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Within- and between-host dynamics of producer and non-producer pathogens

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Abstract

For infections to be maintained in a population, pathogens must compete to colonise hosts and transmit between them. Within the host, much research has been conducted into pathogen interactions, yet less is known about whether within-host interactions can affect between-host transmission. In this study, we use an experimental approach to investigate within-and-between host dynamics using the pathogen *Pseudomonas aeruginosa* and the host *Caenorhabditis elegans.* Within-host interactions often involve the production of goods, that are beneficial to all the pathogens in the local environment but susceptible to exploitation by non-producers. We exposed the nematode host to 'producer' and two 'non-producer' bacterial strains (specifically for siderophore production and quorum sensing), in single infections and coinfections, to investigate within-host colonisation. Subsequently, we introduced infected nematodes to pathogen-naive populations, to allow natural pathogen transmission between hosts. We find that producer pathogens are consistently better at colonising hosts and transmitting between them than non-producers during coinfection and single infection. Non-producers were poor at colonising hosts and between-host transmission, even when coinfecting with producers. Understanding pathogen dynamics across these multiple levels will ultimately help us to predict and control the spread of infections and contribute to explanations for the persistence of cooperative genotypes in natural populations.

Key words: pathogen transmission, quorum sensing, coinfection, *Pseudomonas aeruginosa*, social behaviour

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Introduction

To be successful, pathogens must be able to compete to colonise a host and transmit between new hosts (Anderson and May 1986). Theory has highlighted the trade-offs that can arise between these two levels as a result of counterbalancing selective pressures within and between hosts (Anderson and May 1982; Koella and Antia 1995; Walther and Ewald 2004). Within the host, pathogens must balance replication and transmission to new hosts (Reece *et al*. 2009), in an analogous trade-off between reproduction and growth within multicellular organisms (Bell 1980; Clutton-Brock 1984; Greischar *et al*. 2016). Studying pathogens at these two levels has mainly been theoretical (Anderson and May 1986; Coombs *et al*. 2007; Mideo *et al*. 2008; Handel and Rohani 2015), leaving an outstanding data gap in disease ecology (Coombs *et al*. 2007; Mideo *et al*. 2008; Handel and Rohani 2015). Understanding this interplay between different levels of infectious disease is crucial for understanding the fitness and transmissibility of pathogens, which could help contribute to developing effective intervention strategies (Hellriegel 2001; Handel and Rohani 2015) for clinically important pathogens, such as *Plasmodium sp* (Greischar *et al*. 2016) to those having major effects on ecosystems, such as the fungi *Batrachochytrium dendrobatidis* (Pedersen *et al*. 2007; Voyles *et al*. 2009; Kilpatrick *et al*. 2010; Wilber *et al*. 2017).

A pathogen rarely infects a host alone (Petney and Andrews 1998; Pedersen and Fenton 2007; Rynkiewicz *et al*. 2015; Betts *et al*. 2016). The presence of coinfecting strains may alter the within-host dynamics of an individual pathogen (Mideo 2009). For example, in the host there may be competition for resources (Pedersen and Fenton 2007; Mideo 2009), including key nutrients such as iron (Ratledge and Dover 2000; Nairz *et al*. 2010; Kramer *et al*. 2019). These within-host interactions may in turn alter between-host dynamics, as the presence of competitors may mean host exploitation is optimal (Frank 1996; Alizon *et al*. 2013) with reduced selection for transmission to secure a larger proportion of resources within the host

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(McKenzie and Bossert 1998; Greischar *et al*. 2016).

Within-host interactions are of vital importance for understanding infection outcomes (Foster 2005; West *et al*. 2007; Leggett *et al*. 2014; Rezzoagli *et al*. 2020). It has been well established that pathogens can interact cooperatively to successfully to colonise a host, for example via forming biofilms (Griffin *et al*. 2004; Kreft 2004; Foster 2005; Diggle *et al*. 2007b; West *et al*. 2007). Social traits such as the production of public goods into the local environment (West *et al*. 2006, 2007; Leggett *et al*. 2014) are frequently involved in these pathogen interactions. Public goods benefit all the cells in the local environment, but they are costly to produce and thus are susceptible to exploitation by pathogens which may utilise but not produce public goods (non-producers) (Velicer 2003; West *et al*. 2007). For example, the production of compounds known as siderophores, that are used to bind to and uptake iron (Buckling *et al*. 2007; Kramer *et al*. 2019), benefitting all bacteria in the local area as iron is often limited within a host (Ratledge and Dover 2000; Nairz *et al*. 2010). The release of products such as siderophores is regulated by communication between pathogens using small autoinducer molecules in a process known as quorum sensing (Keller and Surette 2006; Williams *et al*. 2007; West *et al*. 2007; Rumbaugh *et al*. 2009). Non-producers for both siderophore production and quorum sensing (QS) have been shown to arise in infections (De Vos *et al*. 2001; Köhler *et al*. 2009; Jiricny *et al*. 2014; Andersen *et al*. 2015). Yet when populations are sampled at random non-producers are rare (Köhler *et al*. 2009; Andersen *et al*. 2015), suggesting that non-producers could be poor at between-host transmission even if they are effective at invading established infections. Non-producers have been shown to be able to invade *in vitro* (Griffin *et al*. 2004; Diggle *et al*. 2007b) and *in vivo* (Rumbaugh *et al*. 2009). Compared to our understanding of how pathogens interact within a host, relatively little is known about how within-host processes contribute to variation in pathogen transmission between hosts (Brown *et al*. 2009; Handel and Rohani 2015; VanderWaal and Ezenwa 2016;

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Stephenson *et al*. 2017).

