

Genetic influence on disease spread following arrival of infected carriers

Simon Fellous,^{1*} Alison B. Duncan,¹
Elsa Quillery,¹ Pedro F. Vale² and
Oliver Kaltz¹

Abstract

Epidemiology in host meta-populations depends on parasite ability to disperse between, establish and persist in distinct sub-populations of hosts. We studied the genetic factors determining the short-term establishment, and long-term maintenance, of pathogens introduced by infected hosts (i.e. carriers) into recipient populations. We used experimental populations of the freshwater ciliate *Paramecium caudatum* and its bacterial parasite *Holospora undulata*. Parasite short-term spread (approximately one horizontal transmission cycle) was affected mainly by carrier genotype, and its interactions with parasite and recipient genotypes. By contrast, parasite longer term spread (2–3 horizontal transmission cycles) was mostly determined by parasite isolate. Importantly, measures of parasite short-term success (reproductive number, R) were not good predictors for longer term prevalence, probably because of the specific interactions between host and parasite genotypes. Analogous to variation in vectorial capacity and super-spreader occurrence, two crucial components of epidemiology, we show that carrier genotype can also affect disease spread within meta-populations.

Keywords

Carrier host, genotype-by-genotype interaction, infectious disease, meta-population, microcosm, parasite introduction, R_0 .

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INTRODUCTION

Parasite emergence and spread largely depends on host population structure, the potential for parasites to move between populations and their ability to colonise new populations of uninfected hosts (Anderson *et al.* 1986; May *et al.* 2001; Cross *et al.* 2005, 2007; Ostfeld *et al.* 2005; Cook *et al.* 2007). The latter, the parasite's ability to spread within a host population, is classically assessed using the disease's basic reproductive number, R_0 , which reflects the number of secondary infections caused by a single infected individual in a totally susceptible population (May *et al.* 2001; Cross *et al.* 2005, 2007; Matthews & Woolhouse 2005; Cook *et al.* 2007). This theory has been developed to reflect a variety of parasite life-cycles, including vertical transmission or horizontal transmission either by direct contact or autonomous infectious forms (e.g. Anderson & May 1992; Lipsitch *et al.* 1995; Matthews & Woolhouse 2005). Regardless of the infection cycle, this theoretical framework is most commonly used to estimate the effective reproductive rate (R) of the parasite, that is, the rate of increase in the number of infections per unit time.

There is an increasing appreciation that pathogen reproductive rate and invasion success depend on complex interactions between the biotic interactors and the abiotic environment in which they exist (Prentis *et al.* 2008; Lazzaro & Little 2009; Wolinska & King 2009). It is especially well-documented that in many host–parasite systems the success of infection (a crucial initial step for successful invasion) depends strongly on the genotypes of host and parasite [genotype-by-genotype ($G \times G$) interactions (reviewed in Lambrechts *et al.* 2006b; Salvaudon *et al.* 2008; Lambrechts 2010)]. Invasion success can

thus be better understood by considering the effect of the genetic background of the various interacting organisms: the recipient host population and the invading parasite. Moreover, the arrival of a parasite into a previously uninfected population is frequently the product of the immigration of an infected vector, or of a carrier of the same or a different host species. This adds an additional biotic interactor, whose genetic identity has until now been neglected. Thus, the dynamic of the spread of a parasite within an uninfected population can encompass several levels of genetic interactions which should be considered together ($G_{\text{recipient}} \times G_{\text{carrier}} \times G_{\text{pathogen}}$). This inherent biological complexity adds to the difficulty in recording transmission events in wild populations, making it very challenging to understand the factors that influence parasite spread in real epidemic scenarios.

