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Host heterogeneity affects both parasite transmission to and fitness on subsequent hosts

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Summary

Infectious disease dynamics depend on the speed, number and fitness of parasites transmitting from infected hosts (‘donors’) to parasite-naïve ‘recipients’. Donor heterogeneity likely affects these three parameters, and may arise from variation between donors in traits including: (i) infection load; (ii) resistance; (iii) stage of infection; and (iv) previous experience of transmission. We used the Trinidadian guppy, *Poecilia reticulata*, and a directly transmitted monogenean ectoparasite, *Gyrodactylus turnbulli*, to experimentally explore how these sources of donor heterogeneity affect the three transmission parameters. We exposed parasite-naïve recipients to donors (infected with a single parasite strain) differing in their infection traits, and found that donor infection traits had diverse and sometimes interactive effects on transmission. First, although transmission speed increased with donor infection load, the relationship was non-linear. Second, while the number of parasites transmitted generally increased with donor infection load, more resistant donors

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transmitted more parasites, as did those with previous transmission experience. Finally, parasites transmitting from experienced donors exhibited lower population growth rates on recipients than those from inexperienced donors. Stage of infection had little effect on transmission parameters. These results suggest that a more holistic consideration of within-host processes will improve our understanding of between-host transmission and hence disease dynamics.

Introduction

Understanding how multiple within-host processes interact to determine variation in between-host parasite transmission remains a fundamental and largely outstanding challenge in epidemiology and disease ecology [1, 2]. Epidemics such as HIV/AIDS, gonorrhoea and SARS, in which a minority of 'superspreading' infected hosts ('donors') are responsible for the majority of transmission events, highlight the importance of such heterogeneity between donors [3-9]. In the context of host-to-host parasite transmission, variation in at least four 'infection traits' can contribute to donor heterogeneity: infection load, resistance, stage of infection, and previous experience of transmission. These components of donor heterogeneity have the potential to affect the speed at which transmission occurs ('transmission speed') [10-12], the number of parasites transmitting ('transmission load') [9, 12-16], and the fitness of transmitted parasites (defined here as the instantaneous population growth rate) [17], and thus the progression of epidemics. These infection traits are also fundamental for evolutionary dynamics, determining the strength of selection, the evolutionary response and thus the evolutionary trajectories of both host and parasite [18-20]. It is therefore important to investigate how the potentially interactive effects of donor infection traits, driven by within-host processes, contribute to variation in these between-host transmission parameters [1, 2].

While still poorly understood, variation in infection load is the best-studied and most intuitive source of donor heterogeneity [1]. In order to quantify infection load, some studies use an instantaneous measure (e.g. [10, 17]), whereas others use the area under the curve of infection load over the whole course of an individual's infection ('infection integral' e.g. [11]). Although these two metrics may sometimes be highly correlated, we argue that for many disease systems, they describe different, potentially uncorrelated, aspects of within-host processes: donors with low instantaneous loads could go on to develop heavy loads, and vice-versa. We therefore here explore the contribution of both the donor's instantaneous infection load ('donor infection load'), and its infection integral (as a measure of resistance, following [21]) to variation in transmission parameters.

Both donor infection load and infection integral are often positively correlated with transmission speed [10-12], and load [12, 14-16], although the shapes and generality of these relationships remain unclear [1]. Intuitively, the more parasites a host has, the larger the number that can potentially transmit to a new host. However, in many systems this relationship may be more nuanced, for example because parasite dispersal rates may depend on individuals balancing the costs of density-dependent resource competition with the benefit of increased mating opportunities [22-24]. Similarly, donor infection integral (our measure of 'resistance') may often be positively correlated with transmission load, but can also be seen as a measure of a host's quality from the parasite's perspective. Parasites may be less likely to transmit from a less resistant host that provides the quantity or quality of resources necessary to sustain high parasite growth rates [23, 25][Forbes et al., this issue], but such a relationship is likely only detectable while controlling for a donor's instantaneous infection load.

The fitness of transmitted parasites, defined here as the instantaneous population growth rate, may also be affected by the infection load or resistance of the previous host. For example, donors with

heavy infection loads could be infected with and therefore transmit faster growing parasite strains [7, 12, 17], or they may transmit less fit parasites due to increased resource competition [26, 27]. Resistant donors may transmit slower growing parasites: those that were directly damaged by the host's immune response [13], or parasite genotypes that have reduced fitness associated with the cost of avoiding damage from that immune response [17].