In this study, we use the well-established model bacterial pathogen, *Pseudomonas aeruginosa* (Griffin *et al*. 2004; Buckling *et al*. 2007; Diggle *et al*. 2007b) to investigate the role of public goods production on within-host colonisation and between-host transmission. Each of the two non-producer strain we used were unable to produce a public-good; one strain is unable to produce a type of siderophore, and the other is not able to quorum sense. Both of these traits are well-established model traits for investigating cooperative interactions between bacterial cells (Griffin *et al*. 2004; Diggle *et al*. 2006) with consequences for virulence in the case of QS (Rumbaugh *et al* 2009). We predicted that producer (potentially cooperative) strains would be better able to transmit even if they were outcompeted by exploitative non-producers (potential cheats). Investigating pathogen transmission between hosts can be logistically challenging as it requires large population sizes (Handel and Rohani 2015). We thus used the model *Caenorhabditis elegans* as the host, enabling us to have large populations with natural between-host faecal-oral transmission (Kenney *et al*. 2005; Diaz and Restif 2014). We began by tracking the within-host dynamics of producers and non-producers, in single infections and coinfections, over time. We varied the order of exposure of the different types of pathogen to determine whether priority effects altered within-host interactions (Goodman and Ross 1974; de Roode *et al*. 2005; Jackson *et al*. 2006; Clay *et al*. 2018). We then introduced infected nematodes into uninfected populations and measured rates of between-host transmission. We investigated whether differences in pathogen strain transmissibility were due to variation in host preference for ingesting a particular pathogen strain or in host shedding into the environment. Overall, our study demonstrates that, in contrast to expectations from *in vitro* competition assays (Griffin *et al*. 2004; Diggle *et al*. 2007b; Kümmerli *et al*. 2009), producer pathogens are superior to non-producers at within-host colonisation and between-host transmission in this species interaction.

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Materials and methods

Bacterial Pathogen and Nematode Host

C. elegans is a nematode species whose natural diet is composed of microorganisms (Hope 1999). The gut of *C. elegans* can be colonised by a variety of microbes, including pathogens (Clark and Hodgkin 2014). We used two strains of *C. elegans N*2 and CB5584 (*mIs12 II*). CB5584 express fluorescence in their pharynx making them distinguishable from N2 under a fluorescent microscope. We selected CB5584 as it enabled us to identify nematodes from different populations, those exposed to pathogens (N2) and pathogen-naive populations (CB5584) without any difference in pathogen susceptibility (Wang 2020). Nematodes were maintained at 20°C on a lawn of food (*Escherichia coli* (OP50)) on nematode growth medium (NGM) plates. To synchronise life cycles, nematodes were treated with bleach (NaClO and sodium hydroxide) which kills everything except unhatched eggs (Hope 1999). After bleaching, nematodes were synchronized overnight in M9 buffer and maintained for two days.

We exposed nematodes to the gram-negative pathogen, *P. aeruginosa,* an opportunistic pathogen of plants and animals, including humans (Tan *et al*. 1999). On our selected media, *P. aeruginosa* is a slow killing pathogen of *C. elegans* (Tan *et al*. 1999). We used three strains of *P. aeruginosa* in our experiments with the same genetic background (PAO1 strain background). The 'producer' strain was: PAO1 WT::GFP (WT labelled with GFP, which we herein refer to as the producer) and two 'non-producer' strains: PAO1 ΔLASR::mCherry (quorum sensing non-producer labelled with mCherry, which we herein refer to as non-producer A); and PAO1 Δpvd::mCherry (siderophore (pyoverdine) non-producer strain labelled with mCherry, which we herein refer to as non-producer B) (Rezzoagli *et al*. 2019). The non-producer strains differed. Non-producer A lacked the ability to communicate with other bacteria via quorum sensing (Keller and Surette 2006; Williams *et al*. 2007; West *et al*.

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2007; Rumbaugh *et al*. 2009) which also affects a variety of other traits (e.g. biofilm development (Diggle *et al*. 2007b; Williams *et al*. 2007)). Comparatively, non-producer B had only lacked the ability to produce the single siderophore pyoverdine (Buckling *et al*. 2007; Visca *et al*. 2007). Each strain had a visually distinct colony morphology which enabled colony counting in the two-stage exposure experiment.

For our investigation of nematode preference, we also used two strains of *Pseudomonas fluorescens:* a producer (CHA0) and a non-producer (CHA019) of *gacS* defensive toxins (Jousset *et al*. 2009). *C. elegans* have previously been shown to preferentially graze on the non-producer, CHA019 (Jousset *et al*. 2009), and so we used it as a positive control. All bacteria were stored at -80ºC in a 1:1 ratio of sample to 50% glycerol solution in cryotubes. Strains of *Pseudomonas* bacteria were grown overnight at 37^{0}C in Lysogeny broth (LB) shaking at 200 r.p.m. OP50 was grown under the same conditions at 30ºC.