Here, we exploited the power of microbial microcosms (Jessup *et al.* 2004; Buckling *et al.* 2009) to carry out experimental epidemiology under a scenario where infected 'carriers' introduce a parasite into an initially uninfected population of 'recipient' hosts, thereby mirroring Anderson and May's original concept of parasite reproductive rate (Anderson & May 1992). We used the freshwater ciliate host, *Paramecium caudatum*, and the bacterial parasite, *Holospora undulata*. Following a full-factorial design, we set up different combinations of host resident genotypes, carrier genotypes and parasite isolates to dissect the genetic determinants of disease spread. In this system, horizontal transmission occurs by the dissemination of infectious forms into water and their ingestion by new hosts, whereas vertical transmission occurs with the asexual division of infected hosts (see Figure S1 for a detailed description of the life-cycle). We therefore

¹Institut des Sciences de l'Evolution, UMR CNRS-UM2-IRD 5554, CC 065, University of Montpellier 2, Place Eugène Bataillon, 34095 Montpellier Cedex 05, France

²Centre d'Ecologie Fonctionnelle et Evolutive, UMR CNRS 5175, 1919 route de Mende, 34293 Montpellier Cedex 05, France

*Correspondence: E-mail simonfellous@free.fr

measured the effective reproductive rate (R) of the parasite taking into account these two modes of transmission, and we studied how this estimate of short-term spread is related to long-term infection prevalence. Our results show that the genetic identity of the carrier host is essential for initial parasite establishment, whereas longer term maintenance appears mainly dependent on the genotype of the parasite.

MATERIALS AND METHODS

Biological system

The protozoan *Paramecium caudatum* inhabits stagnant freshwater bodies in the Northern hemisphere (Wichterman 1986). Our laboratory cultures are maintained at 23 °C, in a medium prepared from dried organic lettuce supplemented with the food bacterium *Serratia marcescens* (Strain A173; Institut Pasteur, Paris, France). Under permissive conditions, *P. caudatum* divides 1–2 times every 24 h provided regular dilution.

The obligate parasite *Holospira undulata* (alpha-proteobacteria) develops in the host micronucleus (Fokin 2004). Horizontal transmission follows ingestion of infectious forms of the parasite during food uptake (see Figure S1 for a detailed description of the life-cycle). Ca. 24 h post infection, the long S-shaped infectious forms (10–15 µm) differentiate into the short round-shaped (5 µm) reproductive forms that begin to multiply and fill out the micronucleus. After 7–10 days, reproductive forms differentiate into infectious forms. Established infections are characterised by heavily swollen micronuclei, filled with a mix of hundreds of reproductive and infectious forms. At this stage, infection reduces host division rate and survival (Nidelet *et al.* 2009). Infectious forms are released during host division or after host death. Vertical transmission occurs when reproductive forms segregate into the daughter nuclei of a mitotically dividing host. During the first few days after infection, the micronucleus is not yet entirely filled with reproductive forms, and this allows new infections to be distinguished from established infections.

Origins of hosts and parasites

We used two host genotypes as carriers (VEN and K8) and three host genotypes as recipients (VEN, K8 and CRA). The VEN genotype originates from Italy (Venice), the K8 genotype from Japan and the CRA genotype from Poland (Krakow). Sequencing of the CO1 mitochondrial gene (Barth *et al.* 2006) confirmed the genetic difference between these genotypes (Duncan *et al.* 2010).

We used three parasite origins for the experiment (P.VEN, P.K8 and P.CRA). They were generated by culturing the parasite on the VEN, K8 and CRA host genotypes for 7 months. Previous experiments showed that this parasite can evolve differently when cultured on different host clones (e.g. Nidelet & Kaltz 2007). At the end of this 7-month period – that is, 2.5 months prior to the experiment – infectious forms from each of the three parasite origins were extracted and used to infect paramecia of the VEN and K8 clones, following a standard infection protocol (Nidelet *et al.* 2009). We thus had six origins of infected hosts (three parasite origins \times two host clones) as carriers. Two weeks before the experiment, we isolated and propagated single infected individuals from each of the six host–parasite combinations, to obtain stocks of > 95% infected hosts to be used as carriers.

