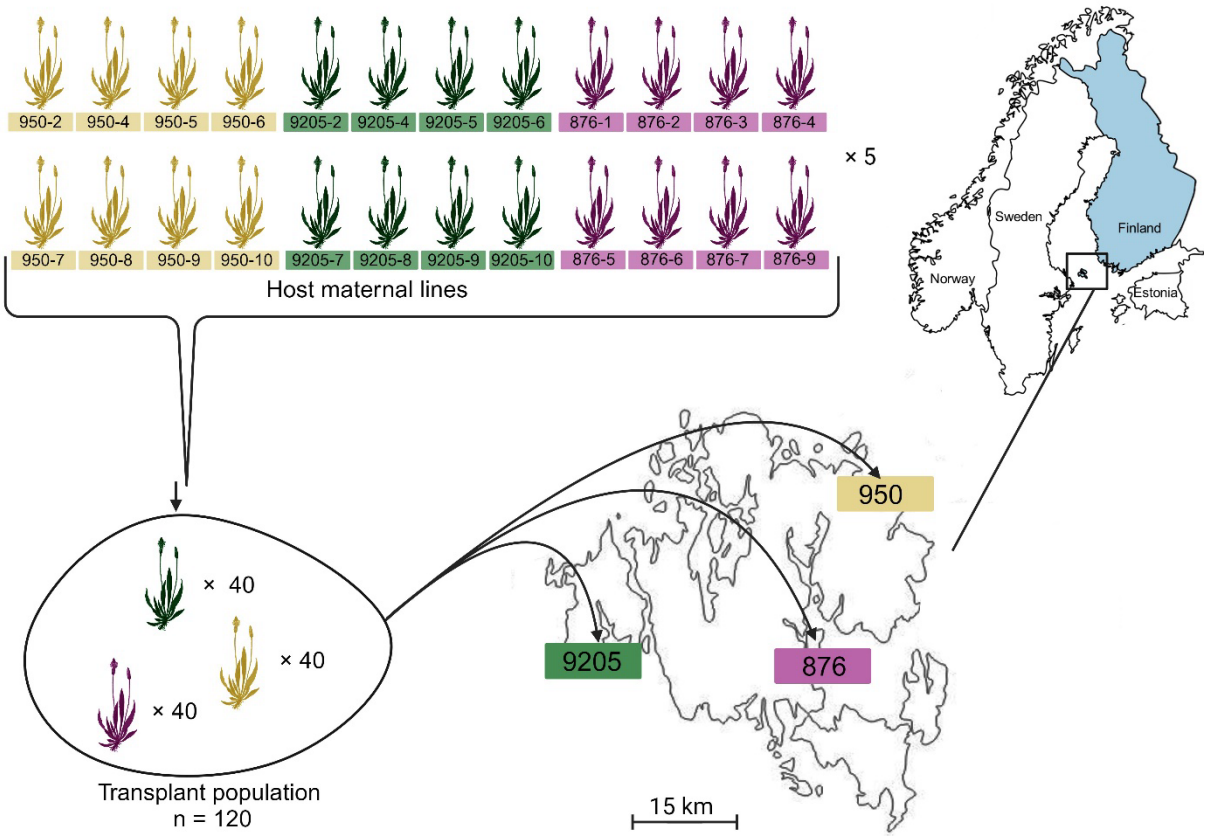
Preparation of host plant material and field experiment

To investigate the role of local adaptation in viral community assembly, we conducted a reciprocal transplant experiment in three *P. lanceolata* populations (ID: s: 9205, 876, and 950) in the Åland Islands. In autumn 2020, seeds were collected from eight individuals per studied population and germinated in early April 2021 with the aim of obtaining up to 15 offspring per maternal line. The seeds from 24 maternal lines (Supplementary table 1), were sown in peat pots with a 3:1 mixture of potting soil and sand and then placed in a growth chamber with a 16:8 h light-dark cycle. After three weeks, the seedlings were transferred to the greenhouse and replanted into 10 cm ×10 cm pots filled with a 1:1 mixture of potting soil and sand. The plants were watered as needed and, when large enough, fertilized weekly with NPK fertilizer (7:2:2). During the growth period in the greenhouse, leaf samples were collected for PCR screening to confirm that each maternal line was virus-free of PlLV, *Plantago latent caulimovirus*, *Plantago betapartitivirus*, *Plantago enamovirus,* and *Plantago closterovirus,* all of which are among the most common viruses in the Åland Islands populations (39,40). Two weeks prior to the transplant experiment, the plants were treated with fungicide (Bordeaux mixture).

In early June 2021, the greenhouse-grown naïve plants were taken to the Åland Islands and placed in their transplant populations. For each maternal line, five offspring were placed

in their sympatric *P. lanceolata* population and five in each of the two allopatric populations (Figure 1). For four maternal lines with fewer offspring, priority was given to sympatric placement, and the remaining individuals were distributed among the two allopatric populations (Supplementary table 1). Finally, the experiment consisted of 348 plants across the three transplant populations (Supplementary table 1). The plants were randomly placed among the natural vegetation and kept in pots placed inside plastic boxes (approximately 13 cm × 11 cm) to isolate them from the local soil. To minimize within-population spatial effects, we shuffled the plants among the plastic boxes three times per week for the duration of the experiment. The plants were watered as needed.

