The direction of local adaptation on pathogen exploitation rates reverses with environmental context

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ABSTRACT

Host diversity and spatial heterogeneity are both predicted to impact parasite evolution, and these processes may interact in nature to impact local adaptation. Here, we experimentally evolve granulosis virus in microcosms of its \textit{Plodia interpunctella} host with varied spatial structure and host genetic diversity. We find that virus evolves specific interactions with its locally familiar host genotype in both homogeneous and spatially heterogeneous host populations, but that the impact of local adaptation depends on the spatial structure. In one treatment, exploitation rates are higher on the familiar host, while, in the other, they are lower. While we find that food viscosity had unexpected impacts on the spatial structuring of contacts, spatially structured transmission may have led to the virus evolving more prudent exploitation rates on only the familiar host, leading to locally maladapted high exploitation rates on foreign hosts. Additionally, we also find that virus in heterogeneous microcosms is more locally adapted when there are higher contact rates between the hosts. Our experiment demonstrates that trade-offs optimizing exploitation rates at intermediate values, like those governing pathogen infectivity in spatial structure, may interact with trade-offs determining niche breadth in ways that can reverse the impact of local adaptation on pathogen phenotypes. [200/200]
INTRODUCTION

When trade-offs between different phenotypes act upon an organism, they can lead to a variety of evolutionary outcomes including the evolution of intermediate optimal strategies, evolutionary branching and niche partitioning, and cyclical dynamics [1–4]. Thus, trade-off theory underpins a wide array of ecological and evolutionary theory on adaptation and diversification [5,6]. One area where trade-offs are of particular importance is in explaining how biotic diversity is generated and maintained [7–9]. The idea here is that ‘jacks-of-all-trades are the master of none’, meaning that trade-offs between adaptation to different abiotic environments or biotic partners require an organism to choose between being a fit specialist or mediocre generalist [10]. For host-parasite interactions, this fundamental problem of niche breadth evolution becomes a question of host range evolution [11–13] and has implications for theories ranging from the Red queen hypothesis of sex [14,15], Janzen-Connell effects [16,17], Geographic mosaic theory [18], and zoonotic emergence [19,20].

However, it is an obvious fact that any organism is subject to multiple environmental pressures and thus a single phenotype may be simultaneously subject to multiple, potentially interacting trade-offs [21]. For example, while an organism’s growth rates on different environments might trade-off with each other, that organism’s growth rate might also be subject to life history trade-offs that select for intermediate optimal strategies [22]. Less appreciated is how the specific geometries and interactions between trade-offs can lead to different evolutionary outcomes for a system [22–25]. Depending on the order in which we consider our trade-offs in the above example, we could hypothesize that: 1) growth rates could be lower than maximal across the system, but still highest for the specialist on its familiar environment or 2) growth rates could be at the intermediate optimal level for both the specialist on its familiar environment and the ‘mediocre’ generalist on both. While the first type of interaction does mean that the outcome of evolution under multiple trade-offs is more consistent with the predictions of theory based on individual trade-offs and is thus more naturally assumed, there is no reason to expect that it should actually be more common. It is therefore necessary to better understand how trade-offs interact to determine how predictions of single trade-off theory scale into more ecologically realistic scenarios.

Here, we experimentally evolve granulosis virus in microcosms of its host Plodia interpunctella, the Indian meal moth (Hübner), host that vary in their host genetic diversity and spatial structuring. Altering host genetic diversity allows us to examine the consequences of trade-offs between host types on virus evolution [26], while altering the spatial structure of infectious contacts selects on pathogen exploitation rate [27]. Additionally, the spatial structuring of genetic diversity might further influence host range evolution due to differences in contact rates between hosts [28–30]. Thus, our experiment examines how pathogen evolves when experiencing a combination of selection pressures that are governed by separate, but potentially interacting, trade-offs.

On its own, spatially structured transmission is predicted to select for less infective, more prudent pathogens as higher proportions of local, rather than global, transmission mean that the pathogen competes with more closely related strains to deplete its local susceptible population and ‘self-shade’ [27,31–33]. Previous evolution experiments in the Plodia interpunctella and PiGV
system support this theory [34], as do experiments in microbial systems [35]. On its own, host heterogeneity is also predicted to select for less infective and virulent pathogens, but, in this case, the lower exploitation rates are due to evolutionary constraints where adapting to two host types mean that the pathogen is predicted to be less able to specialize on either one [8,26,36,37]. Some previous experiments in empirical systems confirm these hypotheses [38–43], but such constraints are not consistently observable and their exact nature is still unclear [37,44–48]. Our experiment therefore examines how an optimizing life history trade-off mediated by susceptible density and acting at the population level interacts with a constraint-based trade-off acting at the individual level.