Within-host colonisation and dynamics

Part 1 (I): Single Exposure

Approximately 1000 nematodes at the L4 stage were transferred onto a lawn of either the producer, non-producer A, or non-producer B for 24 hours. The plates were saturated with bacteria such that the nematodes had a continuous grazing source. For the first stage of the experiment, a sample of 4-5 nematodes were removed from the single pathogen treatment after 24 hours and washed according to the droplet method (Ford *et al*. 2016) (Figure S1). This involves passaging worms through five droplets of M9 buffer using a platinum wire to remove external bacteria. Treatments for this experiment consisted of five biological replicates, and the whole experiment was replicated four times.

Part 1 (II): Two Stage Exposure

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The two-pathogen treatment involved the pathogen strain being switched after 12 hours exposure, with an additional washing stage between transfers (See Figure S1). We exposed nematodes to the pathogen treatments sequentially. Simultaneous exposure would have resulted in competition outside of the host, which we wanted to avoid. There were four different treatments according to the order of primary and secondary pathogen exposure: *i)* producer to non-producer A; *ii)* producer to non-producer B; *iii)* non-producer A to producer; and *iv)* non-producer B to producer. This exposure method allowed us to investigate whether the order of exposure affected within-host interactions.

To obtain an estimate of pathogen load, we calculated the number of colony-forming units (CFUs) per nematode. We placed 4-5 clean nematodes in 90µl of M9 in a 1ml Eppendorf tube (containing microbeads). The tube was placed in a bead-beater for one minute at 2800rpm. After crushing, serial dilutions were plated onto Kings Broth (KB) media (Ghoul *et al*. 2016) and grown overnight at 30ºC. This media was not iron-deficient. The number of colonies was counted. Treatments for both stages of the experiment consisted of five biological replicates, and the whole experiment was replicated four times.

For details of an additional two-stage exposure analysis see supplementary materials. In this analysis we conduct time controls using the nematode food (OP50) and demonstrate that the time of exposure (i.e., 0 hours or after 12 hours) does not affect colonisation ability of any of the pathogen types.

Between-host dynamics

Part 2: Between-host transmission

To investigate between-host dynamics, we used nematodes from the within-host assay (see Figure S1). After the nematodes' exposure in the first instance to a single pathogen (either non-producer or producer) and in the second stage exposure to a two-pathogen treatment, a sample

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of 10 clean nematodes were transferred to a pathogen-naive population of GFP labelled nematodes (see Figure S1, Part 2). After 24 hours, we took 4-5 pathogen-naive nematodes from each plate to calculate pathogen load by counting the CFUs within nematodes. We were able to distinguish, and selectively pick, the pathogen-naive nematodes using a fluorescent microscope. The pathogen-naive worms were distinguished by their green pharynx.

Preference Assay

To determine whether nematodes preferred to consume producers or non-producers, we conducted a preference assay. Bacterial cell density was measured at an absorbance of 600 nm (A_{600}) and then standardized to a density of 1 using M9. We used these standardised cultures to make equidistant 20µL spots on a 90mm KB media (Ghoul *et al*. 2016) plate (see Figure S3A). Each KB plate contained one spot of producer and a spot of either non-producer A, non-producer B, or the control *P fluorescens* (CHA0 or CHA019). We left the bacteria to dry and then incubated the plates overnight at 30°C prior to the aversion assays taking place. We introduced approximately 40 clean nematodes to the centre of to the prepared plates with equidistant producer and an alternative pathogen (non-producer A, non-producer B, CHA019 or CHA0) (as in (Abada *et al*. 2009)). We recorded the approximate time of bacteria drying as 0 hours and left the plates for 6 hours (by this time nematodes are likely to have established their preference (Shtonda and Avery 2006; Ballestriero *et al*. 2016)). Treatments consisted of six biological replicates and the whole experiment was replicated five times. After 6 hours we recorded the number of nematodes on the producer, non-producer or neither colony.

Host shedding of pathogens into the environment assay

To investigate how many pathogens were shed from the nematode host into the environment we exposed nematodes to either a producer or non-producer *P. aeruginosa* (non-producer A or

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non-producer B) for 12 hours. These exposed nematodes were cleaned and transferred to an empty NGM plate (with a minimal lawn of OP50). After 12 hours, these nematodes were picked off the plate and the pathogen cells liberated using M9 buffer. The bacteria from the plate were then grown and counted for comparison (Figure S3B). Pathogen colonies were phenotypically distinguishable from OP50.

Statistical analysis

All of the statistical analysis and data presentation was carried out in R version 3.6.2 (R Core Team 2019) using RStudio (RStudio Team 2021) and the packages 'dplyr' (Wickham *et al*. 2017), 'ggplot2' (Wickham 2009), 'multcomp' (Hothorn *et al*. 2008), 'cowplot' (Wilke 2020) , 'stargazer' (Hlavac 2018), 'broom' (Robinson *et al*. 2021), 'lme4' (Bates *et al*. 2015) and 'lmerTest' (Kuznetsova *et al*. 2017). Results were considered to be statistically significant when $p<0.05$.

Within-host colonisation and dynamics part 1 (I): Single Exposure

To compare pathogen loads within hosts exposed to either a non-producer or producer, we fit a linear model with 'CFU per nematode' as the response variable and 'pathogen' (three treatments: *i*) producer; *ii*) non-producer A; and *iii*) non-producer B) as the explanatory variable. We then carried out an ANOVA with this model and ran post-hoc Tukey tests.

