Abstract

Niche breadth coevolution between biotic partners underpins many theories of diversity and co-existence, and also influences patterns of disease emergence and transmission in host-parasite systems. Despite these broad implications, we still do not fully understand how the breadth of parasites' infectivity evolves and the nature of any associated costs. Here, we serially passage a granulosis virus on multiple inbred populations of its *Plodia interpunctella* host to explore the dynamics and outcomes of specialization. Uniquely, we explore the dynamics of host genotype specialization in multiple fitness components at multiple time points throughout the course of experimental evolution. We find that the *Plodia interpunctella* granulosis virus consistently evolves increases in overall specialization, but that our two fitness components evolve independently such that lines specialize in either productivity or infectivity. This highlights the importance of measuring multiple fitness components when testing for specialization or local adaptation. Furthermore, we found that specialization in our experiment is not explained simply by costs through trade-offs or mutation accumulation, suggesting a combination of both evolutionary mechanisms. These results are important for understanding the evolution of specialization in host-parasite interactions and its broader implications for co-existence, diversification, and infectious disease management.

Key Words: Specialization, Host Range, Experimental Evolution, Host-Parasite Interactions

Introduction

The question of why some species are specialists and others are generalists has been central to evolutionary biology since its inception (Darwin, 1859). This co-existence of strategies is commonly explained by there being some cost to generalism such that specialists are favored under certain ecological conditions (Futuyma and Moreno, 1988) because "jacks-of-all-trades are the masters of none" (MacArthur 1984). The theory of costly generalism has been extensively applied in the host-parasite eco-evolutionary literature to explain parasite niche breadth and specialization at the levels of both host species and host genotype (Gandon and Poulin, 2004; Osnas and Dobson, 2012; Regoes et al., 2000). Niche breadth at the level of host species has important implications for pathogen emergence (Guth et al., 2019; Woolhouse and Gowtage-Sequeria, 2005), species invasions (Strauss et al., 2012), and species co-existence (Connell, 1971; Janzen, 1970); while

niche breadth and specialization at the genotype level underpins the monoculture effect (Elton, 1958), local adaptation (Kawecki and Ebert, 2004), and the Red Queen Hypothesis of Sex (Jaenike, 1978). Despite these broad implications for niche breadth evolution in antagonistic coevolutionary systems, there is still debate about how universal costs of generalism are in different biological systems (Jaenike, 1990; Remold, 2012) and open questions about the ecological conditions that bias heterogenous host populations towards selecting for specialists or generalists (Gibson, 2019).

Specialism presupposes that there are genetic differences between host populations that parasites can specifically adapt to exploit (Agrawal and Lively, 2002). There are two main methods to determine whether parasites can specialize on different hosts: experimental cross inoculation across different hosts with their natural parasite populations (Greischar and Koskella, 2007) and the experimental evolution of parasites on different hosts (Bono et al., 2017; Turner and Elena, 2000). Experimental cross-inoculation studies allow tests of naturally evolved differences between populations, while laboratory experimental evolution directly examines the evolution of specialism under controlled conditions. In particular, experimental evolution approaches are powerful in that they allow for gene-by-gene interactions to be distinguished from gene-by-environment interactions (Kawecki et al., 2012).

Experimental evolution approaches have been applied to questions of host genotype specialization with great success, but in a relatively limited number of host-parasite systems including mice and RNA virus (Kubinak et al., 2012), mosquitos and microsporidia (Legros and Koella, 2010), daphnia and bacteria (Little et al., 2006), protists and bacteria (Nidelet and Kaltz, 2007), *C. elegans* and bacteria (Schulte et al., 2011), and wheat and fungus (Zhan et al., 2002). Generally, these studies find that serial passage on a single host genotype increases fitness on that host genotype while decreasing or at least resulting in smaller fitness gains on other genotypes. This is important since it directly demonstrates a potential benefit of specificity in these host parasite systems. Given the important implications of such specificity in host-parasite interactions, however, it is important to continue to broaden the range of systems that have been studied. In that way, we can better assess whether specialism evolution is typical in host-parasite systems, which would imply that host generalism is constrained by costs.

Furthermore, most studies only examine the degree of specialization at the end of the experiment, so we do not have an understanding of the dynamics of specialism evolution over time. This is particularly important at the start of the experiment, where we need to distinguish

evolution from ecological strain sorting. It is also the case that, often, experiments have only examined parasite traits related to infection ability on different host genotypes. Therefore, there is a lack of data on the evolution of specialism across the range of parasite fitness traits; it could be the case that specialization is typical only of the infection process.