Other, largely neglected, sources of donor heterogeneity may contribute to the variation in transmission parameters. One such is the timing of the transmission event during the donor's infection (e.g. early or late stage of infection) which, for many infections, encompasses variation in the strength of the donor's immune response, infection load, symptoms and behaviour, as well as the demography of the infecting parasites [10, 13, 17, 28-31]. This potentially important source of donor heterogeneity remains poorly studied, but does appear to affect transmission: the time between trypanosome infection of donor bumblebees and transmission to the recipient affects parasite establishment success on the recipient [13]. Simiarly, entomopathogenic nematodes extracted from caterpillars early in infection are larger and better able to establish infection in new hosts than those extracted late in infection [27]. Additionally, experience of transmitting an infection ('transmission experience') may contribute to donor heterogeneity by changing the interaction between the donor and its parasites, and the behaviour of both organisms in ways that alter the speed, number, or fitness of the parasites transmitting during subsequent transmission events. The number of transmission events experienced by an individual will depend on the rate at which it contacts others, which is highly variable in natural populations [3, 5, 32-35]. Highly connected individuals, simply by virtue of these connections, may give rise to superspreading events that accelerate epidemics [4, 5, 7, 36]: superspreaders do not necessarily differ from the rest of the population in their infection characteristics [36] (although this is common [7]). Despite the obvious importance of these superspreaders, the present study is, to our knowledge, the first to quantify how multiple transmission parameters are affected by donor experience; previous studies

using a ‘contact tracing’ approach have considered only binary outcomes (i.e. transmission or no transmission [5, 8, 35, 37]).

Donor heterogeneity may thus result from variation in at least four related components: infection load, resistance, stage of infection, and donor experience. We used the guppy *Poecilia reticulata*-*Gyrodactylus turnbulli* host-parasite system to experimentally explore how these four components affect transmission speed and load, and the subsequent fitness of transmitted parasites. This system has a number of features that make it ideal for studying transmission. First, ectoparasitic *G. turnbulli* feed and reproduce on host skin, and their abundance is easily monitored through time using non-destructive methods [30, 31]. Second, because the parasite can reproduce asexually, experimental strains can be founded by single individuals, meaning variation among experimentally infected donors in their infection traits, and the fitness of transmitted parasites, is unlikely caused by profound genetic differences between the parasites. Third, individual guppies differ markedly in their ability to limit the population size and growth rate of *G. turnbulli* [30, 38, 39]. Fourth, transmission events are experimentally tractable because individual parasites move between hosts during social contact [30, 40]. In this experiment we took advantage of these features to expose parasite-naïve recipients to donors (all infected with a single parasite strain) differing in their infection traits. Our results reveal that donor infection traits have important and, in some cases, interactive effects on parasite transmission.

Materials and Methods

General experimental design

We experimentally explored how heterogeneity between donors in four infection traits (infection load, resistance, stage of infection and transmission experience) contributes to variation in three transmission parameters: transmission speed, transmission load, and transmitted parasites fitness

(figure 1). The experiment was built around natural variation in donor resistance, which we quantified as the integral of infection load over the course of the infection (or the observation period if this was shorter). The infection integral thus captures in a single value both the duration and intensity of infection [21]. For donor infection load we used the number of parasites on the donor on the day of transmission, and both donor stage of infection and transmission experience were experimentally manipulated. We infected naïve donors, monitored their infection load through time, and exposed them to naïve recipients during the late stage of infection (single donors), or at both early and late stages of infection (double donors; figure 1). Thus, during the late stage of infection, double donors had previous experience of transmission whereas single donors did not; this comparison allowed us to test for an effect of transmission experience. We measured transmission speed as the number of days before transmission occurred, and transmission load as the number of parasites transmitting from donors to recipients. We estimated the fitness of the transmitted parasites by calculating the instantaneous growth rate of the parasite population on the recipient during the first 12 days of its infection. Instantaneous growth rate was calculated as $r = \frac{\ln N_{Day\,12} - \ln N_{Day\,1}}{12}$, where N is the number of parasites on the recipient [31].

Fish origin and maintenance

The experimental fish were laboratory-bred, parasite naïve descendants of guppies collected from the Lower Aripo River, Trinidad in 2007, and maintained at the University of Exeter, UK. In 2012, approximately 1000 fish were used to found a population at Cardiff University, UK, where they were housed at 25°C±1°C, on a 14h Light:10h Dark schedule (overhead fluorescent lighting), and fed daily with live *Daphnia* sp. and flake food (Aquarian®).