Within-host colonisation and dynamics part 1 (II): Two Stage Exposure

When nematodes were exposed to both a non-producer and producer (four 'pathogen' treatments, written in order of exposure: *i)* producer to non-producer A; *ii)* producer to non-producer B; *iii)* non-producer A to producer; and *iv)* non-producer B to producer), we fit a quasipoisson generalised linear model to compare pathogen loads (with either 'non-producer

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CFU per nematode' or 'producer CFU per nematode' as the response variable and 'pathogen' as the explanatory variable). To assess the significance of 'pathogen', we carried out a likelihood ratio test with this model and a second model from which the 'pathogen' term had been dropped. We then ran post-hoc Tukey tests using the 'glht()' function from the multcomp package (Hothorn *et al*. 2008) to assess pairwise differences.

Between-host dynamics part 2: Between-host transmission

To investigate between-host dynamics within the pathogen-naive nematode populations (introduced to nematodes that had been singly infected with either a producer or a non-producer) we compared pathogen loads across treatments by fitting a linear model with 'CFU per nematode' as the response variable and 'pathogen' (three treatments: *i)* producer; *ii)* non-producer A; and *iii)* non-producer B) as the explanatory variable. We carried out an ANOVA to calculate F ratios for the explanatory variable 'pathogen' and ran post-hoc Tukey tests. To compare the pathogen loads from the pathogen-naive populations, where the introduced infected nematodes had been exposed to both producers and non-producers (four 'pathogen' treatments, written in order of exposure: *i)* producer to non-producer A; *ii)* producer to non-producer B; *iii)* non-producer A to producer; and *iv)* non-producer B to producer), we fit a quasipoisson generalised linear model (GLM). For the GLM the response variable was either the pathogen load of non-producers ('non-producer CFU per nematode') or that of the producer ('producer CFU per nematode') and 'pathogen' as the explanatory variable. To assess the significance of the explanatory variable 'pathogen', we carried out a likelihood ratio test with this model and a second model from which the 'pathogen' term had been dropped. We then ran post-hoc Tukey tests.

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Preference Assay & Host shedding of pathogens into the environment assay

To investigate whether nematodes displayed a preference for either producers or non-producers, we calculated a choice index:

$$Choice\ Index = \frac{Number\ of\ Nematodes\ in\ Producer\ Colony - Number\ of\ Nematodes\ in\ Alternative\ Colony}{Total\ number\ of\ Nematodes}$$

Where a value of one indicates complete preference for the producer, 0 indicates a lack of preference and -1 indicates complete preference for the alternative strain (all non-producers except for CHA0). We tested whether the choice indexes were significantly different from zero (indicating no preference) using one sample t-tests. Finally, to compare the number of CFUs present on the lawn of a plate after an infected population of nematodes had been removed, we fit a quasipoisson generalised linear model with 'CFU per nematode' as the response variable and 'pathogen' as the explanatory variable. We conducted a likelihood ratio test with this model and a second model from which the 'pathogen' term had been dropped and ran post-hoc Tukey tests between the pathogen treatments.

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Results

Within-host dynamics: Producers are better than non-producers at colonising nematode hosts

Part 1 (I): Single Exposure

The producer was better able to colonise the nematode host than both non-producers, as the pathogen load (CFUs per nematode) was significantly higher for producers than non-producer A or non-producer B (Post-hoc Tukey tests; non-producer A – producer: mean difference = -11200, $p < 0.001$; non-producer B – producer: mean difference = -8870, $p < 0.01$, see Figure 1, Table S1). There was no difference between the pathogen loads for nematodes exposed to

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non-producer A or non-producer B (Post-hoc Tukey test; non-producer B – non-producer A: mean difference = -2336, $p = 0.19$, Figure 1, Table S1), but overall, there was a significant effect of 'pathogen' treatment ($F_{2,57} = 39.8$, $p < 0.001$, Table S1, Figure 1).

Part 1 (II): Two Stage Exposure

When nematodes were exposed to both producers and non-producers, producers colonised at a similar level across pathogen treatments within the nematode host (No effect of treatment: Likelihood ratio: deviance = 2500, $p = 0.365$, Figure 2, Table S2B). There was a significant effect of pathogen treatment for non-producer CFUs (deviance = 100414, $p < 0.001$, Figure 2, Table S2A). The pathogen load of both non-producer B treatments (producer to non-producer B and non-producer B to producer) were significantly higher than both non-producer A treatments (producer to non-producer A and non-producer A to producer), regardless of the order of exposure, with non-producer B to producer having a significantly higher number of CFUs per nematode than all the other treatments (Figure 2, Table S2A).

Between-host dynamics: Producers are better at between-host transmission than non-producers

Part 2: Between-host transmission

When infected nematodes that were exposed to only a single type of pathogen were introduced to the pathogen-naive population, the producer was best able to spread to new hosts with a significantly higher pathogen load in the pathogen-naive worms than both non-producer pathogens ($F_{2,57} = 7.73$, $p = 0.001$, Post-hoc Tukey tests; producer to non-producer A: mean difference = -3440, $p = 0.030$; producer to non-producer B: mean difference = -5040, $p = 0.0009$, see Figure 3A, Table S5).

When nematodes were infected by producers and non-producers, the pathogen load of

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producer in the pathogen-naive populations of nematodes remained consistently high (Figure 3B). Although pathogen loads were similar across treatments, there was a significant effect of 'pathogen' (Likelihood ratio test: 'producer' deviance = 11100, $p = 0.01$, Table S6B) likely driven by the statistically significant difference between the loads of the producer to non-producer B and non-producer A to producer treatments (Tukey post-hoc test of difference: mean of 'non-producer A to producer' – 'producer to non-producer B' = 0.358, $p = 0.026$). The non-producer pathogen loads of nematodes were low and close to zero across treatments (Figure 3B), except for the non-producer B to producer treatment where there was a significantly higher number of CFUs per nematodes than all the other types of non-producer (Table S6). This higher number of CFUs per nematode for non-producer B to producer treatment seemed to drive a significant effect of 'pathogen' (Likelihood ratio test: 'non-producer' deviance = 94200, $p < 0.001$).