Protocol

An experimental replicate population consisted of 20 uninfected paramecia (recipients) of a given genotype, to which we added two infected carrier individuals from a given host–parasite combination. Carrier hosts were washed twice in fresh medium before releasing them into the experimental populations. Thus, all new infections resulted from infectious forms released by the carriers.

The 22 individuals were transferred to 300 µL of fresh medium in a single well of a 48-well plate (NuncTM, VWR, France). The replicate populations of each treatment were divided in two groups, one group being destructively sampled after 6 days, and the other group 21 days after the start of the experiment. Plates were kept in an incubator at 23 °C. For each sampled population, we counted the number of living paramecia under a dissecting microscope; a cell sample was then fixed with lacto-aceto-orcein (Görtz & Dieckmann 1980) and their infection status determined under an optical microscope ($\times 1000$, phase contrast). We distinguished between established infections and new infections, with their micronucleus partially filled with reproductive forms (Figure S1). This permitted clear distinction for the first time point (day 6) between carrier individuals and their daughter cells (both with established infections) and newly infected *Paramecium* from the resident population (harbouring early infection stages).

On the second time point (day 21) it was not possible to distinguish if infections were of carrier or recipient origin, as the first cohort of infected recipients had likely developed fully established infections or produced new cohorts of horizontal infections. Note that at any stage of a *H. undulata* epidemic, hosts harbouring new infections are either (1) previously uninfected cells that recently ingested infectious forms or (2) daughter cells resulting from the division of such hosts. Because *P. caudatum* hosts can divide several times between the moment they ingest the parasite and the moment it starts producing new infectious forms, distinguishing between these two processes is technically and conceptually difficult.

The experiment was set up in a full-factorial design, using the three recipient host genotypes (VEN, K8, and CRA), two carrier host genotypes (VEN and K8) and three parasite isolates (P.VEN, P.K8 and P.CRA). For each recipient/carrier/parasite combination, we prepared 12 replicate populations ($3 \times 2 \times 3 \times 12 = 216$ populations in total), distributed randomly across four different plates, of which two were destructively sampled for each time point. As controls, we established 12 populations with 20 individuals from each of the three recipient genotypes in to which we introduced two non-infected individuals of the VEN or K8 genotype (i.e. $12 \times 3 \times 2 = 108$ populations).

Statistical methods

We estimated the rate of increase in the number of infections (R) after 6 days. Because of the 7 to 10 day latency time of infection (see above and Figure S1), all new infections detected on day 6 could be attributed to horizontal transmission by the carriers. Consequently, this value represents the effective reproductive rate of the parasite during no more than one cycle of horizontal transmission (eqn 1). R is therefore a measure of parasite spread starting from two initial infected individuals, similar to R_0 (Anderson & May 1992). We also measured infection prevalence (i.e. proportion of infected hosts) for days 6 and 21.

$$R = \frac{I_N + (I_E - 2)}{2}, \quad (1)$$

where I_N is the estimated number of hosts with new infections, and I_E is the estimated number of hosts with an established infection. We subtracted from I_E the two initial carrier hosts that harboured an established infection. Because vertical transmission cannot be negative, we set the term $(I_E - 2)$ to 0 if I_E was inferior to 2. The number 2 in the denominator accounts for the initial presence of 2 infected carriers. We estimated the absolute number of infected host in every microcosm by multiplying the proportion of infected paramecia in our sample by the total number of paramecia in the microcosm. Note that, on day 6, hosts with an established infection (I_E) represent carrier cells and their vertically infected descendants. Conversely, hosts with a new infection (I_N) represent the newly horizontally infected cohort and, if they have divided after infection, their vertically infected descendants (see Figure S1).