We use experimental evolution to test for host genotype specificity in the *Plodia interpunctella* (Hübner) and Plodia interpunctella granulosis virus (PiGV) laboratory model system. *Plodia interpunctella*, the Indian meal moth, is a stored grain pest that has been extensively used to test eco-evolutionary dynamics in the lab (Boots, 2011; Boots et al., 2009; Boots and Mealor, 2007). In particular, it has proven to be an amenable system for testing evolutionary trade-offs, as the trade-off between resistance and development time in this system is amongst the best characterized in evolutionary biology (Bartlett et al., 2018; Boots, 2011; Boots and Begon, 1993). However, the system has not yet been tested for host genotype specificity. Here, we determine whether PiGV evolves to specialize multiple fitness traits on the *Plodia interpunctella* genotype that it is serially passaged on. We serially passage virus on one of three inbred host populations for nine passages and measure viral infectivity and productivity on the familiar (the genotype that the virus evolved on) and unfamiliar (genotypes that the virus was unexposed to) host genotypes at multiple time points of evolution. We find that serially passaging virus leads to consistent increases in specialization on familiar host genotypes through the course of experimental evolution.

Methods

Study System

The study system is *Plodia interpunctella* (Hübner), the Indian meal moth, and the Plodia interpunctella granulosis virus (PiGV). *Plodia interpunctella* is a pest that lives in grain stores (Mohandass et al., 2007). During its five larval instar stages, it develops within its food medium before pupating and emerging into an adult moth. The adult moth stage disperses, mates, and lays eggs in a semelparous breeding event but does not feed (Boots and Begon, 1993; Gage, 1995; Silhacek and Miller, 1972). Previously, Bartlett et al. (2018) evolved a number of inbred moth populations by mating individual brother-sister pairs for more than 27 generations. At this point,

inbred populations should represent near-clonal populations of a single genotype that was randomly selected from the genetically diverse founder population via drift.

Plodia interpunctella granulosis virus (PiGV) is a dsDNA baculovirus that is an obligate killer (Vail and Tebbets, 1990). The natural life cycle is as follows: a larvae ingests virions in the occlusion body form, the virions shed their protein coats and infect gut epithelial cells, the virions either pass through the gut to establish a successful infection or are cleared during molting (freeing the larvae to carry out the rest of their life history), the virus begins to proliferate through the entire body of the larvae, and, once at a critical mass, packages into the protein-coated occlusion body form and kills its host (Rohrmann, 2013). It can then be transmitted to susceptible larvae when they cannibalize infected cadavers and ingest occlusion virus. Critically, the virus must kill its host in order to transmit and larvae can only pupate and become adult moths if they were not successfully infected (Boots and Begon, 1993).

Host Selection and Maintenance

We selected three inbred *Plodia interpunctella* populations with similar overall levels of resistance for this experiment, as measured by a preliminary resistance assay of all twelve of the inbred populations (unpublished data). The chosen inbred populations (Lines 2, 9, and 17) represent genotypes with similar medium overall levels of resistance (Supplemental Table 1). Populations of these genotypes were maintained in 1000mL straight-side wide-mouth Nalgene jars (ThermoFisher Scientific, U.K.) with 200 g of standard food medium in a single incubator at 27±2 °C and 35±5% humidity, with 16:8hr light:dark cycles. Standard food was made with 250g 'Ready Brek' (Weetabix Ltd., U.K.), 150g wheat bran (Bob's Red Mill, U.S.A.), 100g rice flour (Bob's Red Mill, U.S.A.), 100g brewer's yeast (MP Biomedicals, U.S.A.), 125ml glycerol (VWR, U.S.A), 125ml organic honey (Dutch Gold Honey Inc., U.S.A.), 2.2g methyl paraben (VWR, U.S.A.), and 2.2g sorbic acid (Spectrum Chemicals, U.S.A.). We mixed the food medium in batches and froze it for 24 hours before use. To maintain these populations, we moved about fifty adult moths onto fresh food jars as new adult moths emerged about monthly. Crucially, these host populations were maintained in the absence of the virus.

Setting Up Experimental Evolution

Virus evolution was initiated with a single genetically diverse virus stock that we diluted to a passaging dose that would cause high mortality (~7.5x10^8 occlusion bodies per mL). We counted the concentration of this passaging dose on a Petroff-Hauser counting chamber with a darkfield microscope at 400x magnification. This dilution was combined with 2% sucrose (ThermoFisher Scientific, U.S.A.) and 0.2% Coomassie Brilliant Blue R-250 dye (ThermoFisher Scientific, U.S.A.). The sucrose encourages the larvae to consume the virus solution and the dye allows us to recognize larvae that have consumed half their body length of virus solution and are therefore considered successfully inoculated.

We set up three replicate evolving lines of virus on each of the three inbred host genotypes. For each virus line, we collected 100 third instar larvae of the appropriate genotype in a petri dish and starved them under a damp paper towel for 2 hours. We then syringed tiny droplets of our virus-sucrose-dye solution onto the petri dish for the larvae to orally ingest. After about an hour, we moved 50 successfully inoculated larvae into two 25-cell compartmentalized square petri dishes (ThermoFisher Scientific, U.S.A.) with standard food. The grid plates were then transferred to a single incubator for 20 days.