Donor infection and parasite screening

On Day 0 of the experiment, 65 female guppies (mean standard length [mm]±SE: 17.5±0.4) were haphazardly selected and infected. The experimental *G. turnbulli* strain (*Gt3*) used was founded by

a single parasite from an ornamental guppy in 1997, and has since been maintained on an inbred ornamental guppy stock ('culture fish'). To infect experimental donors, culture fish were killed using an overdose of tricaine methanesulfonate (MS222; PHARMAQ UK, Ltd.). Donor fish were anesthetized with 0.02% MS222. Under a dissecting microscope, the tails of the culture and donor fish were placed in close proximity until two individual parasites, each pregnant with a mid-term embryo [31], had transmitted. Infected donor fish were revived, placed in individual 1 L tanks, and maintained under standard conditions (as above). Water in each tank was changed every other day. We monitored the infection trajectory of experimental donor and recipient fish by mildly anesthetizing each fish (0.02% MS222) and counting the number of *G. turnbulli* every other day throughout the course of infection ('screening'). This method also exposed the parasites to MS222, but the frequency of exposure was standardised across fish for all experimental factors, and previous work indicates that such brief exposure to low doses of anaesthetic has negligible effects on *Gyrodactylus* spp. parasites ([41] and JC, unpublished data).

Experimental procedure

Building upon natural variation in resistance among the 65 experimental donors, we incorporated donor infection load at time of transmission, stage of infection and donor experience into the experimental design as follows. We divided the donors into two groups. One group transmitted parasites to recipients only at the 'late' stage of their infection, while the other group transmitted to recipients at both the 'early' and 'late' stages of their infection. Two time points were selected as representative of these infection stages: Day 5 and Day 12. On Day 5 in this system the parasite is established but infection loads tend to be low and relatively uniform, whereas by Day 12 infection loads are highly variable among hosts (e.g. [42]). For 'double donors' (n = 48), a naïve recipient fish was added to the tank on Day 5 and Day 12, whereas for 'single donors' (n = 17), a naïve recipient fish was added to the tank on Day 12 only (figure 1). At Day 5 (n = 48) and Day 12 (n = 57; all donors minus double donors that had lost their infection by Day 12 [n=3], or were

accidentally omitted [n=3]), naïve female recipients were size matched within 2 mm (recipient mean standard length [mm]±SE: 17.5±0.4) to the donor and placed in the donor holding tanks. We excluded data from four experimental pairs in which the recipient did not become infected (two pairs at Day 5, two at Day 12). Each pair of fish was screened for transmission every 24 hours, but because of the generation time of *G. turnbulli* (24-48 hours at 25°C; [31]), these data could not indicate the number of parasites lost from the donor. Further, the data could not be used to discriminate between the number of parasites transmitting directly from the donor, and those born on the recipient within 24 hours of transmission. As variation in the population growth rate was not associated with the number of parasites transmitting or donor stage of infection (as described in the results section), however, we consider this uncertainty to affect all experimental pairs equally. When transmission occurred, the recipient was isolated, its experimental time set to Day 1, and it was screened every other day up to Day 30.

Data Analysis

All statistical analyses were conducted in R (3.0.2; [43]), and we provide the data, script and output of the analyses in electronic supplements S1 & S2. During data exploration, the highest correlation coefficient we found between our continuous dependent variables was $r = 0.35$ (for donor integral and donor infection load), and we therefore include all of these in our starting models. Although donors had significantly higher infection loads in late than early infection (mean difference = 14.29; $t_{59.1} = 4.26$; $p < 0.001$), we included both stage of infection and infection load in the starting models to test whether there were any effects of stage of infection on our response variables that could not be explained by infection load alone. There was no difference in infection load between experienced and inexperienced donors at day 12 ($t_{44.34} = -0.77$; $p = 0.44$).

We used transmission speed (number of days until transmission occurred), transmission load (the number of parasites transmitting from the donor to recipient), and fitness of the transmitted

parasites (instantaneous population growth rate over the first 12 days of the recipient's infection) as response variables in models with the four components of donor heterogeneity as explanatory variables. Transformation of the explanatory variables, the error family and link function were chosen to optimise the fit of each model independently (see table 1). For donor load, resistance and stage of infection, we used the data from all transmission events (labelled A in figure 1), and ran either general or generalised linear mixed models (GLMM, depending on error family and link function; in the lme4 [44] and glmmADMB packages [45]) with donor identity as a random effect to account for non-independence of early and late transmissions by double donors. To test for the effects of donor experience (controlling for both donor load and resistance) on each transmission parameter we ran either general or generalised linear models (GLM, again depending on error structure, using R [43] and the MASS package [46]) using only data from transmission events from donors late in infection (labelled B in figure 1).