For all analyses, R and prevalence were $\log_{10}(x + 1)$ -transformed so as to better comply with the model's assumptions (Crawley 2007). These variables were analysed with generalised linear models assuming a Poisson distribution, using the log link and controlling for overdispersion. Repeating these analyses using linear mixed models, with normal error distributions, gave the same qualitative results (Table S1). The host genotypes and parasite isolates were treated as fixed factors because they were not randomly pooled out of a population of genotypes (as random factors are), but chosen for their known phenotypic differences. Experimental plate was included in all models. In alternative models we included population density as a covariate to investigate the influence of host population dynamics on epidemiology. Finally, we employed linear regression models to investigate the relationship between short and longer term parasite spread. All analyses were carried out using JMP 6.0.3 (SAS Institute 2006).

RESULTS AND DISCUSSION

Short-term dynamics show carrier genotype effect

After 6 days (no more than one round of horizontal transmission), most populations had gone through 2–3 doublings (average population size = 96.6 ± 23 SD). The parasite effective reproductive rate (R) ranged between 0 and 11.2 among all microcosms, indicating considerable variation among replicate populations in the initial spread of the parasite. All three experimental factors – carrier genotype, recipient genotype and parasite isolate – influenced R , although not independent of each other (Fig. 1, Table 1). Overall, the short-term spread of the parasite was greatest with VEN carriers, but this depended on the identity of the recipient host and the parasite isolate. Indeed, estimated R was greatest for recipient VEN populations when exposed to VEN carriers, but lowest when exposed to K8 carriers (Fig. 1a). Similarly, R was highest for P.K8 parasites when carried by VEN 'carriers', but lowest when carried by K8 hosts. This highlights that the carrier genotype is the determining factor for R , hence for the initial establishment of the parasite. Analyses of proportion of infected hosts (i.e. prevalence) gave similar results because R and prevalence after 6 days were highly correlated (Figure S2). Comparison with parasite-free controls (mean 101.8 ± 21.8 SD) showed that parasite presence had no detectable

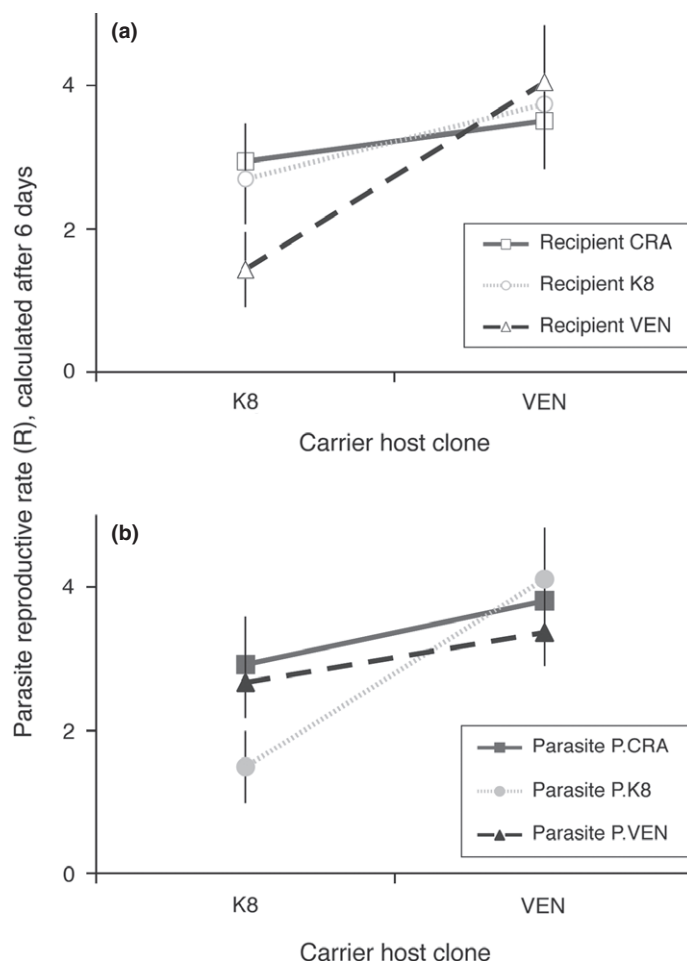


Figure 1 Parasite reproductive rate (R), reflecting its short-term spread, as a function of carrier host clone and (a) the recipient host clone and (b) parasite isolate. Note that proportion of infected hosts (i.e. prevalence) produce similar patterns. Symbols indicate means, vertical bars are standard errors.