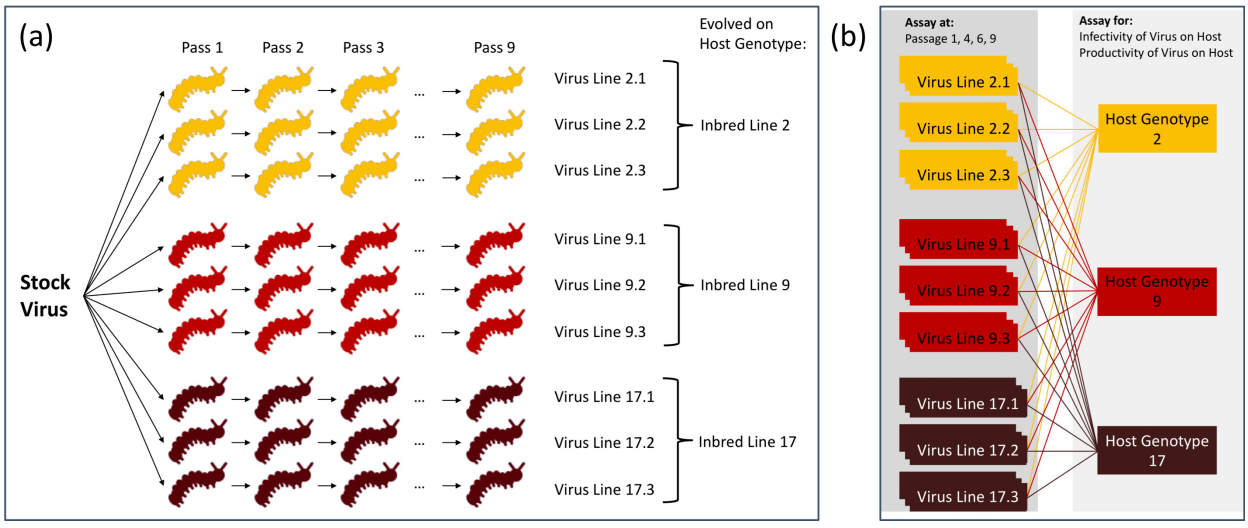

Figure 1. (a) Passaging scheme for virus experimental evolution lines. Stock virus was used to initiate nine virus evolution lines, 3 per host genotype. Virus was serially passaged through larvae for 9 passages. (b) Assay scheme for virus experimental evolution lines. The starting virus stock

and every virus line at passages 1, 4, 6, and 9 was assayed for infectivity and productivity on each host genotype.

Serial Passage

After 20 days, we harvested virus from each virus line under sterile conditions by collecting up to 10 virus killed cadavers from each line and transferring these to sterile 15 mL disposable tissue grinders (ThermoFisher Scientific, U.S.A.). Infected larvae were recognizable by their opaque, chalky, white coloration. We were not able to collect 10 infected cadavers from all virus lines at all passes, so, when we could not find 10 infected cadavers, we collected every infected cadaver that we could find (See Supplementary Table 2). To extract virus from infected cadavers, we added 2mL of sterile DI water to the tissue grinders and homogenized the solution until all cadavers had been thoroughly crushed. We then transferred 1mL of the supernatant to a sterile 1.5mL Eppendorf tube and centrifuged the solution for 1 minute at 3,000 rpm to remove larger particulate matter from the supernatant. We transferred 600uL of this solution to a sterile 1.5 mL Eppendorf and centrifuged this for 3 minutes at 13,000 rpm to pellet the virus. We removed the supernatant from the pellet and resuspended in 1mL sterile water.

After extracting the virus, we resuspended the virus pellets in water, diluted the solution 10x, and added 600uL of the dilution to a .65 micron filter spin column (Millipore Sigma, U.S.A.) that we centrifuged at 13,000rpm for 3 minutes to semi-purify the virus of possible bacterial and fungal contaminants (See Supplement Table 3). We counted each of the semi-purified virus solutions and diluted them to the passaging dose concentration of ~7.5x10^8 occlusion bodies per mL in 2% sucrose and .2% dye to form our final passaging solutions for each virus line. These virus dilutions were then used to infect the next set of third instar larvae of the appropriate genotype following the protocol above. Virus was serially passaged for nine passages (corresponding to nine transmission steps or about 6 months).

Assaying

We assayed each virus line at multiple passages to track evolution over the course of the experiment. We assayed the starting population of virus as well as virus harvested from passages 1, 4, 6, and the final passage 9. By assaying passage 1, we were able to check for the ecological effects of strain sorting from our genetically diverse starting virus population. For each assay, we inoculated all 3 host genotypes with all 9 virus lines at both the passaging dose and 10% of the

passaging dose. We inoculated 25 larvae for each host genotype x virus line x dose combination using the standard inoculation protocol above. Because of time constraints, inoculations for each passage were conducted across three days with one host genotype each day being inoculated with all of the virus lines. By assaying all the virus populations from each of the evolutionary histories on all of the host genotypes, we were able to measure how the evolving virus line changed in fitness on the host genotype that it was being selected on and on both of the foreign genotypes.