After six weeks of exposure, a 3 cm^2 piece of leaf tissue was collected for RNA extraction and snap-frozen in liquid nitrogen. At this time, we also recorded host characteristics that prior work suggests could affect viral infections on *P. lanceolata*. Plant size was measured as $n \times A$, 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 (14,19).



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130 Figure 1. Reciprocal transplant experiment where *Plantago lanceolata* individuals from 24
131 maternal lines originating from three populations were transplanted into their sympatric and
132 two allopatric populations during natural viral epidemics. We placed 40 individuals into
133 sympatric population and ~80 individuals into two allopatric populations, with a total of 348
134 plant individuals across three populations in the Åland Islands SW Finland.

135

136 RNA extraction and RNA purification

137 Total RNA was extracted using a modified acid phenol-chloroform extraction protocol (44). A
138 3 cm^2 leaf tissue sample was ground in liquid nitrogen, after which 800 µl of warm extraction
139 buffer was added and mixed thoroughly. The extraction buffer consisted of 2%
140 hexadecyltrimethylammonium bromide (Sigma-Aldrich USA), 2% of polyvinylpyrrolidone K-
141 30 (MW 40 000, Sigma-Aldrich USA), 100 mM of Tris-HCl (pH 8.0, Thermo Fisher Scientific,
142 USA), 25 mM of Ethylenediaminetetraacetic acid (pH 8.9, Sigma-Aldrich, USA), 2.0 M of
143 NaCl (Sigma-Aldrich, USA) and 2% of β-mercaptoethanol (Sigma-Aldrich, USA). Next, 800
144 µl of acid phenol-chloroform-isoamyl alcohol (IAA; 25:24:1) was added, and the sample was

centrifuged at 13500 rpm for 15 minutes at RT. The supernatant was transferred to a clean tube, mixed with 1 ml phenol-chloroform-IAA and centrifuged under the same conditions. RNA was precipitated by adding 160 µl of 10 M LiCl and incubating overnight at +4 °C. The following day, samples were purified with chloroform-IAA (24:1) purification step and washed twice with ethanol. The RNA pellet was resuspended in 25 µl of nuclease-free water and treated with Ambion® DNA-*free*™ DNA removal Kit (Invitrogen, USA). RNA concentration was measured using Nanodrop 2000 (Thermo Fischer Scientific, USA) and Qubit (Thermo Fischer Scientific, USA), and RNA was stored at -80 °C.

Small-RNA sequencing and bioinformatic pipeline

To identify the viral communities present in the sentinel plants, we assigned the samples to small-RNA (sRNA) sequencing. From the 348 sampled experimental plants, we randomly selected samples from three individuals from each maternal line from each transplant population to be assigned for sRNA sequencing. From maternal line 876-4, we sequenced three samples from the sympatric transplant population but only one sample from one of the allopatric populations, resulting in 211 samples assigned for sRNA sequencing. The RNA extracted from the selected samples was diluted with nuclease-free water and sent to the sequencing facility according to the sequencing company's instructions (Fasteris SA, Switzerland).

The sRNA sequencing and library preparation were carried out at Fasteris SA (Switzerland). Small-RNA cDNA libraries were prepared using QIAseq miRNA Library Kit (Qiagen) according to Fasteris SA Small RNA-Seq Gel-free protocol with 100 ng of total RNA. Sequencing was performed using Illumina NovaSeq 6000 (Illumina Inc, San Diego, California, USA) and targeted insert sizes from 0 nt to 43 nt with an average library yield of 1779 Mb.

Inserts with sizes from 20 nt to 25 nt were selected for bioinformatic analyses. Sequencing adapter removal was done using Trimmomatics software (45), and the reads were de novo assembled to contigs using VirusDetect software (46). VirusDetect software conducts BLASTX and BLASTN searches against curated plant virus database (vrl_Plants_248_U100) of VirusDetect for each sample separately. We used default parameters BLASTX and BLASTN searches, default similarity 25 % and p-value 1e-5. We then assigned the obtained contigs to virus family level for the statistical analyses (Supplementary table 2).