Finally, the spatial structuring of host genetic diversity has important consequences for how pathogens are expected to evolve in metapopulations and locally adapt to different patches [49–51]. Theory suggests that contact rates between hosts, or migration between patches, should alter how pathogens are selected to specialize on their familiar host or become generalists. Higher contact rates between host types should select for generalists [28–30], but can also lead to greater local adaptation when migration increases the amount of genetic variation that selection can act upon and increases local competition for patches [52–54].

Our experiment therefore explores how trade-offs mediated by spatial structure and host heterogeneities interact and how the spatial structuring of host heterogeneity effects its impact on pathogen evolution. We find that manipulating the spatial structure of a population can reverse the impact of local adaptation on pathogen phenotypes so that they are less exploitative of their familiar host than of foreign hosts. We also find that virus in heterogeneous microcosms is more locally adapted when there are higher migration rates between the host types. These results imply that interactions between trade-offs mean that local adaptation might not always correlate with higher exploitation rates on the familiar host when optimizing life history trade-offs are simultaneously selecting for lowered exploitation rates.

METHODS

Study system

*Plodia interpunctella*, or the Indian meal moth, is a stored grain pest that naturally lives at high population density within its cereal food medium for 5 larval instar stages before it pupates into an adult moth that disperses, mates, and lays eggs, but does not eat [55,56]. It is naturally infected by the *Plodia interpunctella* granulosis virus (PiGV), a dsDNA baculovirus that transmits when larvae directly cannibalize infection killed cadavers or consume virus that was released into the environment from such cadavers [57,58]. Thus, the virus is an obligate killer that will only transmit if it kills its host. Notably, the virus can only infect *P. interpunctella* during the larval stages and, if exposed larvae clear the infection, they can pupate and carry out the rest of their life history as normal [59]. Infection killed cadavers are recognizable because successful infection turns larvae an opaque, chalky white color due to the high density of viral occlusion bodies [60]. The system has proved a powerful tool to examine the effects of spatial structure on pathogen exploitation rates and, more recently, host genotype specialization [34,61].
Host line selection and maintenance

For this experiment, we select 2 inbred populations of *P. interpunctella* that were previously generated in the lab by brother-sister mating individuals for >27 generations [62]. Each population should therefore be essentially a clonal population of two distinct host genotypes (Il-9 and Il-17). These lines were chosen because they had similar levels of general resistance [61]. We have previously shown that virus evolved in homogeneous populations of these specific inbred lines can specialize on its familiar host genotype by increasing their infectivity and/or productivity [61].

We maintain these lines in wide mouthed Nalgene pots (ThermoFisher Scientific, U.K.) in the absence of infection with 200g ‘Standard’ food medium (made in batches of 500g cereal mix (50% Earth’s Best oatmeal, 30% wheat bran, 20% rice flour), 100g brewer’s yeast, 2.2g sorbic acid, 2.2g methyl paraben, 125ml honey, and 125ml glycerol) in incubators set at 27±2 °C and 35±5% humidity, with 16:8hr light:dark cycles [63]. To maintain populations, ~50 adult moths are moved into a new pot with new food when they emerge monthly. Thus, host lines are maintained in the absence of infection and do not evolve throughout the experiment.