After 20 days, we froze the grid plates and counted the number of infected and uninfected individuals in each grid. We collected all the infected larvae from each assay grid that had been inoculated with the higher dose and froze them in a pooled sample per grid plate. We extracted virus from these samples via tissue grinding and the two centrifugation steps (without filtering) and counted the virus in a Petroff-Hauser counting chamber as above. From these virus counts and the number of infected larvae, we were able to determine how many occlusion bodies each virus line produced on average per infected cadaver when infecting each host genotype. Finally, we multiplied the average number of virions produced per infected cadaver by the proportion of larvae infected to get a composite measure of fitness for each virus line on each host genotype.

Statistical Analyses

We analyzed all data using a linear mixed modelling approach in R (v.3.4.4 – "Someone to Lean On") (R Core Team 2018), accounting for our mixed design using the 'afex' package (Singmann et al., 2019) which relies on the 'lme4' linear mixed modelling engine (Bates et al. 2015). Where appropriate, we followed this with post-hoc testing using the 'emmeans' package (Lenth, 2019) to identify pairwise differences between levels of interacting fixed effects, with *p*-values corrected for multiple comparisons using a Bonferroni correction. Our response variables were either fitness, infectivity, or productivity of the virus line. We used a binomial error structure for models of infectivity and poisson error structures for models of productivity and fitness (see annotated R script associated with this manuscript).

The first part of our analysis looked at data from the end of the evolution experiment (passage 9). We tested for an effect of specialization by using a 'self' factor that was either true (virus was assayed on same host genotype it was evolved on) or false (virus was assayed on a host genotype it was not evolved on). We first included this as a fixed effect alongside 'assay genotype' and 'evolution genotype' (the host genotype used for the assay and that the virus was evolved on,

202 respectively). In the case of the 'infectivity' data analysis, 'dose' was also included as a fixed
203 effect. Our random effects were 'evolution genotype' and 'virus line', with 'virus line' nested
204 under 'evolution genotype' to account for our experimental structure. We further used the same
205 modelling approach to test for a correlation between a virus line's virion production and its
206 infectivity by including virion production as an additional fixed predictor in a separate model of
207 infection likelihood at the highest dose.
208 We further analyzed the effect of 'self' by including it as a fixed term in models the same
209 as specified above, however with 'virus line' replacing 'evolution genotype' as a fixed effect (no
210 change to the random effects), and a potential interaction between 'self' and 'virus line' to see if
211 there were differences in the ability of each virus selection line to evolve any specialism. We used
212 pairwise comparisons between lines to investigate these differences; further, we used these
213 pairwise differences to test for a correlation between each virus line's fitness on a familiar
214 genotype and its fitness on a foreign genotype.
215 We also analyzed our fitness data across the whole experiment, including data for passages
216 0, 1, 4, 6, and 9, to interrogate how specialization evolved with time. We used the same general
217 approach as detailed above, where fixed effects were 'assay genotype', 'self', 'passage number',
218 and an interaction between 'passage number' and 'self'. Our error structure included 'evolution
219 genotype', 'virus line' and 'passage number', with 'virus line' nested under 'evolution genotype'
220 as above, and 'passage number' nested under 'virus line' to account for multiple generations acting
221 as repeated measures.
222 We used the 'ggplot2' (Wickham, 2009) package to plot graphs of our results.
223
224 **Results**
225 **Specialization of Viruses at the Final Passage**
226 After nine passages of experimental evolution, we find good evidence that viruses evolved
227 to specialize on their familiar host genotype, indicated by a significantly positive effect of 'self'
228 on viral infectivity ($p = 0.01$), productivity ($p < 0.001$), and overall fitness ($p < 0.001$) (See Figure
229 2). Therefore, the evolved virus lines infected relatively higher proportions of individuals,
230 produced more virions per infection, and therefore had higher fitness when infecting the host
231 genotype that they had evolved on than when infecting foreign host genotypes. As expected, we

found a significant effect of 'assay genotype' (host) across all three response variables ($p < 0.002$ in all cases) and a significant positive effect of 'dose' ($p < 0.001$) when analyzing infection likelihood. We did not find a significant effect of 'evolution genotype' on infection likelihood ($p = 0.69$) or fitness ($p = 0.12$), meaning that specific host genotypes did not lead to the evolution of generally more infectious or higher fitness virus populations when averaged across all three assay genotypes. However, we did find a significant effect of 'evolution genotype' on viral productivity ($p = 0.03$), driven by the higher productivity of virus lines evolved on host genotype 17 (See Supplemental Model Tables).

We found evidence that virus lines differed both in overall fitness and in specialization when we tested for an effect of 'virus line' and an interaction between 'virus line' and 'self'. We found a significant effect of 'virus line' and a significant interaction between 'virus line' and 'self' when analyzing both the fitness and viral productivity data ($p < 0.001$ in both cases), but not when analyzing the infectivity data ($p = 0.06$ for 'virus line', $p = 0.25$ for 'virus line':'self' interaction). That is, virus lines differed in their overall fitness and productivity (but not infectivity) across all the assay genotypes and, while lines generally showed higher fitness and productivity on their familiar host genotype, this effect varied significantly amongst lines.