All six starting models (using either all data or only data from late infection transmission events, and one for each transmission parameter [speed, load, transmitted parasite fitness]) contained donor infection load at time of transmission and donor resistance (the infection load integral) as continuous fixed effects. Because fish size is often identified as an important determinant of infection dynamics in this system [42, 47], and the size difference between fish often affects how they interact [48, 49], we additionally included the standard length of the recipient, and the size difference between the donor and recipient as continuous fixed effects in all models. All analyses began with a full model with two-way interactions between fixed effects. The full models were reduced using backward stepwise deletion of non-significant terms to minimise Akaike's Information Criterion (AIC), following the drop1 function in the lme4 package [44].

Results

Our results reveal that donor heterogeneity has strong effects on the three transmission parameters: transmission speed, load and transmitted parasite fitness. The more heavily infected a donor on the day of recipient exposure (donor load), the faster transmission occurred, but the relationship was non-linear (models 1 [all data] and 4 [late infection transmission events only] in table 1; figure 2). We confirmed that this result is not simply a sampling artefact associated with the Poisson distributions of the predictor and response variables (further analyses described in S1). The data additionally suggest a 'transmission threshold' of ca. 40 parasites; transmission took longer than one day in 12.5% of trials above this donor infection load threshold, compared to 55.7% of trials below this threshold (figure 2).

The number of parasites transmitting depended principally on the donor's infection load at transmission, but this effect varied with donor resistance (models 2 and 5 in table 1; figure 3). While more resistant donors transmitted more parasites with increasing infection loads, less resistant donors (those with high infection integrals) tended to transmit relatively few parasites, regardless of their loads at the time of transmission. We also found weak evidence that donors transmitted more parasites at the later stage of infection (model 2 in table 1).

Among late-stage transmission to recipients added at Day 12, donors with transmission experience transmitted more parasites than those without experience (model 5 in table 1; figure 4a). Although this result is only marginally significant ($p = 0.03$), the effect size is substantial: in the raw data, experienced donors transmitted on average 3.1 parasites more than inexperienced donors. Donor experience is also the only variable that explains a significant amount of variation in the fitness of the transmitted parasites (models 3 and 6 in table 1). Parasites transmitted by experienced donors were significantly less fit (showed slower population growth over the first 12 days on the recipient) than those transmitting from inexperienced donors (model 6 in table 1; figure 4b). This effect was dramatic: parasite populations transmitted by experienced donors were equally likely to increase or

decrease in size, but those from inexperienced donors almost exclusively increased over the first 12 days on the recipient (figure 4b). We found no evidence that the size of the recipient, or the difference between donor and recipient size affected any of our transmission parameters (all $p > 0.05$).

Discussion

Our results reveal that donor heterogeneity arising from variation in infection load, resistance, stage of infection, and transmission experience, affect transmission speed, transmission load and the fitness of transmitted parasites in complex ways. Heavily infected donors transmitted infection more quickly, but the relationship was not linear (figure 2). The donor's instantaneous infection load also predicted the number of parasites transmitting ('transmission load'), but this relationship was more nuanced than commonly assumed: the least resistant donors (those with the highest infection integrals) transmitted fewer parasites, and their transmission loads increased little with infection load (figure 3). This result suggests that the widely held assumption that infection load and transmission load are positively correlated may actually depend on donors' ability to limit parasite population growth. Additionally, we found that donors with transmission experience transmitted more parasites, but that the parasites transmitted by such hosts were less fit on the recipient (figure 4). We discuss the potential mechanisms and implications of these three results in turn.

Transmission speed increased with donor infection load, but the relationship was not linear. This nonlinearity indicates that the increase in infectiousness was not simply a result of there being more parasites and thus a higher probability of some transmitting. Instead, it appears that the host-parasite interaction changes, encouraging parasites to transmit, once a certain infection load is reached. In our data, there appeared to be a threshold of *ca.* 40 parasites, above which transmission rarely took longer than one day. Hendrichsen et al [50] found a similar pattern among Atlantic salmon infected with *G. salaris*. The existence of a threshold infection load above which transmission is rapid may