Table 1 Final minimal statistical models, after backward elimination of non-significant terms

Trait	Factors	d.f.	χ^2	P value
Parasite reproductive rate (R) after 6 days ($\log_{10} X + 1$ transformed)	Experimental plate	8	19.5	0.012
	Carrier clone	1	8.61	0.003
	Recipient clone	2	9.43	0.009
	Parasite isolate	2	3.05	0.218
	Carrier clone \times recipient clone	2	12.7	0.002
	Carrier clone \times parasite isolate	2	11.8	0.003
Proportion of infected hosts after 21 days ($\log_{10} X + 1$ transformed)	Experimental plate	8	16.4	0.037
	Carrier clone	1	13	< 0.001
	Recipient clone	2	13	< 0.001
	Parasite isolate	2	26.3	< 0.001
	Parasite isolate \times recipient clone	4	21.8	< 0.001
	Parasite isolate \times carrier clone	2	15.2	< 0.001

effect on host population density ($P > 0.1$), which was only significantly explained by recipient genotype ($F_{2,146} = 78.1$; $P < 0.001$), but not by parasite isolate or carrier genotype.

The introduced carriers transmitted the parasite both vertically and horizontally (see Figure S1). On day 6, we detected daughter cells of the carriers with vertically transmitted infections (2.8%) and horizontal infections resulting from the ingestion of infectious forms by recipient hosts (5.1%). That is, about a third of the transmission originating from carriers was vertical, to which may be added the vertical transmission due to the (unquantified) division of horizontally infected hosts. In our system, these vertical components of parasite transmission are thus non-negligible, especially during population growth. However, including population density in models explaining R did not change the results. This indicates that variation in growth rate did not explain the observed differences in R . Moreover, it is important to note that our results are robust to analyses using only horizontally infected *Paramecium* to estimate R , i.e. analyses that excluded vertically infected *Paramecium* arising from division of the carriers (Table S2).

Our study demonstrates how the genetic identity of the infected host that carried the parasite to the parasite-free population can influence short-term parasite spread. The role of such carriers relates to several other epidemiologically important phenomena. Carriers can be compared with vectors of infectious diseases since they both ensure the transfer of parasites between patches of resources. However, carriers are from the same species as the recipient whereas vectors are from a different species, which potentially follow different demographic and evolutionary trajectories. Like carriers here, vectors can exhibit genetic variation in their ability to transmit parasites (e.g. Yan *et al.* 1997; Lambrechts *et al.* 2006a, 2009) with obvious implications for disease dynamics (Schmid-Hempel & Koella 1994; Salvadon *et al.* 2008; Wolinska & King 2009). Our results also parallel a study on plasmid transfer within bacterial populations, where the presence of bacterial strain with a high donor rate (called ‘amplifier strain’) accelerated the spread of conjugative plasmids within heterogeneous communities of bacteria (Dionisio *et al.* 2002). In addition, the influence of carrier genetic identity can also be compared with situations where most transmission is caused by relatively few individuals (so-called ‘super-spreaders’ or ‘super-shedders’) resulting in greater rates of parasite transmission than would be predicted by using mean R_0 values (Lloyd-Smith *et al.* 2005; Matthews & Woolhouse 2005). Taking into account such heterogeneities in transmission potential among hosts, and their origins, allows for a better understanding of parasite epidemiology and informs on potential strategies for disease control (Woolhouse *et al.* 1997; Lloyd-Smith *et al.* 2005; Matthews & Woolhouse 2005; Cook *et al.* 2007). In some cases, variation in individual transmission has been shown to be due to the network structure of social interactions (e.g. Lurie *et al.* 2003; Drewe 2010). Here, our results shed new light on some of the potential genetic underpinnings of such variation, namely the role of carrier genotype.