Using a Bonferroni-corrected pairwise comparison analysis, we find that most lines differ from each other in fitness: only 4/36 pairwise comparisons between lines showed no significant differences in fitness on a familiar host, and only 8/36 showed no difference on a foreign host. The large majority of lines therefore differed from all other lines in their fitness on both hypothetical average familiar and foreign hosts. Only one pair of lines (9.2 and 2.2) showed no difference from one another on both foreign and familiar host genotypes. Looking at the effect of 'self' for each line, our pairwise comparisons also explain that the effect of 'self' differs between lines. Line 9.1 was significantly less fit on a familiar host compared to a foreign one ($p < 0.001$), while lines 9.2 and 2.2 showed no significant differences in their fitnesses on familiar compared to foreign hosts ($p = 0.50$ and $p = 0.28$ respectively). All other lines were significantly more fit on a familiar host than a foreign one, though there were differences in the magnitude of this effect. Notably, these virus line differences and their interaction with 'self' were due to increased virion production; lines didn't significantly differ in their ability to infect an 'average' host and didn't differ in how much more likely they were to infect a familiar host.

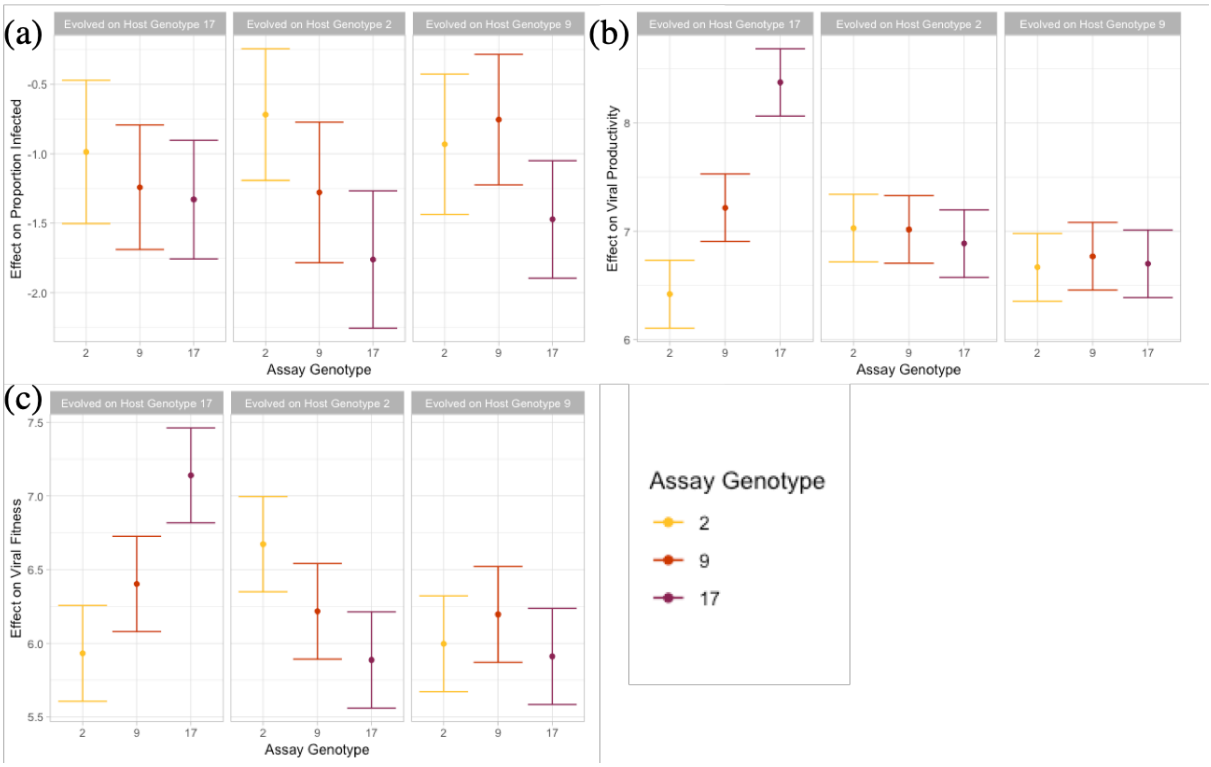

Figure 2. Specialization of Virus at Final Passage. Paneled plots show the effect of the virus's evolutionary history on its (a) infectivity, (b) productivity, and (c) composite fitness when infecting each of the assay lines. Fitness is the proportion infected x the average number of virions produced per infected cadaver. Virus lines are significantly more infective, productive, and fit on familiar genotypes. Y-axis effect sizes and errors are taken from the GLMM models using the 'emmeans' package.