Preference Assay

C. elegans did not display a preference for either the non-producer or producer strains of *P. aeruginosa* (see Figure 4A, Table S7A). The choice index was not significantly different from zero for either producer v non-producer A (Mean choice index = 0.0468, $t = 0.733$, $p = 0.45$) or producer v non-producer B (Mean choice index = -0.0333, $t = -0.683$, $p = 0.5$). As a positive control, we found *C.elegans* showed a preference for the non-producer *P. fluorescens* strain CHA019 (Mean choice index = -0.405, $t = -12.28$, $p < 0.001$, Figure 4B) as in (Jousset *et al.* 2009). There was no preference for the producer *P. protegens* strain CHA0 (Mean choice index = 0.00641, $t = 0.188$, $p = 0.85$, Figure 4B).

Host shedding of pathogens into the environment assay

Nematodes shed significantly more producers into the environment than either type of non-

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producer (overall effect of 'pathogen' Likelihood ratio test: Deviance = 64,

.100, $p < 0.001$; Post-hoc Tukey tests; producer – non-producer A: difference = -0.825, $p<0.001$; non-producer B – producer: difference = -0.284, $p = 0.007$, Figure 4C, Table S7B). There was also a significant difference between the number of CFUs present on a lawn between the two non-producer strains, with more non-producer B shed into the environment than non-producer A (non-producer B – non-producer A = 0.541, $p<0.001$, Figure 4C, table S7B).

Discussion

We revealed that public-good producers showed superior ability to establish infection within- and between-nematode hosts compared to non-producers. There were consistently more producers present in the nematodes when the hosts were directly exposed to the pathogens as well as when the pathogens were spread naturally between hosts. Non-producers were poor at both within-host colonisation and between host transmission, even when coinfecting hosts with producers. Our results suggest that a higher pathogen load for producers is related to an increase in their ability to transmit between hosts. This positive relationship between pathogen load and transmissibility is a common assumption in theoretical studies (see Handel and Rohani 2015 and references therein), but with few empirical examples (guppy [*Poecilia reticulata*] ectoparasite [*Gyrodactylus turnbulli*] system in Stephenson *et al*. 2017).

The counterbalancing selection pressures experienced by pathogens at the level of within-host colonisation and between-host transmission can lead to trade-offs (Anderson and May 1982; Koella and Antia 1995; Walther and Ewald 2004). Within a host, the ability to reproduce rapidly may be advantageous to outcompete competitors. However, pathogens that reproduce more slowly may be able to persist for longer periods in the external environment. This could be a beneficial trait for transmission, but disadvantageous for colonisation (Walther and Ewald 2004). These trade-offs can be affected by multi-species interactions within a host (Mideo

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2009; Alizon *et al*. 2013), and by transmission mode (Walther and Ewald 2004; Antonovics *et al*. 2017). Counter to our expectations, we do not find evidence for an existing trade-off between within-host colonisation and transmissibility for producer pathogens.

We predicted that in the absence of producers, non-producers would be less able to colonise the host. Non-producers lack the ability to either produce iron-scavenging siderophores (non-producer B (pyoverdine)) (Buckling *et al*. 2007) or induce the las quorum sensing pathway (non-producer A) (Keller and Surette 2006; Diggle *et al*. 2007b, a; West *et al*. 2007). Non-producer A was relatively poor at colonising and transmitting between hosts compared to non-producer B, an outcome likely due to the more severe fitness consequences experienced by non-producer A than non-producer B. Non-producer A is unable to communicate with other pathogens via quorum sensing (Venturi 2006; Keller and Surette 2006). This is a crucial trait to the success of *P. aeruginosa* that controls behaviours, such as biofilm development and the production of virulence factors (Diggle *et al*. 2007b; Williams *et al*. 2007). While non-producer B does not produce the primary siderophore pyoverdine (Buckling *et al*. 2007; Visca *et al*. 2007), which can negatively impact the fitness of the strain in an iron-limited host environment (Ratledge and Dover 2000; Nairz *et al*. 2010) it can produce other siderophores and can take up iron from the environment in other ways (Buckling *et al*. 2007; Visca *et al*. 2007). In addition, nematode hosts did not preferentially consume pathogenic producers over non-producers. There was no difference in the intake of pathogen type. More producers, however, were shed into the local environment than non-producers, making them more successful at between-host transmission than non-producers.