Manipulating spatial structure

We manipulate spatial structure in the experiment by changing the viscosity of the food medium, as in [34,64,65]. Because larvae live within their food medium, this alters individuals’ dispersal patterns and thus the spatial structure of their infectious contacts. We make food of two viscosities, ‘loose’ and ‘sticky’, which differ solely in the amount of glycerol added. ‘Loose’ food contains 500g Earth’s Best oatmeal, 100g brewer’s yeast, 2.2g sorbic acid, 2.2g methyl paraben, and 175ml glycerol. ‘Sticky’ food contains 500g Earth’s Best oatmeal, 100g brewer’s yeast, 2.2g sorbic acid, 2.2g methyl paraben, and 450ml glycerol. Food viscosity does not affect development time or mass at pupation (Figure S1). ‘Loose’ and ‘sticky’ food is then added to wide-mouthed Nalgene pots and frozen overnight to kill any insect eggs potentially contaminating the food. The mass of food added to these pots (300g ‘loose’ and 550g ‘sticky’ food) was determined by the volume needed to entirely fill half of one of our plastic microcosm containers.
Figure 1: Experimental design and passaging scheme. (Left) Each microcosm is set up by adding 2 pots of food containing 11-day old larvae to opposite vertical ends of a plastic microcosm with a 3x5 grid. The 3rd row of the grid is therefore a contact zone between the sides. There are 3 replicates for each of the 8 microcosms. There are 4 different treatment types of homogeneous or heterogeneous hosts by 'loose' or 'sticky' food. Each homogeneous treatment is set up with both host types and each heterogeneous host treatment is set up alternating which host is on 'top' or 'bottom' of the grid. Microcosms are consistently oriented in the growth chamber so that the 'bottom' side is closer to the door. (Right) After 20 days incubating with virus, microcosms are frozen and dissected into grid squares. All infected larvae from each grid square are harvested and added to a 1.5mL Eppendorf tube (1 square per tube) and homogenized to release occlusion bodies. 500mL of the virus solution is then pipetted in droplets over the same grid square of a new microcosm of the same treatment set-up with fresh 11-day old hosts. Therefore, the spatial force of infection is preserved across passages. We experimentally evolve virus for 10 passages.

Setting up host populations for microcosms

After thawing the pots of ‘sticky’ and ‘loose’ food, we add ~50 similarly aged adult moths to each pot from either our Il-9 or Il-17 host maintenance populations and incubate them for 10 days as in Figure 1. These single-genotype adults mate and lay eggs into the ‘sticky’ or ‘loose’ food. 10 days after adding adults, these pots containing Il-17 larvae in ‘loose’ food, Il-9 larvae in ‘loose’ food, Il-9 larvae in ‘sticky’ food are used to set up microcosms. Therefore, pots are set up with the same number of adults, but may not contain the same number of larvae.

Microcosms are set up in 9.25” x 12.08” x 0.9” inch plastic A4 document cases (Daiso Japan, U.S.A.) that have been marked with an even 3 x 5 grid of 3.08” x 2.42” squares. We set up 8 microcosms per replicate: 1) homogeneous Il-9 hosts in ‘loose’ food; 2) homogeneous Il-17 hosts in ‘loose’ food; 3) homogeneous Il-9 hosts in ‘sticky’ food; 4) homogeneous Il-17 hosts in ‘sticky’ food; 5) heterogeneous hosts (Il-9 on top / Il-17 on bottom) in ‘loose’ food; 6) heterogeneous hosts (Il-17 on top / Il-9 on bottom) in ‘loose’ food; 7) heterogeneous hosts (Il-17 on top / Il-9 on bottom) in ‘sticky’ food; and 8) 7) heterogeneous hosts (Il-9 on top / Il-17 on bottom) in ‘sticky’ food (Figure 1). Each microcosm therefore has a single food type (either ‘sticky’ or ‘loose’),
according to its treatment. To arrange hosts in the microcosm, we add 2 pots of the food containing 10-day old larvae to opposite vertical ends of the grid, so that food fully fills the plastic containers. For homogeneous host treatments, we add 2 pots containing larvae of the same genotype (either both II-9 or both II-17) and, for heterogeneous treatments, these 2 pots contain larvae of different genotypes. Thus, homogeneous genotype microcosms have a single genotype of host throughout the grid, while heterogeneous microcosms have two genotypes separated vertically across the grid with a contact zone at the 3rd row. To balance our treatments and account for potential effects due to position within the grid, we set up 2 microcosms of each of the 2 heterogeneous treatments and alternate which genotype is on ‘top’. There are therefore 8 unique microcosm types.

These microcosms are then incubated overnight, as above, so that virus can be added to them on day 11. We repeat this process each passage to set up new microcosms so that evolving virus can be added to a fresh microcosm. This allows us to maintain 2 non-interbreeding and non-evolving host genotypes in our microcosms and preserve the spatial structuring of such genotypes across passages.

Experimental evolution

We set up 3 replicates of each of our 8 unique microcosm types (24 serially passaged microcosms total). Experimental evolution is initiated by pipetting 5ml of $10^{-2}$ stock virus solution in small droplets evenly over the top of each microcosm. Each microcosm is then incubated for 20 days and then frozen overnight to kill larvae for harvesting. Passage lengths (10 days before set-up, 1 day overnight, 20 days with virus) therefore roughly correspond to the host’s natural ~monthly cyclical demography [65]. We stagger the replicates’ passaging schedules weekly due to the time intensive nature of passing.