Statistical analysis

All statistical analyses were conducted in R (version 4.2.2; (47). To test whether local adaptation influenced host infection status (infected by any studied virus= 1, not infected by any studied virus = 0), we fitted generalized linear mixed models (GLMM) using the "glmmTMB" R-package (48) with binomial distribution and logit link function. Specifically, we constructed GLMMs to test the two key metrics of local adaptation: i) local *vs.* foreign and ii) home *vs.* away (49,50). For the local *vs.* foreign model (LF), a categorical variable representing sympatry or allopatry, nested within transplant population, was included as a fixed effect. Seed origin population and plant size were included as additional fixed effects and maternal line nested within seed origin population was included as a random effect to account for genetic variation among hosts. For the home *vs.* away model (HA), the model structure was identical, except that the categorical variable of sympatry or allopatry was nested within seed origin population and included as a fixed effect. Model assumptions were assessed using R-package “DHARMa” (51). The significance of the main effects were evaluated using Wald X^2 tests (function “Anova” in R-package “car”; (52). For significant effects, pairwise comparisons of the estimated marginal means were performed using functions “contrasts” and “emmeans”

193 from the R-package “emmeans” (version 1.8.8; (53), applying Tukey’s method for multiple
194 comparisons.

195 To investigate the effects of local adaptation, population and host characteristics on
196 within-host viral diversity, while also accounting for viral (co-)occurrence patterns in the
197 transplant experiment, we implemented Joint Species Distribution Modelling (JSDM) using
198 the hierarchical modelling of species communities (HMSC) framework (54,55). HMSC is a
199 hierarchical generalized linear mixed model with Bayesian inference and allows the analysis
200 of multiple species’ responses to ecological variables while incorporating species- and
201 community-level parameters and accounting for covariation among species. Th response
202 variables in our HMSC model were the occurrences of the three most prevalent virus families:
203 *Caulimoviridae*, *Partitiviridae* and *Pospivirodae*. As fixed effect predictors, we included 1)
204 maternal line ID, 2) seed origin population, 3) sympatry/allopatry, 4) plant size, and 5) signs
205 of herbivory. Transplant population was included as a random effect. Including
206 sympatry/allopatry as a fixed effect allowed us to directly estimate the effect of local adaptation
207 in our model. We used four separate Markov chain Monte Carlo (MCMC) chains to sample the
208 posterior distribution. Each chain was run for 1 875 000 iterations, and the first 625 000 were
209 discarded as burn-in. Subsequently, the remaining iterations were thinned by 5000, resulting in
210 250 posterior samples per chain. Finally, we obtained a total of 1000 posterior samples across
211 all four chains. The model fit was evaluated by examining explanatory and predictive
212 performance via ten-fold cross-validation, using Tjur’s coefficient of determination (Tjur R^2)
213 and area under the curve (AUC), respectively. The HMSC analyses were ran using the R-
214 package “Hmsc” (version 3.0-14).