The importance of carrier variability is obvious in a meta-population context, as carriers transport infection between populations. We found here that some carrier genotypes spread infection more than others when arriving in a new population. However, this may only highlight one of multiple facets of the identity of carrier genotype. Indeed, genetic variation can also exist for infected carriers leaving a population (i.e. dispersal) (Fellous *et al.* 2011). Carriers more likely to disperse may not necessarily be better at travelling long-distances or better spreaders upon arrival in another population. Therefore, knowledge about the genetic variation and covariation of carrier properties will be important for understanding disease spread in spatially structured populations.

Longer term dynamics show the importance of parasite genotype

Between the first and second time point (day 21), the parasite had the opportunity to complete 1–2 further cycles of horizontal transmission (both from infected carriers and infected recipients) in addition to the vertical transmission due to the division of carriers and horizontally infected recipients. The resulting prevalence, the proportion of infected hosts, on day 21 was strongly influenced by parasite isolate as demonstrated by populations with P.CRA parasites containing the greatest proportion of infected hosts (Table 1, Fig. 2). To some degree, the influence of parasite isolate depended on the recipient genotype. Indeed, prevalence with P.CRA parasites was highest in VEN recipients, intermediate in K8 recipients and lowest in CRA recipients (Fig. 2a). The parasite isolate effect also interacted with carrier genotype (Table 1, Fig. 2b), although this effect turned out to be non-significant ($P > 0.1$) when host population density was added to the statistical model (all other terms remained significant). Overall, density was still not significantly different between infected and uninfected populations ($P > 0.1$).

Although the short-term dynamics revealed a significant role of carrier genotype, the longer term patterns were characterised by the emergence of parasite isolate effects and $\text{Genotype}_{\text{Recipient}} \times \text{Genotype}_{\text{Parasite}}$ interactions. This shift towards such well-known genetic

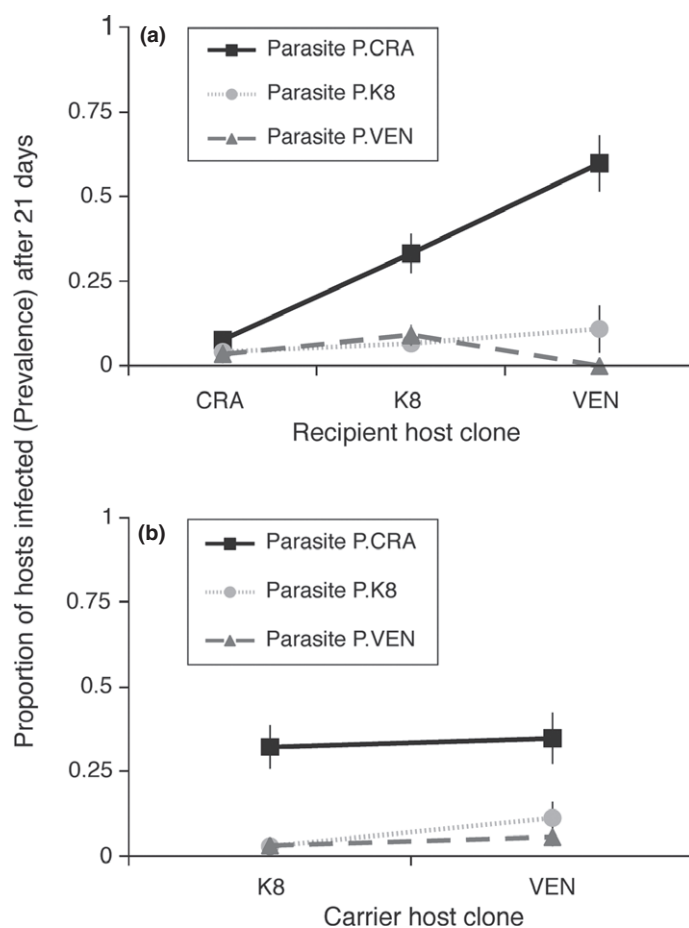


Figure 2 Longer term spread of the parasite estimated by the proportion of infected hosts after 21 days (i.e. prevalence) as a function parasite isolate and (a) recipient host clone and (b) carrier host clone. Symbols indicate means, vertical bars are standard errors.

influences (Carius *et al.* 2001; Lambrechts *et al.* 2006b; Nidelet & Kaltz 2007; Salvaudon *et al.* 2007; Lambrechts 2010) illustrates that the influence of the carrier may be transient and most influential on the initial establishment of infection in a population.