To harvest virus, we dissect each microcosm by grid square and separately collect infection killed cadavers from each square in sterile 1.5ml Eppendorf tubes (Figure 1). We add 1ml sterile, MilliQ water to each square’s tube and use a sterile pellet pestle (Fisher Scientific, U.S.A.) to manually burst larvae and release viral occlusion bodies. We droplet 500ul of this solution from each square onto the same square of that treatment’s freshly set-up microcosm to infect the next passage and freeze the rest of the solution. Therefore, we preserved the force of and spatial structure of infection within the grid across passages. We record the number of infected cadavers in each square at each passage to track the spatial ecology of infection in the experiment.

The microcosms newly infected with passaged virus were then placed back into incubators for 20 days to allow the virus to infect larvae for the next passage. We experimentally evolve virus for 10 passages, a number standard for microbial evolution experiments. There are several occasions where we had to use the frozen virus solutions reserved during passing to re-set up passages due to low numbers of infected cadavers or contamination with another pathogen. Contamination was recognizable because the most common contaminant, *Bacillus thuringiensis* (Bt), turns infected larvae black rather than white [66]. We could generally expect any co-infected larvae to display the black phenotype since Bt kills larvae more quickly (Yitbarak, unpublished data), so re-setting up passages when Bt was visually detected would clear the virus population. Congruously, we did not see any signs of persistent contamination within our passages nor in any of our assays.
Assay

After passage 10, we collect and virus infected cadavers from each square as above. We next pool the virus from the 3 squares in each row of the 3x5 grid in equal proportion so that we have 5 virus samples per microcosm. Each pooled sample is therefore the virus population at a certain vertical position within the microcosm, along the gradient of heterogeneity for the heterogeneous host microcosms. We purify virus by centrifuging for 1 min at 3000 rpm to remove larger particulate matter and then 3 min at 13000 rpm to pellet virus. We run these samples through a .65 micron filter to semi-purify our virus of larger bacterial and fungal contaminants, as in [61]. Next, we quantify the concentrations of each sample by counting occlusion bodies on a Petroff-Hauser counting chamber with 400x darkfield microscopy and dilute each to an assay dose concentration of ~7.5x10^8 occlusion bodies per mL in 2% sucrose and .2% dye [61]. The sugar entices the larvae to ingest the virus and the dye allows the experimenter to determine which have ingested half their body lengths of solution and are considered exposed.

We assay each pooled virus sample on both of our 2 host genotypes, Il-9 and Il-17, to determine the proportion of hosts each virus sample infects on each genotype and the average number of occlusion bodies each virus sample produces per infection on each genotype. With 5 virus samples for each of the 8 microcosms that have 3 replicates, this results in 240 virus sample x assay line combinations. We batch these assays to infect 1 genotype with all 5 virus samples for 4 of the microcosms of 1 replicate each day. Since we can only assay half of a replicate’s microcosms each day, we balance the batches so that the 4 microcosms assayed each day have equal homogeneity/heterogeneity, Il-9/Il-17 proportions, and loose/sticky food. Because only 1 assay genotype is assayed per day, we include assay genotype in all statistical models to account both for general differences in resistance and assay day effects.

To set up infectivity assays, we first move ~70-80 adult moths of the appropriate inbred line for the batch into new pots with 200g ‘standard’ food. Eleven days after setting up assay pots, we collect 100 third instar larvae for each assay combination in a petri dish and stave them under a damp paper towel for 2 hours. After starvation, we syringe tiny droplets of the appropriate virus-sucrose-dye solution onto the petri dish for the larvae to consume. We add 50 larvae that have orally ingested half their body lengths of virus solution to 2 25-cell compartmentalized square petri dishes (ThermoFisher Scientific, U.S.A.) with ‘standard’ food and incubate them for 20 days [61]. Assay grids are labelled with random identifiers to blind assay combinations and prevent bias. After 20 days, assay grids are frozen and destructively sampled to count the number of infected and uninfected individuals in each grid. Infected cadavers from each assay grid are then saved in a pooled sample for virus quantification.