Even in the presence of producers, non-producers were less able to colonise hosts and transmit between hosts. This inability of non-producer strains to exploit producers (i.e. their inability to cheat effectively) has previously been found in *in-vivo* studies of a nematode host (Rezzoagli *et al*. 2019), as well as in larvae of the wax moth (*Galleria mellonella*) (Harrison *et*

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al. 2006) and the diamondback moth (*Plutella xylostella*) (Zhou *et al*. 2014). We expect that this is likely due to several factors. Firstly, within the nematode gut there is a relatively low pathogen density (Rezzoagli *et al*. 2019). Non-producers are most likely to benefit from producers when they exist at a low density (Ross-Gillespie *et al*. 2007; Rumbaugh *et al*. 2009) within a high density of producers (Ross-Gillespie *et al*. 2009) (negative frequency dependence). Secondly, the spatial heterogeneity within the host may not be conducive to cheating (Harrison *et al*. 2006; Leggett *et al*. 2014; Rezzoagli *et al*. 2019). The *in vitro* environment is more homogenous (Pedersen and Fenton 2007; Mideo 2009), allowing non-producers to mix with producers and potentially utilise the products released into the environment (Griffin *et al*. 2004; Diggle *et al*. 2007b; Kümmerli *et al*. 2009). Whereas *in vivo,* the environment is likely to have more structure, preventing mixing. Therefore within the host, non-producers may be more likely to be surrounded by their non-producer relatives (Frank 1998), and thus will be less able to directly benefit from the public goods of the producers (Harrison *et al*. 2006; West *et al*. 2006; Rezzoagli *et al*. 2019). Finally, the nematode host itself may impact the non-producer's colonisation ability; limited iron availability may remove any benefit of not producing the costly siderophores (Ratledge and Dover 2000; Nairz *et al*. 2010).

Understanding how social traits affect within- and between-host dynamics requires further experiments (Mideo *et al*. 2008; Handel and Rohani 2015). Our study, among others (Diaz and Restif 2014), highlights the benefits of *C. elegans* as a model host for naturally studying between-host dynamics without major logistical challenges (Handel and Rohani 2015). A future avenue for exploration within this system could involve experimentally evolving non-producers de-novo within the nematode that can successfully cheat (Ghoul *et al*. 2014) to determine how within-host cheating impacts pathogen transmissibility. Cheats have been shown to evolve in natural populations, for example quorum sensing cheats are found in the lungs of cystic fibrosis patients (Diggle *et al*. 2007). It is predicted that successful within-host

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cheating could negatively impact the transmission dynamics of an infection, and it could be explored as a potential disease intervention strategy (Brown *et al*. 2009; Jiricny *et al*. 2014; Leggett *et al*. 2014).

We suggest that within-host pathogen interactions affect between-host infection dynamics. We provide empirical evidence linking processes occurring at different scales, in a field dominated by theory (Coombs *et al*. 2007; Mideo *et al*. 2008; Handel and Rohani 2015), investigating the role of pathogenic social traits important in disease. Taking a multilevel experimental approach to within- and between-host dynamics is an outstanding and major challenge within evolutionary biology (Mideo *et al*. 2008). Addressing this challenge will improve our ability to predict and control infection in less well understood systems, such as disease-induced population extinctions (Pedersen *et al*. 2007; Smith *et al*. 2009; Wilber *et al*. 2017) and may also allow us to harness pathogen interactions for our own benefit (Smith and Holt 1996; Mideo 2009; Leggett *et al*. 2014; Rezzoagli *et al*. 2020).

Supplementary material.

The supplementary material for this article can be found at:

Data availability.

The data and code from this study have been uploaded with the supplementary material.

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Author's contribution.

V.L.P, K.C.K and A.S.G conceived and designed the study. V.L.P. performed the experiments and analysed the data, with guidance from K.C.K. V.L.P. and E.S. wrote the manuscript, with guidance from K.C.K. All authors have approved the manuscript.

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Competing interests.

The authors declare there are no conflicts of interest.

Ethical standards.

Not applicable

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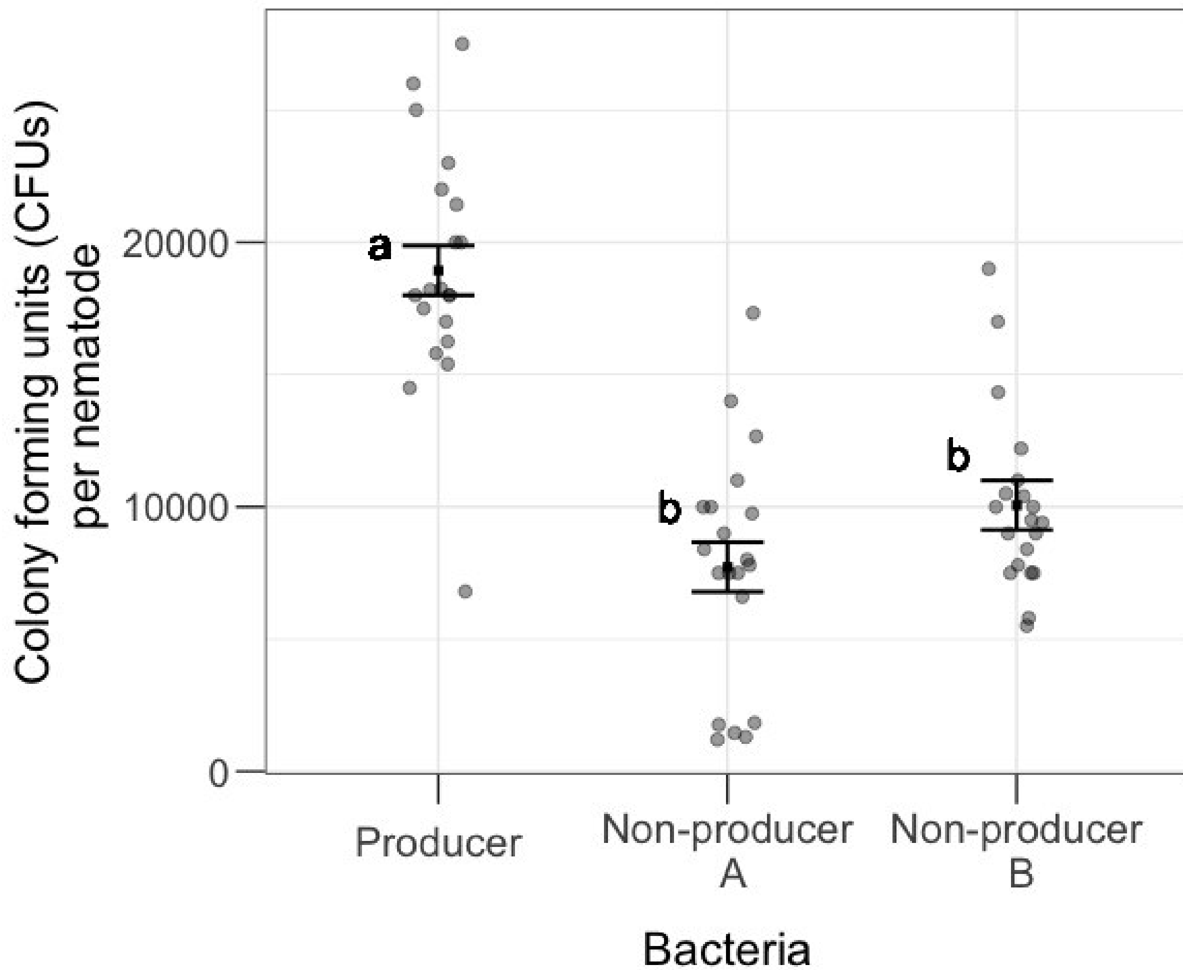