Evolution of Specialization over Time

Our experiment is unusual in that it tracks evolution over time. Our analysis of fitness data across all passages (0,1,4,6,9) showed a significant effect of passage number on virus fitness ($p < 0.001$), and a significant interaction between passage number and 'self' ($p < 0.001$). Virus lines decreased in fitness from passage 0 to passage 1 and generally increase in their overall fitness from passage 1 to passage 4 (with no further meaningful overall change from passages 4-6 and 6-9). The initial decrease in fitness from passage 0 to passage 1 could be due to the different storage and extraction methods of the starting virus stock from experimental virus solutions, and analyzing fitness without passage 0 data confirms the passage 1-9 trends. However, viruses increase their fitness on their familiar host genotype **relative to their foreign host genotype** in every case (from

0-1, 1-4, 4-6, and 6-9), suggesting either a gain in fitness on the familiar genotype, a loss of fitness on the foreign genotype, or both (see Figure 3). Interestingly, when we analyze the infectivity and productivity data separately, we do not see this steadily increasing effect of self across the time course of the experiment.

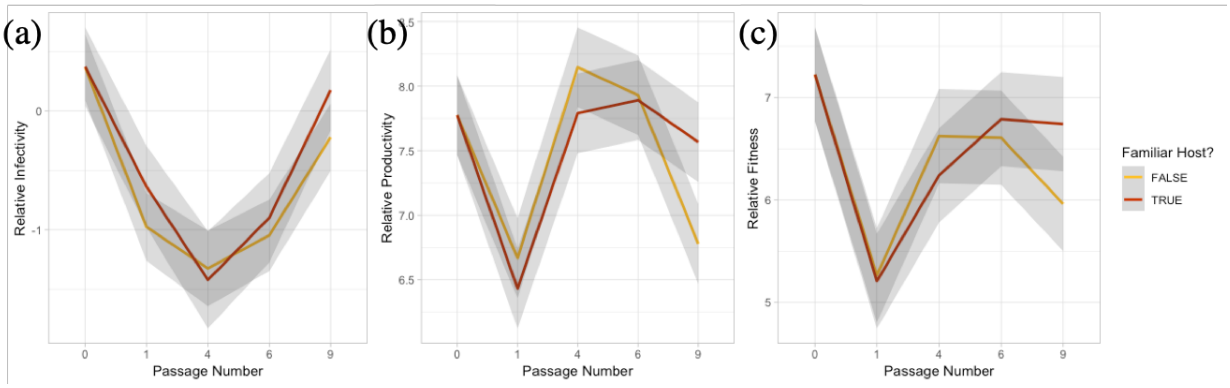

Figure 3. Evolution of Specialization over Time. Panelled plot showing the effect of whether the virus was assayed on its familiar host genotype (red) or on a foreign one (yellow) on viral (a) infectivity, (b) productivity, and (c) fitness over time. Virus lines evolve significantly higher relative infectivity, productivity, and fitness on familiar lines over the experiment. Y-axis effect sizes and errors are taken from the GLMM models using the 'emmeans' package.

Relationship between Viral Productivity and Infectivity

We find a significant negative correlation between virus productivity and infection likelihood ($p < .001$) in the passage 9 dataset. However, when we analyzed the full dataset with all passages, we find that virus productivity and infectivity were significantly positively correlated ($p<.001$), such that more productive virus lines are also more infective. Therefore, we fit and test a model with an interaction effect between 'self', 'productivity', and 'passage number' and find a significant interaction between these three metrics ($p<.001$) such that the direction of the relationship between viral productivity and infectivity changes from positive to negative depending on the passage number and whether the virus is infecting a familiar or foreign host (see Figure 4).

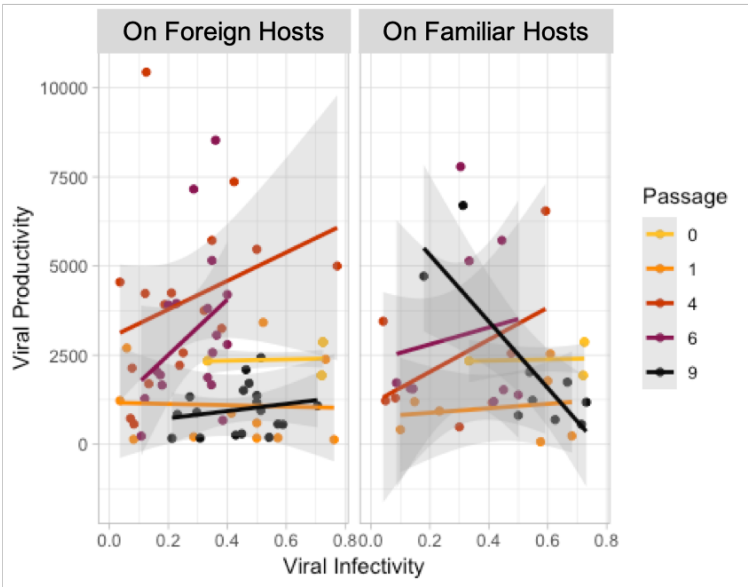

Figure 4. Relationship Between Viral Productivity and Infectivity. Paneled plot showing the relationship between viral productivity and infectivity on both familiar (right panel) and foreign hosts (left panel) at each passage. Infectivity is defined as the proportion of hosts infected and productivity is the average number of virions produced per infected host. The direction of the relationship between viral productivity and infectivity significantly changes from positive to negative depending on the passage number and whether the virus is infecting a familiar or foreign host.