215

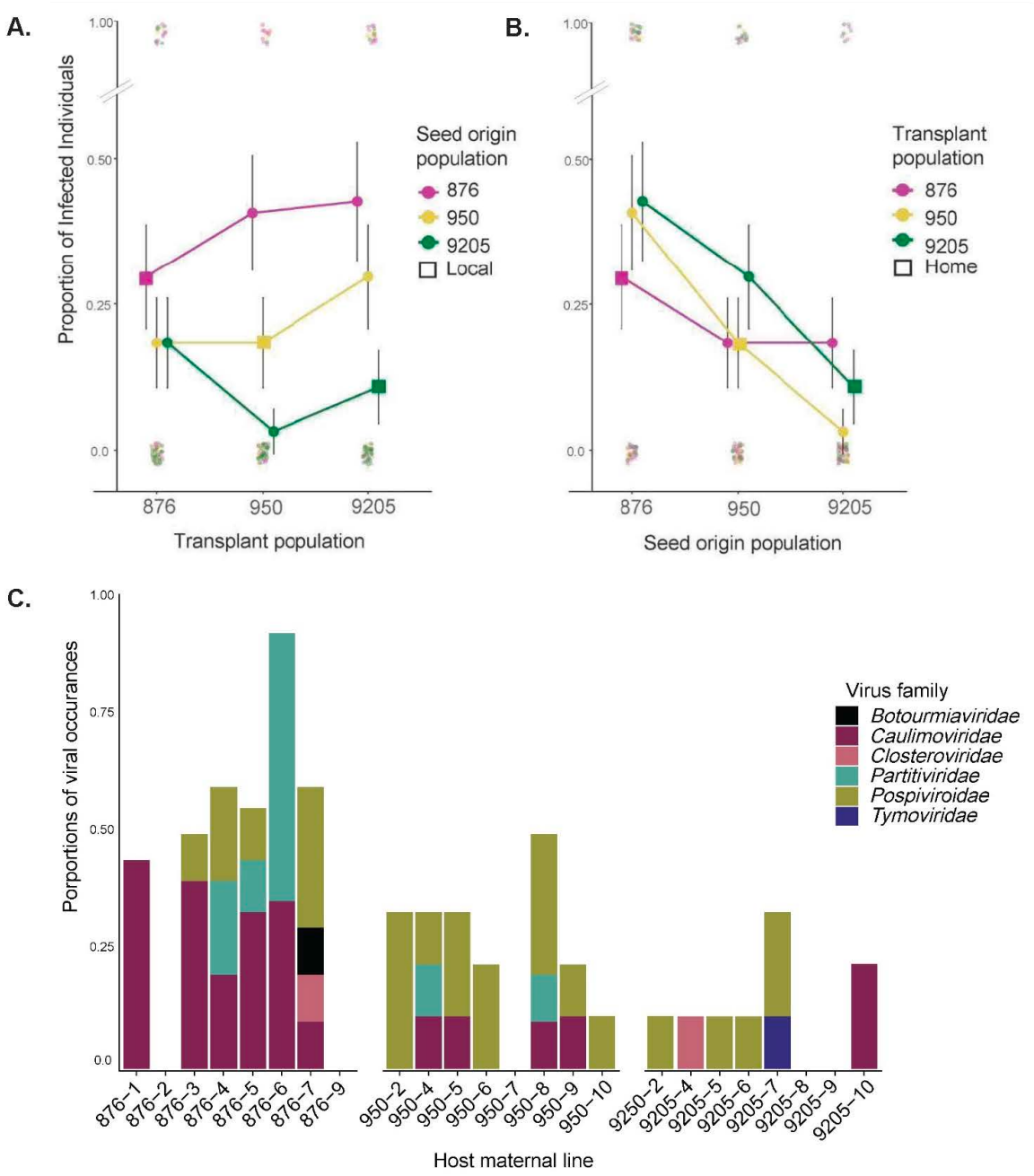
216

Results

Description of the sRNA sequencing data

From the 211 sequenced individuals, the sRNA sequencing yielded on average 23,799,485 reads per plant tissue sample (min 17,364,260; max 49,152,805; SD 7,738,962). The VirusDetect pipeline assembled 2374 contigs ranging from 41 to 2080 nt in length (mean length of 159 nt and SD 163 nt). Of these, 11% of contigs had virus-specific BLASTN hits with 80–100% identity (mean 93%), while 89% had BLASTX hits with 22–100% identity (mean 67%).

In total, we assembled 1151 plant virus-associated contigs across the 211 individuals, representing six plant virus families: *Tymoviridae, Botourmiaviridae, Closteroviridae, Partitiviridae, Caulimoviridae* and *Pospiviroidae* (Figure 2C, Supplementary table 2). From each family, we identified 1 to 3 virus genera and 3 to 842 contigs for each genus. At the species level, we acquired BLAST hits to 1 to 15 species, depending on the virus genus (Supplementary table 2). Overall, 26% of the host individuals were infected, and of those 86% were colonized by one virus family and 14% by two virus families. The most prevalent families were *Caulimoviridae* and *Pospiviroidae* (both in 43% of the infected individuals), whereas *Tymoviridae* and *Botourmiaviridae* were the rarest (both in 2% of the infected individuals; Figure 2C).



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235 Figure 2. Proportions of virus infected *Plantago lanceolata* host individuals in a reciprocal
236 transplant experiment using (A) local vs. foreign, (B) home vs. away metrics of local
237 adaptation, and (C) infection pattern across host maternal (n =24) line grouped by seed origin
238 population. In panel A, colours indicate the seed origin populations and the squares mark the
239 local host. In panel B, the colours represent the transplant populations and the squares mark
240 the home habitat of the host (purple = seed origin/transplant population 876 yellow = seed
241 origin/transplant population 950, and green = seed origin/transplant population 9205). In panel
242 C colours represent the six virus families detected with sRNA sequencing.

243

244 Analysis of viral local adaptation: local *vs.* foreign

245 Using the local *vs.* foreign criterion, we observed indications of viral local adaptation in
246 transplant population 876, where local hosts had higher infection rates than foreign hosts. A
247 similar trend was observed in population 950, where local hosts showed the second-highest
248 infection rates (Figure 2A). Conversely, in population 9205, local hosts harboured fewer
249 infections than foreign hosts – suggesting viral maladaptation. However, the GLMM (LF) did
250 not provide statistical support for these trends (Wald X^2 = 1.53, P = 0.673; Table 1). Seed
251 origin populations differed significantly in infection rates (Wald X^2 = 7.37, P = 0.025; Table
252 1), with individuals originating from population 9205 having significantly fewer infections
253 than those originating from population 876 (Figure 2A, Supplementary table 3A; estimate =
254 2.071, SE = 0.778, z-ratio = 2.661, P = 0.021).

255 Table 1. Type II Wald X^2 test for Generalizer linear mixed model estimating the effects of plant
256 size, seed origin population, transplant population and local *vs.* foreign metric of local
257 adaptation on host infection status (1=infected, 0=uninfected) in a reciprocal transplant
258 experiment in the Åland Islands (model LF).