What constitutes a good *Holospira* carrier?

If initial establishment of the parasite is contingent on carrier genotype, knowledge about hosts that are the best carriers may be helpful for limiting disease spread between populations. In the present case, two hypothetical mechanisms may underlie variation in carrier success, both related to the quantity of infectious forms released (in the environment) for horizontal transmission.

First, as the infectious forms of the parasite are released when infected hosts divide, populations in which infected hosts divide more would have the greatest parasite spread. However, estimates of division rate of infected carriers were not correlated with R on day 6 (Figure S3). Second, carriers in which the parasite produces higher parasite loads may release more infectious forms. We estimated parasite load by measuring the size of the parasite-filled micronuclei of hosts with established infection (e.g. Restif & Kaltz 2006; Nidelet *et al.* 2009). This variable differed significantly among parasite isolates ($F_{2,27} = 7.16$; $P = 0.003$), but there was no significant correlation between parasite load and R after 6 days (Figure S4).

These results do not support the idea that short-term parasite spread (R) was greater when the shedding of infectious forms was more frequent (i.e. no correlation with carrier division rate) or when the parasite produced more infectious forms (i.e. no correlation with parasite load). These two potential mechanisms would have influenced transmission intensity with respect to the *quantity* of propagules released per infected carrier. Alternatively, carrier success may have been affected by the *quality* of the propagules (i.e. their intrinsic properties). Evidence for this possibility comes from a study by Magalon *et al.* (2010), investigating the evolution of vertical and horizontal transmissibility in *H. undulata*. They found that one selection treatment produced parasites with smaller parasite loads; however, a dose-controlled inoculation experiment showed that these parasites were also more infectious on a *per-capita* basis. These observations suggest that transmission efficiency in this system can indeed be increased through selection on the quality of the infectious forms, not only on to their quantity.

An interesting possibility, potentially related to the question of propagule quality, is that parasite strains may adapt to their carrier hosts before they arrive in recipient populations, hence affecting their transmission success from their carrier. We tested this hypothesis, but found no supporting evidence. A preliminary experiment (E. Quillery, A. Duncan, S. Fellous and O. Kaltz, unpublished data) showed significant differences in infectivity among the parasite isolates according to their original hosts encountered during the 7-month period of divergence ($F_{2,22} = 8.26$, $P = 0.0021$). However, there was no effect of the carrier clone, on which these isolates were cultured for 2.5 months prior to our experiment ($F_{1,14} = 1.34$, $P = 0.2664$), and thus no evidence for adaptation to the carrier. The significant main effects of parasite origin in the present experiment (Table 1) further support the idea that parasite variability was shaped by the host clone they originally infected and not by the clone of the carrier.

Relationship between short- and long-term dynamics

As shown above, host and parasite identities had contrasting effects on short- and long-term dynamics (Table 1, Figs 1 and 2). We further tested for a quantitative link between these two aspects by regressing the mean prevalence per treatment after 6 and 21 days (Fig. 3). The relationship was positive, but not significant ($F_{1,16} = 2.3$, $P = 0.15$, $R^2 = 0.13$), thus showing that the initial processes captured by our analysis after 6 days had a limited influence on the long-term fate of the parasite. This was also confirmed by a more detailed analysis, in which both time points were included in the same model and that revealed interactions between the time point and the genetic factors (Tables S3 and S4).