Correlation between Fitness on Familiar and Foreign Hosts

We next determined the correlation between a virus line's fitness on their familiar host genotype and on the foreign host genotypes. A negative correlation would mean that the virus lines with the highest fitness on their familiar genotype had the lowest fitness on foreign genotypes and indicate a strict trade-off. At the final passage of experimental evolution, there was no significant effect of fitness on familiar genotypes on fitness on foreign genotypes (t_7 = - 0.48, p = 0.65). Extending this analysis to include passages 4, 6, and 9, we continue to find no significant correlation between fitness on familiar and foreign genotypes (t_{23} = 1.7, p = 0.11) (See Figure 5).

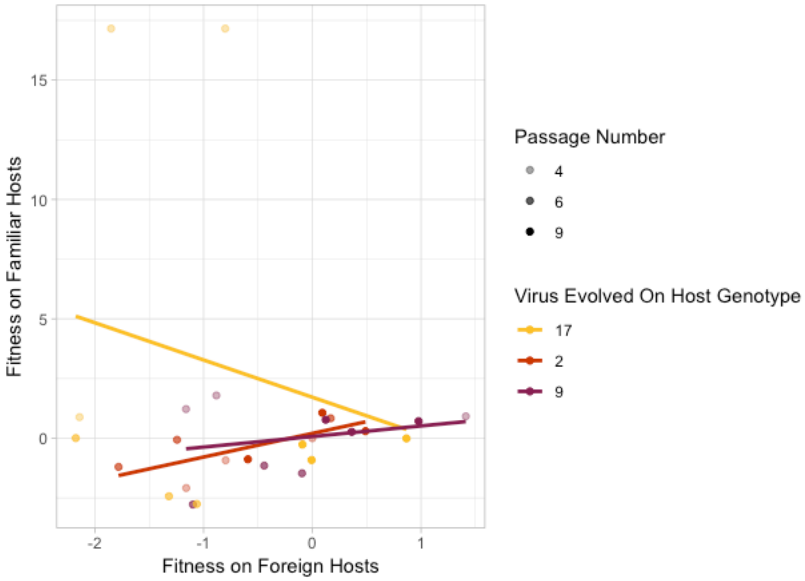

Figure 5. Correlation Between Fitness on Familiar and Foreign Hosts. Plot showing the correlation between each virus line's fitness on familiar and foreign hosts at passages 4, 6, and 9. There are no significant trends between fitness on foreign and familiar hosts across the experiment. Effect sizes are taken from the GLMM models using the 'emmeans' package.

Discussion

Specialization is critical to many of our theories of coevolution and the maintenance of diversity (Futuyma and Moreno, 1988). In particular, specialization between parasites and their hosts is crucial for understanding patterns of disease emergence and spread (Woolhouse and Gowtage-Sequeria, 2005). Here, we use experimental evolution techniques to test whether a granulosis virus can evolve to specialize on specific genotypes of its moth host. We find that the virus evolved to specialize in infectivity, productivity, and overall fitness on familiar host genotypes. The degree of specialization depends on the virus line, but, importantly, the specialization is not purely a component of the infection process. Rather, in our system, the specialization is stronger post-infection in viral productivity on the host. Additionally, the strength of specialization in overall fitness increases steadily though out the course of experimental evolution. We have therefore shown that there are specific genetic interactions between *P. interpunctella* and PiGV, suggesting that larger ecological and evolutionary processes driven by

GxG effects, like the monoculture effect (Elton, 1958; King and Lively, 2012), have the potential to be important in the system.

A key finding is that the virus specializes on familiar host genotypes in viral infectivity and productivity (Figure 2). The relationship between these components is positive at the start of the experiment but, by passage 9, evolves be negative when infecting familiar hosts (Figure 4). This suggests that different virus lines are primarily being selected to increase specialization on familiar host genotypes in either viral productivity or viral infectivity. This finding highlights the importance of measuring multiple fitness components in experimental evolution studies as pathogen populations can use many strategies to increase their overall fitness.

Some previous similar studies have also measured multiple fitness components related to parasite virulence and transmission (Kubinak et al., 2012; Legros and Koella, 2010; Nidelet and Kaltz, 2007; Zhan et al., 2002). Kubinak et al. (2012) found that Friend complex virus evolved both higher viral productivity and virulence on familiar host genotypes and Zhan et al. (2002) found that fungal strains could specialize in both virulence and frequency, though this effect was inconsistent depending on the pathogen strain considered. However, Legros and Koella (2010) found that microsporidia specialized in infectivity, but not productivity, while Nidelet and Kaltz (2007) found that parasites specialized in growth assays, but not horizontal transmission. None of these previous studies have examined the correlations between their fitness components across time, so we do not know how universal our finding of independent evolution of different fitness components is, but their results and ours suggest that researchers should be hesitant to conclude that specialization (or local adaptation) did not evolve unless they have measured multiple axes along which it could be selected.