259

Fixed effect	*Wald* X^2	*Df*	*p-value*
Plant size	1.71	1	0.190
Seed origin population	**7.37**	**2**	**0.025**
Transplant population	1.99	2	0.369
Transplant population : sympatry/allopatry	1.53	3	0.673

260

261 Analysis of viral local adaptation: home *vs.* away

262 Applying the home *vs.* away criterion, we found no evidence of viral local adaptation (Figure
263 2B). Hosts from populations 876 and 950 had lower infection rates in their respective home
264 populations than in their away populations, suggesting viral maladaptation (Figure 2B). Our
265 statistical analysis (model HA) did not detect significant differences in infection rates between

home and away habitats. However, model coefficients for the "sympatry" term nested within seed origin population were lower, suggesting higher infection rates in away habitats (Supplementary table 4). Additionally, seed origin population significantly influenced host infection status (Wald X^2 = 9.09, *P*=0.010; Table 2). Post hoc comparisons showed that individuals from population 876 had significantly higher infection rates than those from population 9205 (estimate = 1.818, SE = 0.649, z-ratio = 2.802, P = 0.014; Supplementary table 3B., Figure 2B and C).

Table 2. Type II Wald X^2 test for Generalizer linear mixed model testing for the effects of plant size, seed origin population, transplant population and home *vs.* away metric of local adaptation on host infection status (1=infected, 0=uninfected) in a reciprocal transplant experiment in the Åland Islands (model HA).

Fixed effect	*Wald* X^2	*Df*	*p-value*
Plant size	1.71	1	0.190
Seed origin population	**9.09**	**2**	**0.010**
Transplant population	1.64	2	0.438
Seed origin population : sympatry/allopatry	1.53	3	0.673

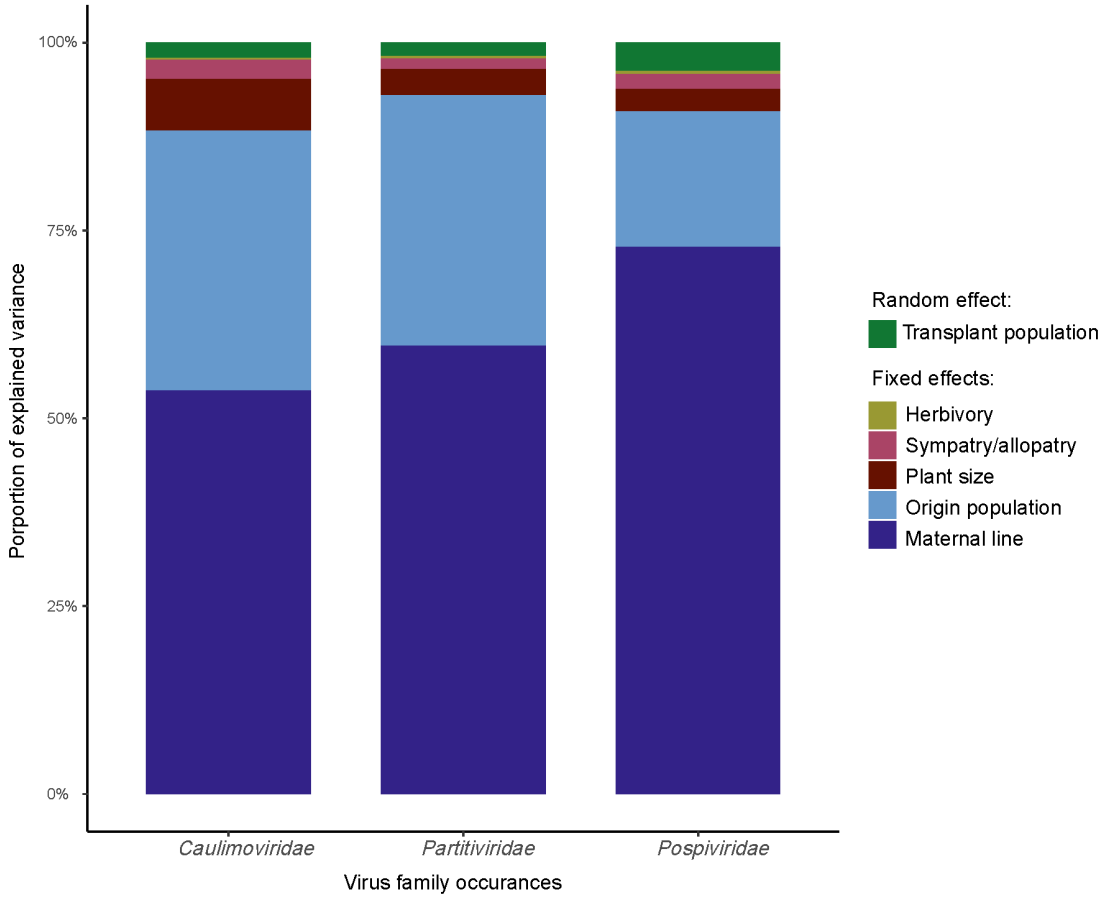
Analysis of viral (co-)occurrence patterns

We applied the HMSC approach to investigate the factors influencing the (co-)occurrence of the detected virus families in a local adaptation experiment. The model predicted virus family occurrences well, although model performance varied among virus families (Supplementary table 5). Tjur R^2 and AUC were used to quantify the explanatory and predictive performance of the model, with a mean Tjur R^2 of 0.27 (range among the detected virus families 0.10-0.47) and a mean AUC of 0.89 (0.80-0.98). The predictive power of the model was based on ten-fold cross-validations, where the mean Tjur R^2 was 0.19 (range 0.01 - 0.4) and the mean AUC was 0.74 (range 0.54-0.88; Supplementary table 5) varying among virus families.