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Figure 1: Within-host colonisation. Difference in the pathogen load, measured by the number of colony forming units (CFUs) per nematode, after exposure to one of the three strains of *P. aeruginosa.* Treatments with different letters are significantly different. Square points with error bars represent mean +/- 1 SE.

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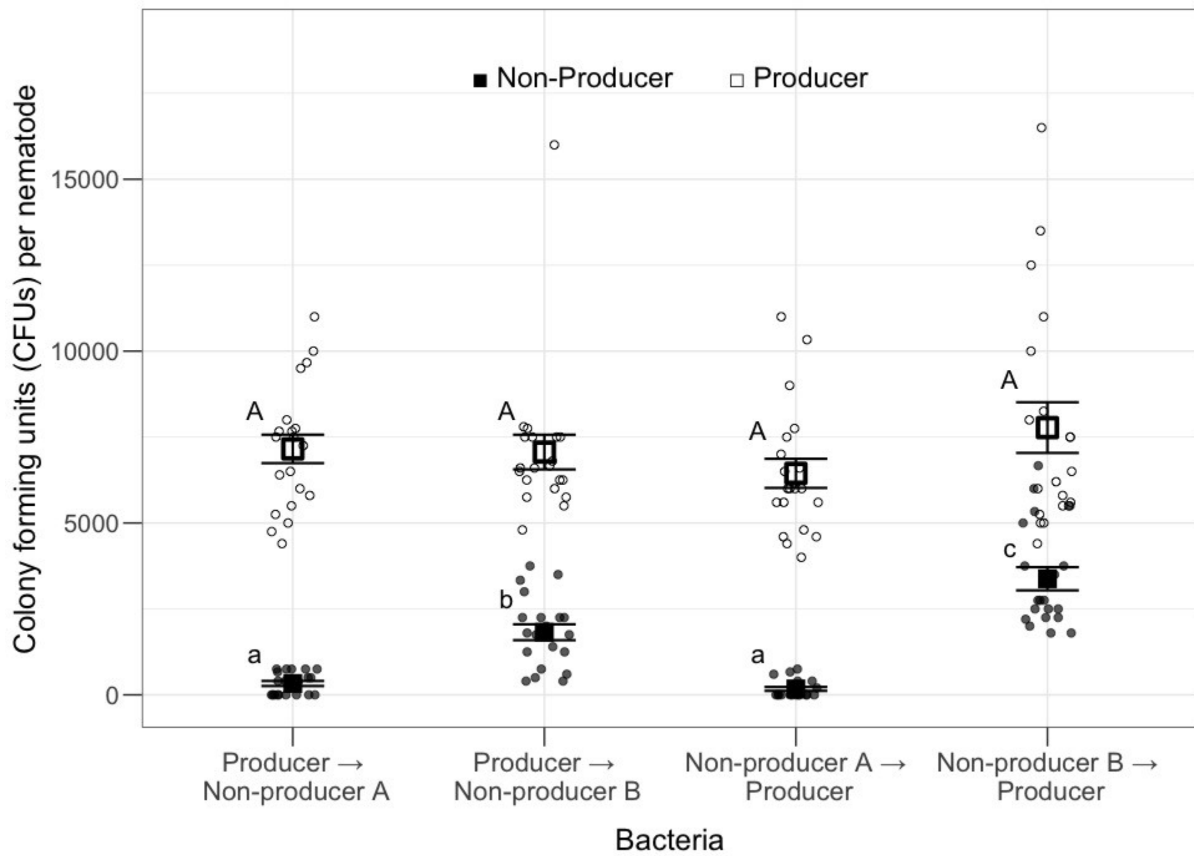

Figure 2: Within-host colonisation and dynamics of producer and non-producer pathogens. Difference in the producer (white) and non-producer (black) pathogen loads, measured by the number of colony forming units (CFU) (per nematode), after the exposure to both pathogen treatments (producer, non-producer A and non-producer B). The x axis labels are given in order of exposure. Points with different letters are significantly different from each other. Square points with error bars represent mean +/- 1 SE.