To determine the average number of occlusion bodies produced per infected individual, we extract virus from the pooled samples from each assay grid using a sterile pellet pestle (Fisher Scientific, U.S.A.) to manually burst larvae and release viral occlusion bodies. We then centrifuge these samples as above, but do not filter them, and count occlusion bodies on the Petroff-Hauser counting chamber. The concentration of occlusion bodies is then divided by the number of infected cadavers that were in the pooled sample to get the average number of occlusion bodies produced per infection for each virus sample on each host genotype.

Statistical analyses
We analyze our data using a generalized linear mixed modelling framework in R [67]. All models were run in R version 4.2.3 (2023-03-15) -- "Shortstop Beagle" [68]. We used packages ‘glmmTMB’ and ‘lme4’ to build models, ‘DHARMa’ to check model residuals, ‘afex’ and ‘car’ to determine significant model terms, ‘emmeans’ to extract effects, ‘tidyverse’ to manipulate data, and ‘patchwork’ and ‘ggplot2’ to plot results [69–77]. We determine error structures for models by testing fitted models with ‘DHARMa’ and then adjusting to best fit residuals. On occasion, observation level random effects are used to correct overdispersion in our models [78]. Where necessary, contrasts are used with ‘emmeans’ to determine effect estimates for treatments. All model tables and annotated code are in the supplement.

Infection time series analysis

We examine the spatial structuring of transmission using the data collected on the number of infected individuals in each square at each passage for each microcosm. To determine whether food viscosity affected the spatial structuring of infection, we built a generalized linear mixed model (GLMM) with a negative binomial distribution (‘nbinom1’) that asked whether the number of infected individuals in a square is significantly predicted by the number of infected individuals in that same square at the previous passage and whether this term interacted with food viscosity. The model also included a fixed effect of the position of the square itself (to account for larval preferences in where they moved) and random effects of passage number, replicate, the pot of larvae used to set up that half of the microcosm (to roughly account for susceptible density), and observation ID.

We additionally run a Levene’s Test for Homogeneity of Variance on the infected count data to determine if our ‘sticky’ and ‘loose’ food treatments differ in their variance, a measure of how ‘clumpy’ infection is within a microcosm.

Assay analysis

Infectivity and productivity assay data was analyzed in a generalized linear mixed modeling framework using the same packages and process as above [67]. We build models treating proportion infected, average number of occlusion bodies per infected individual, and the composite exploitation rate (proportion infected multiplied by the average occlusion body count) as our response variables. We transform average virus counts and composite exploitation rates by multiplying and rounding them to produce integer counts for Poisson and negative binomial distribution assumptions. In each model, we include random effects for batch and the nested effect of virus sample under replicate under treatment.

First, we ask whether virus populations significantly differ in their specialization at the whole microcosm level by building models that include an interaction effect between the assay being on the host that the population was evolved on (‘familiar’) or the foreign host and the type of food the population evolved in. Virus from heterogeneous populations is coded as ‘heterogenous’ as they do not have a familiar or foreign host at the whole microcosm level. We also include assay line as a fixed effect to account for overall differences in resistance between our assay host genotypes. The infectivity model uses a binomial error structure with observation level random effects, the productivity model uses a Poisson error structure with observation level random effects, and the exploitation rate model uses a zero-inflated negative binomial error...
structure with observation level random effects. To better see the effects of specialization in the homogeneous populations, we also run these same models for the homogeneous host treatments only with the infectivity model using a binomial error structure, the productivity model using a Poisson error structure, and the exploitation rate model using a negative binomial error structure with observation level random effects.

Next, we ask whether spatial structure or host heterogeneity have significant effects on viral phenotypes across genotypes (without accounting an effect of local adaptation). We build models for infectivity and productivity with the same random effects as above, and without fixed effects for being assayed on the familiar or foreign host. For host heterogeneity models, we include the assay line, food type, and whether the treatment had homogeneous or heterogeneous hosts as fixed effects, allowing for an interaction between food type and treatment heterogeneity. The infectivity model uses a binomial error structure with observation level random effects and the productivity and composite exploitation models use a zero-inflated negative binomial error structure with observation level random effects. For spatial structure models, we include the food type and assay line as fixed effects with the same random effects as above. The infectivity model uses a binomial error structure, and the productivity and composite exploitation models use a zero inflated negative binomial structure with observation level random effects.