In terms of contributions to the explained variation in our HMSC model, host maternal line was the strongest determinant of viral occurrences, explaining on average 62% of the variance. However, the effect varied among virus families and was most pronounced for *Pospiviroidae* (73%) and less important in explaining *Partitiviridae* (60%) and *Caulimoviridae* 54%) occurrences (Figure 3, Supplementary table 6). For example, maternal line 876-6, displayed the highest infection rates, with 89% of the individuals infected (Figure 2C). Seed origin population was the second most important predictor, explaining on average of 29% of the variance. The effect of host maternal line varied among virus families, with a more pronounced role for *Caulimoviridae* (35%) and *Partitiviridae* (33%), while being less important for explaining the occurrences of *Pospiviroidae* (18%; Figure 3, Supplementary table 6). Consistent with this, individuals from seed origin population 876 harboured 50% of all detected viral infections, whereas individuals originating from population 9205 harboured only 16% of all infections (Figure 2C).

Host plant size accounted for an average of 4% of the variation in viral occurrences, with the strongest effect observed for *Caulimoviridae* (7%). Local adaptation (sympatry/allopatry) had a smaller role in contributing to explained variation, accounting for 2% on average across virus families (Figure 3, Supplementary table 6). Herbivory had minimal effect, explaining only 0% to 0.1% of the viral occurrences. The random effect of transplant population explained on average 2% of the variation across virus families and was slightly more important in explaining *Pospiviroidae* occurrences (4%, Figure 3, Supplementary table 6). Residual correlations among virus families at the random level were not significant, suggesting that after accounting for the effects of the fixed explanatory variables, viral occurrences were not influenced by interactions between virus families.

Figure 3. Variance partitioning of the fixed and random effects in the Hierarchical Modelling of Species Communities model for the three most prevalent virus families (*Caulimoviridae*, *Partitiviridae*, *Pospiviridae*) in the reciprocal transplant experiment. The six variables explaining the occurrences the three virus families were: maternal line, seed origin population, plant size, sympatry/allopatry, herbivory and transplant population (random effect).



Discussion

Here, we used a reciprocal transplant experiment combined with sRNA sequencing and JSDM modelling to investigate the role of local adaptation in shaping within-host viral (co-)occurrences. Although we observed trends suggesting viral local adaptation and maladaptation when applying the local *vs.* foreign and home *vs*. away criteria, the effects were not statistically significant. Instead, we found host maternal line and host seed origin population to be the most important determinants of host infection status and viral community structure. The strength of

these effects varied across virus families, indicating virus-specific assembly processes and variation in the extent to which coevolution shapes these interactions. Jointly our results identify key drivers of viral community assembly and provide insight into how within-host dynamics could scale up to predict the ecological and evolutionary consequences of disease in natural systems.

Using sRNA sequencing, we detected viruses from six virus families, five of which have been previously identified from this system (20,43). Overall, 21% of the sampled sentinel plants were infected, exhibiting a lower infection rate than previously reported from hosts in this system (20,43). Despite the low overall infection prevalence, we found individuals originating from population 876 harbouring significantly higher infection rates than those from population 9205. Viral community composition also varied among seed origin populations and among maternal lines. Individuals from seed origin population 876 harboured viruses from five different virus families (*Caulimoviridae*, *Pospiviroidae*, *Partitiviridae*, *Botourmiaviridae* and *Closteroviridae*), whereas individuals from population 950 were infected by only three virus families (*Pospiviroidae*, *Caulimoviridae* and *Partitiviridae*). The overall lower infection prevalence may be due to differences in exposure time to viral epidemics and additionally, viral prevalence may vary annually due to several factors, such as temperature, humidity and vector behaviour — components of natural systems that are difficult to control in a field experiment (56–58).

Using a reciprocal transplant experimental approach, we were able to apply the two key metrics of local adaptation: local *vs.* foreign and home *vs.* away. While we observed signs of viral local adaptation in transplant population 876 under the local *vs*. away criterion, the pattern was not statistically significant (GLMM LF). Similarly, analysis on the home *vs.* away metric showed no statistically significant effect of local adaptation on host infection status (GLMM HA). In line with these results, when investigating the effects of local adaptation on viral (co-

)occurrence patterns with JSDM in the HMSC framework, we found local adaptation to explain on average only 2.3% of the viral occurrences. However, when using the home *vs.* away criterion (GLMM HA), individuals from seed origin populations 876 and 950 harboured the lowest infection rates in their home populations, suggesting viral maladaptation. Patterns of maladaptation are not unexpected given the dynamic, cyclic nature of coevolutionary interactions between the host and its parasite (59). In the Aland Islands *P. lanceolata* populations are highly fragmented, and the connectivity levels of the populations vary (60,61) and consequently too high or low gene flow between populations could facilitate parasite maladaptation (59,62–64). Previous studies have shown that well-connected host populations are less affected by disease (65,66), a phenomenon that is likely due to higher resistance diversity in these populations maintained by gene flow (61).