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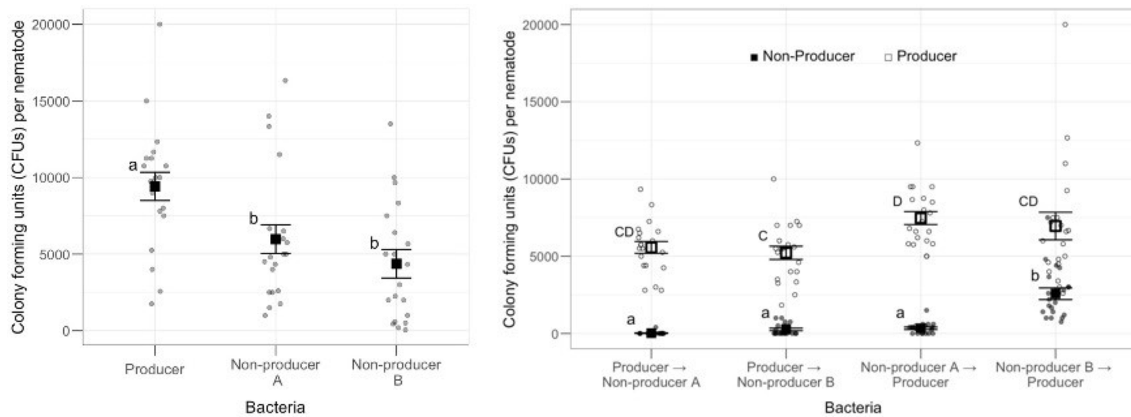

Figure 3: Between-host dynamics of producer and non-producer pathogens. A) Difference in the pathogen load (CFUs per nematode) from the naive population after infected nematodes were introduced. **B)** Difference in the number of producers (white) and non-producers (black) in the colony forming units (per nematode), from the naive population after the infected nematodes are introduced into their population. The x axis labels are given in order of exposure. Points with different letters are statistically significantly different. Square points with error bars represent mean +/- 1 SE.

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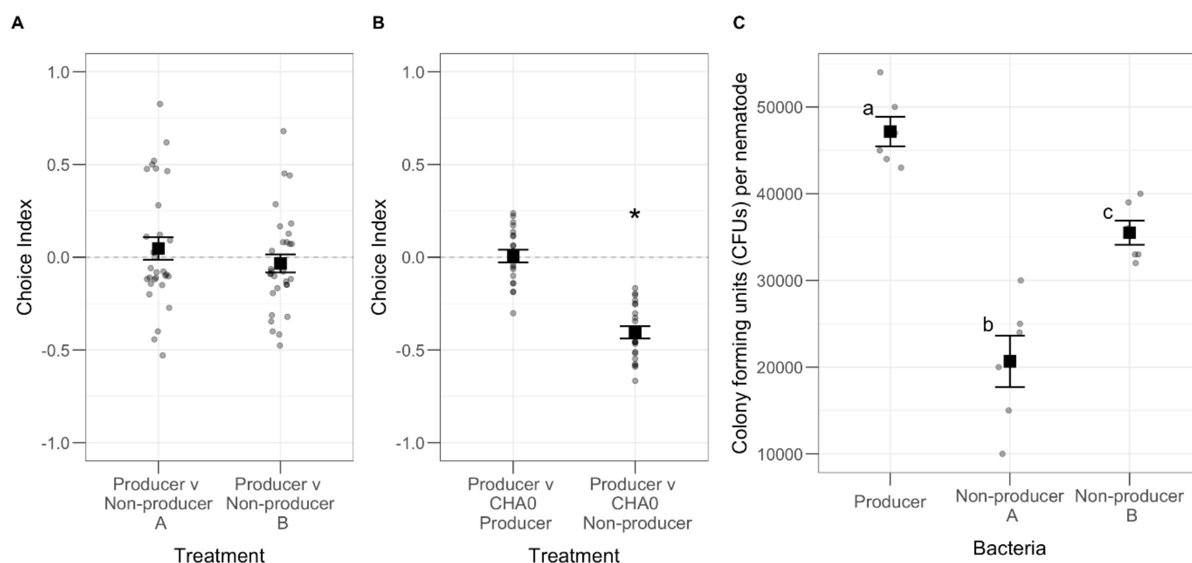

Figure 4: Host preference for picking-up pathogens and host shedding of pathogens in the environment. A&B) Choice Index of *C.elegans* for producer and non-producer strains of *P. aeruginosa*. Where 1 indicates complete preference for the producer, 0 indicates a lack of preference for either and -1 indicates complete preference for the alternative strain (non-producer A, non-producer B, CHAO19 and CHA0). Figure A) shows the choice index for the two focal non-producer strains non-producer A and non-producer B in comparison to the producer whereas B) shows the choice index for *P. fluorescens* in comparison to the producer. * indicates significance. C) Difference the in the number of colony forming units (CFUs) present on a lawn after 12 hours of exposure to infected nematodes. Each of the three points are statistically significantly different from each other denoted by different letters. Square points with error bars represent mean +/- 1 SE.

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