Finally, we ask whether virus phenotypes are spatially structured within the heterogeneous host treatments. First, the local proportion of host genotype 17 is determined for each virus population by its distance from the host contact zone so that the edge of the microcosm where host genotype 17 was added is coded as 1, the next row of the grid is coded .75, the middle contact zone is coded as 0.5, the following row is coded as .25, and the edge of the microcosm where host genotype 9 was added is coded as 0. We build models for infectivity, productivity, and exploitation rate that include the local proportion of host genotype 17, the host genotype of the assay, the food type, and interaction effects between all three fixed terms. We include the same random effects as in the previous models for batch and nestedness, but also include random terms for the specific position and order of lines to account for the fact that we have two heterogeneity treatments that differ in which line is on ‘top’ or ‘bottom’ of the microcosm. The infectivity model uses a binomial error structure, the productivity model uses a Poisson error structure with observation level random effects, and the exploitation rate model uses a zero-inflated negative binomial error structure.

RESULTS

Food viscosity spatially structures of infection

Across the whole experiment, the density of infected hosts in a position positively correlates with the density of infected hosts in that position in the previous passage (Figure 2, estimate = 0.009, p = 0.011). There are also significant effects of the position itself (p < 0.001), where infection is more likely to be found in corner and edge squares of the microcosm and on the side closer to light, and the type of food, where infection is higher in ‘sticky’ food (estimate = 0.26, p < 0.001).

Importantly, there is a significant interaction term between the effect of infected density at a
position in the previous passage and the type of food ($p = 0.043$). However, the predictive power of the previous passage’s density of infected hosts in a position is significantly weaker for ‘sticky’ food that for ‘loose’ food (Figure 2, estimate = -0.009, $p = 0.043$). This means that, contrary to our expectations, ‘loose’ food spatially structures infection at the grid square level more than ‘sticky’ food in our experiment. From experimenter observation, it seems likely that this is because ‘loose’ food packed microcosms more precisely than ‘sticky’ food, leaving fewer gaps for larvae to exit the food into and move unimpeded along. At the same time, however, the food types also differ in the variance of their infected count data ($p < 0.001$), with ‘sticky’ food having more clustered infected individuals (Figure S2).

**Figure 2**: (A) Change in the number of infected cadavers in a square between passages (y-axis) for ‘loose’ and ‘sticky’ food (x-axis). (B) Effect estimates drawn from a GLMM for the interaction between type of food evolved on and the effect of the previous passage’s infection count at a square on the current passage’s infected count on a square.

The impact of local adaptation on virus phenotypes depends on spatial structure

Averaged across host genotypes, the spatial structure manipulation does not affect virus populations evolved per particle infectivity ($p = 0.96$) or per infection virus productivity ($p = 0.58$) (Figure S4). This is in contrast to previous results that only assay virus on genetically diverse local hosts [34]. However, any impact of spatial structure may also depend on local adaptation as evolved virus populations are assayed on both familiar and foreign hosts. When we test whether spatial structure interacts with host genotype specialization, we find that there are significant interaction effects between which host genotype the population was evolved on, the host genotype assayed on, and the type of food evolved on (Figure 3). Specifically, for virus populations evolved in homogeneous host populations, viruses from ‘loose’ food populations are
less infective on their familiar host (estimate = -0.785, p = 0.031) while those from ‘sticky’ food populations do not significantly differ in infectivity between familiar and foreign hosts (estimate = 0.442, p = 0.22) (Figure 3B). Simultaneously, viruses from ‘loose’ food populations are less productive on their familiar host (estimate = -0.451, p = 0.032) while those from ‘sticky’ food are significantly more productive on familiar hosts (estimate = 0.198, p = 0.347) (Figure 3D). Collectively, this means that the effect of being assayed on a familiar or foreign host significantly (p=0.047) depends on food type so that the viruses are more prudent on their familiar hosts in ‘loose’ food and more exploitative on familiar hosts in ‘sticky’ food (loose estimate=-1.29 p=0.037; sticky estimate=1.02, p=0.09) (Figure 3F).

Figure 3: Interactions between local adaptation and the spatial structuring of infection. Panels A, C, E plot raw data and panels B, D, F show model effect estimates drawn from GLMMs for the homogeneous host dataset. Y-axes
represent: (A) proportion infected, (B) effect estimates for the infectivity model for homogeneous host treatments only, (C) average number of occlusion bodies produced per infectious cadaver on a log scale, (D) effect estimates for the viral productivity model for homogeneous host treatments only, (E) composite exploitation rate (proportion infected * average number of occlusion bodies) on a log scale, and (F) effect estimates for the exploitation rate model for homogeneous host treatments only. X-axes represent whether the virus from homogeneous host treatments was being assayed on the host genotype that it evolved on or on the foreign host genotype (A-F) or whether the virus came from a heterogeneous host treatment (A, C, E). Panels show whether the virus came from a treatment with ‘loose’ food or ‘sticky’ food.