Seed origin population was a strong predictor of host infection status. Individuals originating from population 876 were more frequently infected and harboured the most diverse viral communities. In contrast, hosts from population 9205 exhibited high resistance to viral infection and consequently harboured less complex viral communities. Our HMSC analysis mirrored these findings, identifying maternal line and seed origin population as the strongest determinants of viral occurrence across virus families, explaining on average 62% and 29% of the variation, respectively (Figure 3). The variation in infection rates among host origin populations, together with the strong maternal line effects for viral occurrences across virus families, highlights host genetic diversity as a key driver of viral community assembly and composition in this system. Although evidence for viral local adaptation was limited, the variation in infections prevalence among host maternal lines indicates strong potential for coevolution, as genetic variation is a main driver of coevolution (67–69). Moreover, high host genetic diversity in natural populations can mitigate disease risk, a phenomenon known as the monoculture effect (70,71).

Hosts encounter a myriad of parasites throughout their lives (43,72–74), and these interactions can have far-reaching consequences for host-parasite coevolution and population dynamics (75). Despite this, much of the research on local adaptation has focused on pairwise host-parasite interactions (76–78), with little focus on the role of parasite communities in coevolutionary processes. To our knowledge, our study is among the first to study viral local adaptation within a community ecology framework. After accounting for host attributes, we found no evidence of virus-virus interactions shaping within-host viral diversity. Instead, host characteristics, represented by maternal line and host seed origin population, emerged as the most important predictor of viral community structure and host infection status. Our findings highlight the importance of host genetic variation in shaping viral communities and contribute to the growing field of viral community ecology research. Understanding the drivers of complex host-parasite interactions and processes at the community level is essential for predicting how disease dynamics scale up from individuals to populations and understanding the ecological and evolutionary conditions from which novel viral diseases may emerge.

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Author contributions

M.J., H.S. and A.-L.L. designed the study. M.J. performed the field experiment, data collection, and statistical analysis. M.J., H.S. and A-L.L. prepared the manuscript.

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/ViRAL21).

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607 **Supplement**

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609 **Supplementary table 1.** The host maternal lines included in a reciprocal transplant experiment
610 studying viral local adaptation in *Plantago lanceolata* host populations in the Åland Islands
611 during naturally occurring viral epidemics. In the table are included the ID of each maternal
612 line, the ID of the origin population of each maternal line, the ID of the transplant population
613 where the plants were placed during the experiment and the number of individuals, and finally,
614 the number of sequenced individuals.

Maternal line	Seed origin population	Transplant population	No individuals in the experiment	Number of individuals sequenced
876-1	876	876	5	3
876-1	876	950	5	3
876-1	876	9205	5	3
876-2	876	876	5	3
876-2	876	950	5	3
876-2	876	9205	5	3
876-3	876	876	5	3
876-3	876	950	5	3
876-3	876	9205	5	3
876-4	876	876	5	3
876-4	876	950	1	1
876-4	876	9205	0	0
876-5	876	876	5	3
876-5	876	950	4	3
876-5	876	9205	3	3
876-6	876	876	5	3
876-6	876	950	5	3
876-6	876	9205	5	3
876-7	876	876	5	3
876-7	876	950	5	3
876-7	876	9205	5	3
876-9	876	876	5	3
876-9	876	950	5	3
876-9	876	9205	5	3
950-10	950	876	5	3
950-10	950	950	5	3
950-10	950	9205	5	3
950-2	950	876	5	3
950-2	950	950	5	3
950-2	950	9205	5	3
950-4	950	876	5	3
950-4	950	950	5	3
950-4	950	9205	5	3
950-5	950	876	5	3
950-5	950	950	5	3
950-5	950	9205	5	3
950-6	950	876	5	3
950-6	950	950	5	3
950-6	950	9205	5	3
950-7	950	876	5	3
950-7	950	950	5	3
950-7	950	9205	5	3
950-8	950	876	5	3
950-8	950	950	5	3
950-8	950	9205	5	3
950-9	950	876	5	3
950-9	950	950	5	3
950-9	950	9205	5	3

9205-10	9205	876	5	3
9205-10	9205	950	5	3
9205-10	9205	9205	5	3
9205-4	9205	876	5	3
9205-4	9205	950	5	3
9205-4	9205	9205	5	3
9205-5	9205	876	5	3
9205-5	9205	950	5	3
9205-5	9205	9205	5	3
9205-6	9205	876	5	3
9205-6	9205	950	5	3
9205-6	9205	9205	5	3
9205-7	9205	876	5	3
9205-7	9205	950	5	3
9205-7	9205	9205	5	3
9205-8	9205	876	4	3
9205-8	9205	950	5	3
9205-8	9205	9205	5	3
9205-9	9205	876	5	3
9205-9	9205	950	5	3
9205-9	9205	9205	5	3
9250-2	9205	876	5	3
9250-2	9205	950	5	3
9250-2	9205	9205	5	3

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Supplementary table 2. Virus families detected by small-RNA sequencing on *Plantago lanceolata* individuals (n = 211) included in a transplant experiment in the Åland Islands studying viral local adaptation. The genera belonging to each virus family are shown as well as the number of contigs and virus species within each virus family. Reference to the literature describing the detected family listed in the "reference" column [1–3].