Our experiment is, to our knowledge, the first to report on the experiential evolution of parasite host genotype specialization over time (Figure 3). Our time series data allows us to interrogate the dynamics of specialization evolution in our experiment. As passages 1 and 4, virus lines are slightly less fit on their familiar host genotype, suggesting that selection in our experiment was not dominated by the ecological sorting of virus genotypes from the genetically diverse starting population. Instead, the steadily increasing effect of self across the course of the experiment suggests that evolutionary processes were more important.

Additionally, our time series data may suggest that specialization is not driven by fixing a single allele, as this would have resulted in sudden increases in specialization and not the slower increases and wandering that our time series data shows when we look at virus lines individually (Supplemental Figure 1). Instead, our pattern of evolution may suggest that specialization is multi-genic and/or that drift was an important process in our experimental evolution. We also do not see overall fitness continuously increase though the course of the experiment, unlike in most experimental evolution (Ebert, 1998), suggesting that PiGV quickly reached a point of being fairly well adapted to infecting *Plodia interpunctella* under our experimental conditions.

The evolution of host genotype x virus genotype specificity in our experiment could be due to a number of mechanisms. We have shown that there are specific host genotype profiles in *P. interpunctella* that granulosis virus can evolve to specialize on, but we cannot precisely determine the mechanism of this specialization. Several mechanisms for the evolution of specialization have been proposed. The classic trade-off hypothesis expects that increases in fitness on one host negatively trade-off with fitness on foreign hosts so that total fitness across hosts remains constant (Levins, 1968; Regoes et al., 2000). These strict negative trade-offs are not universal though, so several additional theories have been proposed, including host specialization due to weakly positive or neutral genetic correlations leading to asymmetrical fitness gains (Fry, 1996) and host specialization due to the accumulation of deleterious mutations on alternate hosts (Kawecki, 1994; Whitlock, 1996). In the case of weakly positive or neutral genetic correlations between familiar and foreign hosts, alleles that increase fitness on one host may be neutral or less beneficial on other hosts. If this is the case, overall fitness would increase over time as parasite populations can evolve to gain the most fitness on one host type while still having moderate (or no) fitness increases on foreign hosts (Fry, 1996). Alternatively, parasite populations selected on single hosts may just not be able to select against mutations that are only deleterious on alternate host environments (Kawecki, 1994; Whitlock, 1996). Then, overall fitness would decrease over time as fitness on foreign genotypes decays, while fitness on familiar hosts may not change. In this case, fitness on foreign and familiar hosts would be uncorrelated and more dependent on processes like mutation rate and drift.

We did not find a significant correlation between fitness on familiar and foreign hosts, hampering our abilities to make conclusions about the mechanisms driving specialization.

However, we can loosely observe from Figure 5 that the sign of the correlation between fitness on familiar and foreign hosts may differ depending on the evolutionary background of the virus line. There appears to be a negative correlation between fitness on host genotype 17 and foreign genotypes (Figure 5), suggesting that adaptation to this host genotype comes at a cost of decreased fitness on alternate hosts, consistent with the classic trade-off hypothesis. However, the positive correlations between fitness on foreign and familiar host genotypes for host genotypes 2 and 9, suggest that strict negative trade-offs between different host genotypes are likely not universal in our system. Therefore, the possibly negative correlations between fitness on host genotype 17 and foreign genotypes (Figure 5) and the possibly positive correlations between finesses on host genotypes 2 and 9 and foreign genotypes (Figure 5) would suggest that multiple mechanisms contribute to evolving specialism in our system.

Specific interactions between parasites and lepidoptera hosts have only rarely been previously demonstrated. de Roode and Altizer (2010) show that protozoan parasites exhibited GxG interactions with their monarch butterfly hosts in a cross-inoculation experiment. To our knowledge, the only previous demonstration of GxG interactions between baculovirus and lepidopteran hosts is Hudson et al. (2016) who use a cross-inoculation experimental design to demonstrate that baculovirus exhibit GxG interactions with their gypsy moth host (*Lymantria dispar*). They primarily uncover variation in niche breadth of parasite infectivity, which is consistent with gene-for-gene or range models of infectivity. These results do not conflict with ours because Hudson et al. (2016) tested populations of virus that had naturally co-evolved with host populations, while we evolved virus on genetically homogenous host populations and would not have selected for generalism. Thus, if our results and Hudson et al. (2016)'s results are consistent across their somewhat closely related host-parasite systems, we may expect both within range and between range cycling of host-parasite coevolution (Ashby and Boots, 2017).

In conclusion, we used an experimental evolution approach to determine whether a baculovirus could evolve to specialize on specific genotypes of its moth host. We find that virus does evolve higher infectivity, productivity, and overall fitness on familiar host genotypes. This specialization may be driven by a combination of negative and weakly positive correlations between finesses on familiar and foreign host genotypes and mutational accumulation on foreign host genotypes. Time series data shows that specialization in overall fitness evolves directionally

over the time course of the experiment and that the different fitness components of virus lineages may be independently selected on. Our results demonstrate that gene-by-gene interactions are evolvable in the *Plodia interpunctella* and PiGV model system and suggests that the system has promise for experiments on the ecological conditions that shape selection on specialization and niche breadth.

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