Spatially structured host genetic diversity leads to local adaptation

On average, virus populations evolved in heterogeneous host populations do not significantly differ in infectivity (p = 0.60) or composite exploitation (p=0.20) from those evolved in homogeneous host populations, though they are slightly more productive (estimate=0.14, p=0.42). However, heterogeneous host populations have gradients of host mixing within them as the different positions are different distances from the other host genotype. Additionally, we expect spatial structure to impede host movement and thus further alter the degree of host mixing for different positions. Therefore, we explore whether there is an effect host specialization within the heterogeneous host microcosms and whether this effect is altered by the spatial structuring of the population. We find that there is a significant interaction between the local proportion of a host genotype (i.e., the position’s distance from the contact zone) and the bias in exploitation rate (a composite metric of virus infectivity and productivity) that is mediated by the type of food evolved in (p = 0.031, Figure 4). As in homogeneous populations, virus populations are biased towards higher exploitation rates on familiar hosts in ‘sticky’ food populations (estimate=1.714, p=0.03) and somewhat lower exploitation rates on familiar hosts in ‘loose’ food populations (estimate=-0.604, p =0.40).
DISCUSSION

In our experiment, we find that pathogens can evolve to be more exploitative of their familiar host on one food type and less exploitative of their familiar host on the other food type. This effect holds at both the treatment level for virus lines evolved in homogeneous host environments and as a gradient effect in heterogeneous host environments where the degree of local adaptation depends on the distance from the host contact zone (Figure 3, Figure 4). Therefore, our experiment suggests that the multiple selection pressures (host genetic diversity and spatial structure) that our virus populations experience led to their host range and life history trade-offs interacting and reversing the outcome of local adaption on pathogen exploitation rate. This has important implications for eco-evolutionary theory.

A large portion of ecological and evolutionary theory on how host-parasite coevolution can result in biotic diversity depends on specialist interactions and assumes that pathogens have the highest exploitation rates on their familiar or locally common host so that rare hosts have a fitness advantage [6,15,79,80]. This is partially because most of these models focus only on the infectivity component of pathogen specialization and do not consider post-infection processes.
like replication and virulence where high levels might be expected to lower fitness [81]. However, our results suggest that infectivity might also not always be higher on the familiar host when more prudent strategies are fitter [82].

The implications of certain ecological and population structures leading to maladaptively higher exploitation rates on foreign, or rare, hosts have not previously been extensively considered. When transmission between host types is low, maladaptively high virulence on a host would lead to stuttering transmission chains and local extirpation of the pathogen in the rare host and preserve rare genotype advantage [19,83]. However, when transmission between host types freely occurs, selection for lower exploitation rates on the familiar host resulting in maladaptively high exploitation on foreign or rare hosts means that rare genotype advantage is not ubiquitous [81]. This has previously been considered in cases of species invasions (i.e. ‘invading with weapons’) and zoonosis [84,85], but not for host-parasite coevolutionary theory at equilibrium. Therefore, environments selecting for lower exploitation rates may also be less likely to conform to the predictions of eco-evolutionary theory that depends on this rare genotype advantage like the Red Queen Hypothesis of Sex [14] and Janzen-Connell effects [16,17]. Additionally, we should not always expect pathogens to have the highest exploitation rates on their familiar host, even in the absence of co-evolutionary dynamics where the host might be further ‘ahead’ in the arms race [86]. Our data shows that patterns approximating the host being ‘ahead’ in a coevolutionary arms race can be found even in the absence of host evolution.

Our result that virus evolves lower exploitation rates on the familiar host in one of our food types could be understood if food viscosity was spatially structuring infectious contacts so that pathogens were selected to lower their exploitation rates, but that they were only selected to do so on the familiar host. While the pathogen would be selected to have the highest fitness on its familiar host in each treatment, the optimal exploitation rate associated with that peak fitness would depend on the spatial structuring of infectious contacts (because self-shading in spatial structured environments selects for more prudent pathogen strategies) [33]. This means that the pathogens selected in spatially structured environments could actually have higher exploitation rates on foreign host genotypes than familiar.