Family	Genus	Contigs	Species	References
Botourmiaviridae		3	1	
	Gammascleroulivirus	3	1	
Caulimoviridae		896	21	[1,2,3]
	Badnavirus	1	1	
	Caulimovirus	842	15	
	Soymovirus	52	5	
Closteroviridae		13	5	[1,2,3]
	Ampelovirus	1	1	
	Closterovirus	11	3	
	unclassified	1	1	
Partitiviridae		212	11	[1,2,3]
	Betapartitivirus	113	8	
	unclassified	99	3	
Pospiviridae		24	1	[3]
	Pospiviroid	24	1	
Tymoviridae		3	1	[1,2,3]
	Maculavirus	3	1	

Supplementary table 3. Post hoc test comparing the infection status of the host *Plantago lanceolata* from the three seed origin populations in a local adaptation experiment in the Åland Islands during naturally occurring viral epidemics. Pairwise comparison of the estimated marginal means calculated from both generalized linear mixed effects models A) model LF and B) model HA (Table 1 and 2). P-values are Tukey adjusted.

A.

Contrast	*Estimate*	*SE*	*Df*	*Z ratio*	*p-value*
Seed origin population 876 – Seed origin population 950	1.283	0.703	Inf	1.825	0.161
Seed origin population 876- Seed origin population 9205	**2.071**	**0.778**	**Inf**	**2.661**	**0.021**
Seed origin population 950 – Seed origin population 9205	0.788	0.736	Inf	1.070	0.532

B.

Contrast	*Estimate*	*SE*	*Df*	*Z ratio*	*p-value*
Seed origin population 876 – Seed origin population 950	0.703	0.597	Inf	1.176	0.467
Seed origin population 876- Seed origin population 9205	**1.818**	**0.649**	**Inf**	**2.802**	**0.014**
Seed origin population 950 – Seed origin population 9205	1.115	0.664	Inf	1.679	0.213

Supplementary table 4. Model coefficients (model HA) testing for the effects of local adaptation on host infection status using the home *vs.* away metrics of local adaptation. For all variables one levels is a reference level included in the intercept.

Parameter	*Coefficient*	*Std. Error*	*z-ratio*	*p-value*
(Intercept)	0.46958	0.76237	0.61595	0.53792
Plant size	-0.00088	0.00067	-1.31035	0.19008
Seed origin population 950	-1.28293	0.70295	-1.82505	0.06799
Seed origin population 9205	**-2.07082**	**0.77828**	**-2.66078**	**0.00780**
Transplant population 950	-0.47045	0.67071	-0.70142	0.48304
Transplant population 9205	0.28314	0.59902	0.47267	0.63645
Seed origin population 876 × sympatric	-0.86730	0.80862	-1.07257	0.28346
Seed origin population 950 × sympatric	0.29316	0.83422	0.35141	0.72528
Seed origin population 9205 × sympatric	-0.36200	0.92003	-0.39346	0.69398

Supplementary table 5. Explanatory and predictive performance of the HMSC model of viral occurrence in the experimental plant individuals in terms of Tjur R^2 and AUC. The model predictive performance is based on 10-fold cross-validation.

	Model explanatory performance		Model predictive performance with 10-fold cross validation (cv)	
Response variable	**Tjur R^2**	**AUC**	**Tjur R^2 (cv)**	**AUC (cv)**
Caulimoviridae	0.24	0.91	0.16	0.81
Partitiviridae	0.47	0.98	0.4	0.88
Pospivirodae	0.1	0.8	0.01	0.54

Supplementary table 6. Exact values of the HMSC model variance partitioning for the three most prevalent virus families detected in a reciprocal transplant experiment studying local adaptation. Six variables explaining the virus family occurrence in a reciprocal transplant experiment were: maternal line ID, seed origin population ID, sympatry/allopatry, plant size, herbivory and transplant population ID (random).

Model parameter		**Response variable**		
Fixed effects:		*Caulimoviridae*	*Partitiviridae*	*Pospivirodae*
	Maternal line	0.54	0.60	0.73
	Seed origin population	0.35	0.33	0.18
	Sympatry/allopatry	0.03	0.02	0.02
	Plant area	0.07	0.03	0.03
	Herbivory	0	0	0
Random effect:				
	Transplant population	0.02	0.02	0.04

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