It might be expected that the first transmission events favoured the long-term invasion of the parasite, as greater initial infection levels can maximise transmission to the remaining non-infected hosts. However, this was not the case (Figs 3 and S5), possibly because the epidemiological process becomes increasingly complex and is altered by the onset of parasite virulence and specific interactions that occur between host and parasite genotypes (Carius *et al.* 2001; Lambrechts *et al.* 2006b; Salvaudon *et al.* 2007; Nidelet *et al.* 2009; Lambrechts 2010). After the first recipient hosts were horizontally infected by the parasites released by carriers, the following round of horizontal infection was determined by the intensity of parasite transmission achieved by parasites in hosts of the recipient genotype. This potentially explains why the parasite's longer term success was more dependent on parasite isolate and on its interaction with resident genotype ($G_{\text{recipient}} \times G_{\text{parasite}}$ interaction; Figs 2 and 3) than on carrier genotype.

In this study, within-population variation of the host was not considered. However, variation in disease resistance is ubiquitous in the wild (Laine *et al.* 2011). Especially in the context of a meta-populations, where both infected carriers and non-infected hosts disperse between host populations. It is likely that epidemiological outcomes will differ, for example, if resistant hosts or heterogeneous host populations limit pathogen dispersal (Laine *et al.* 2011).

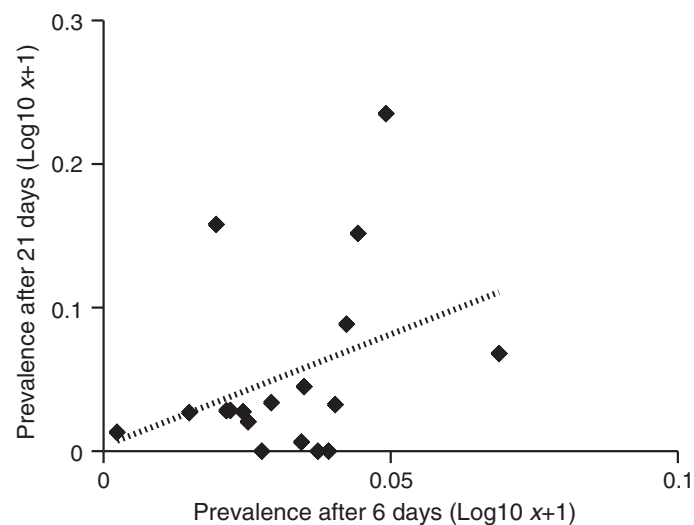


Figure 3 Relationship between proportion of infected hosts (i.e. prevalence) after 6 and 21 days. Statistics for the regression line: $R^2 = 0.13$, $F_{1,16} = 2.3$, $P = 0.15$. A quadratic term did not significantly improve the fit.

Future experiments introducing infected carriers into heterogeneous recipient populations would allow testing these important aspects.

General conclusions

We provide novel empirical evidence that the initial spread of a parasite in an uninfected population depends on the genotype of the infected host that introduces the pathogen. Such carrier hosts, whose role relates to the ones of super-spreaders and vectors, are thus key players when parasite dispersal between patches is based on host movement rather than the displacement of free infectious forms. Previous study in this system has shown that parasite and host genotypes can also affect the dispersal of infected hosts between patches, and thus the frequency of invasion events (Fellous *et al.* 2011). Indeed, the spatial epidemiology of several infectious diseases has been shown to relate to host locomotion (reviewed in Rohani & King 2010). For host species organised in meta-populations, identifying good carriers and dispersers should improve the prediction of disease spread between groups of hosts. Our experiment together with a rapidly expanding number of empirical and theoretical studies, underline the complexity of epidemiological prediction in space and the necessity to simultaneously consider population structure as well as host and parasite genetic properties.

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AUTHOR CONTRIBUTION

SF, ABD, EQ, PFV and OK designed the research; SF, ABD, EQ and OK carried out the experimental work; SF and OK analysed the datasets; SF, ABD, PFV and OK wrote the manuscript.

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