However, our evolution experiment resulted in lower exploitation rates on the familiar host in ‘loose’ food, despite our expectations that ‘sticky’ food would spatially structure the population more. Previous experiments with similar food types used ‘sticky’ food to spatially populations as they, and our preliminary experiments, showed that ‘sticky’ food impedes larval movement (Table S1) [34,64]. Therefore, we were expecting to see lowered exploitation rates in ‘sticky’ food, rather than ‘loose’ food. However, we do have evidence that our food viscosity manipulation behaved in unexpected ways. Our infection ecology data seems to suggest that ‘loose’ food spatially structured infection more than ‘sticky’ food at the level of force of infection within a 3.08” x 2.42” grid square between passages. One reason for the different outcome in our experiment may be that slight differences in ‘sticky’ food viscosity can lead to higher rates of larvae exiting the food to move unimpeded along the surface. While we added enough food to entirely fill our plastic microcosms, the ‘sticky’ food was clumpier and left more air gaps on the surface of the food than the ‘loose’ food. Therefore, larvae in ‘sticky’ food may have been more likely to travel between grid squares along the surface of the food so that infection was more spatially structured in ‘loose’ food treatments. Alternatively, it is possible that the virus had
different rates of environmental decay on the different food types leading to differences in where larvae were infected. Another potential explanation is that eggs and early instar larvae may have been more ‘clumped’ in ‘sticky’ food so that the density of susceptible individuals was more heterogeneous and influenced the local infection density more than the dose of virus added to the square. The fact that ‘sticky’ food microcosms are more heterogeneous in the clustering of infected individuals potentially supports this interpretation. Therefore, the infection ecology results suggest that ‘loose’ food spatially structured infection more than ‘sticky’ food at the grid square level between passages, but we have no way of determining how food structured infection at the sub-square level, when larvae were earlier instar with lower movement rates. It is possible that ‘sticky’ food less structured infection between passages at the square spatial scale, but structured contacts at smaller spatial scales early in each passage. However, greater spatial structuring of infectious contacts in the ‘loose’ food would provide a plausible mechanism for why lower exploitation rates evolved on the familiar host in these lines. It is difficult to think of another plausible hypothesis. As force of infection was higher during passaging in the ‘sticky’ food lines, it is possible that ‘sticky’ lines had stronger selection to specialize on the familiar host, but this could explain why effect sizes of specialization could vary, but not why they would switch directions [87]. Alternatively, it is plausible that infection in ‘sticky’ food infected earlier instar larvae as the high numbers of infected individuals in certain squares during selection passages were observationally correlated with clusters of early instar larvae and ‘sticky’ food moderately slows development (Figure S1). However, we would expect this to lead to lower exploitation rates on the familiar host in ‘sticky’ food as earlier instar larvae are both less resistant and, potentially, more spatially structured [88]. Therefore, the most likely hypothesis to explain these results seems to be that the higher spatial structuring of spatial contacts at the grid-square level between passages in the ‘loose’ food imposed a spatial structuring effect that selected for lower exploitation rates on the familiar host. Another key, but slightly counterintuitive, result of our experiment is that the effects of local adaptation in heterogeneous host environments are stronger in better mixed populations than in more spatially structured ones (Figure 4). As host genotype mixing only depends on spatial structure effects above the square scale, we can better assume that ‘loose’ food more strongly structured contacts between genotypes than ‘sticky’ food. While more contact between host genotypes should, in theory, select more strongly for generalism [28–30], theory suggests that higher migration rates between patches can lead to stronger local adaptation due to migration conferring more genetic diversity to select from and increased competition to strengthen such selection [54]. Our experiment seems to support the latter theory, implying that certain rates of migration between populations may support, rather than homogenize, phenotypic diversity. Our ability to make conclusions is hampered by the fact that our experimental manipulation for spatial structure seemed to have worked in the opposite direction as intended. However, our results do suggest that evolution in response to multiple selection pressures can be governed by interacting trade-offs and result in different locally adapted optimal strategies. Specifically, when optimal exploitation strategies on the familiar host are more prudent, pathogens may have maladaptively high exploitation rates on foreign hosts. Furthermore, our experiment shows that higher pathogen migration rates between host types can lead to stronger effects of local adaptation, suggesting that some population mixing might support metapopulation diversity.
Our result that interactions with other trade-offs can reverse the impact of local adaptation on pathogen exploitation rate has important implications for eco-evolutionary theory on the maintenance of diversity that depends on rare genotype advantage.

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