286 therefore be a pattern common to this genus, and suggests that *Gyrodactylus* spp. transmission is
287 density-dependent.
288
289 The number of parasites transmitting increased with donor infection load, but our results suggest the
290 relationship is more complex than commonly assumed [1][McCallum et al, this issue]. While
291 empirical studies support the assumption that donor infection load and transmission load are
292 positively correlated (e.g. [9, 12, 14-17]), it is becoming increasingly clear that factors other than
293 donor infection load should be considered. For example, pathogen genotype [12, 17], co-infection
294 [51], the donor's stage of infection [13, 27], parasite age [15] and ecological interactions between
295 parasites within a host [22, 24] are all known to affect the number of parasites transmitting. To this
296 list we add the donor's ability to limit parasite growth, i.e. resistance. In our data, for a given
297 infection load, less resistant donors (i.e. those with high infection integrals) transmitted fewer
298 parasites. The distributions of donor loads and integrals underlying this pattern show the over-
299 dispersion typical of host-parasite systems, with relatively few donors exhibiting high infection
300 loads and integrals (figure 3). Given that the few heavily infected hosts in a population are
301 commonly assumed to be the superspreaders, that the number of parasites these hosts transmit is
302 affected by their infection integral is a key result: the sparseness of high load, high integral
303 observations is expected, and should not lead to a downplaying of their fundamental importance.
304
305 The importance of the infection integral over the full duration of a donor's infection (up to 30 days)
306 to the number of parasites transmitting relatively early in infection (mean day of infection on which
307 transmission occurred = 10.7) suggests that the parasites are able to detect and respond to
308 differences in resistance between fish before these are evident in differences in infection load. We
309 found only weak support for donors later in infection transmitting more parasites, which perhaps
310 indicates that these changes happen before Day 5. Potential mechanisms of resistance that could
311 provide cues to the parasite include changes in the pH, chemical composition, or quantity of the

mucous [52]. This result may therefore support the hypothesis that gyrodactylids leave hosts when conditions are, or are likely to become, unfavourable [30], i.e. transmission may be condition- as well as density-dependent. Corroboratively, donors with high infection integrals are those that are most profitable, and hence the parasites are less likely to leave such hosts [23, 25]. These fish may also have been unable to maintain social behaviours that promote transmission, and may have displayed sickness behaviours [33, 53] or released cues that elicited avoidance behaviours in recipients [54]. Such avoidance would reduce the number of parasites able to transmit, as has been demonstrated theoretically [34, 55] and empirically [56, 57].

While it seems likely that heavily infected donors transmit more parasites because more parasites leave these hosts, as described in other systems [9, 12, 13, 51], we cannot rule out an alternative explanation. We were unable to quantify the number of parasites lost by the donor during transmission, so our results may reflect a difference in the quality of these parasites: donors with fewer parasites, or higher infection integrals, may release poorer quality parasites that are less likely to attach to the recipient, and that therefore go unrecorded. Data collected by Scott and Anderson [30] provide partial support for this idea, but further empirical work is needed to rigorously test this hypothesis. Our experiment therefore subsumes the effects of variation in exposure to parasites in our measure of transmission load, but we acknowledge that a recipient's infection load after exposure to a given number of infectious particles is complex, and depends in part on its geno- and phenotype [58, 59]. More generally, considering exposure and susceptibility as separate aspects of disease transmission has been shown to improve the performance of transmission models [60].

We found that donors with transmission experience transmitted more parasites, but that once transmitted to the recipient, these parasites grew more slowly than those from donors without experience. Although we only tested the effect of a single previous transmission event, our result suggests that sequential transmission events may increase the number, but reduce the fitness of

parasites transmitted by donors. The mechanisms driving the effects of donor experience on transmission load and transmitted parasite fitness are unclear. Behaviour may be important: variation in donor behaviour as a result of infection can alter its likelihood of transmitting [33, 61]. In this system donors gain both therapeutic (i.e. a temporary reduction in infection load) and evolutionary benefits (i.e. increased relative fitness) from transmission, so donors may learn to modify their behaviour to increase transmission rates. Indeed, infected guppies often swim in close proximity to others and attempt to initiate body contact ([62], JFS personal observation).

It is possible that changes in the host-parasite interaction resulted in donors with prior experience transmitting more, slower growing, parasites [31, 50]. The extra days with a companion during the experiment may have reduced the stress response of double donors relative to single donors [63], enabling them to mount a more effective immune response [64]. Although during post hoc tests we did not see an effect of the number of experimental days donors spent with recipients on either transmission parameter, a more effective immune response would result in a more hostile environment for the parasite, potentially explaining both why more parasites transmitted, and why parasites from double donors were less fit. Alternatively, in this issue Leggett et al [‘Fast killing..’] demonstrate that low host availability (such as in our single donor treatment) promotes high levels of within-host competition, favouring parasites that maximise host exploitation rather than transmission. Conversely, high host availability favours slower growing, more transmissible parasites [Leggett et al ‘Fast killing..’], which is the pattern we see in the double donor treatment. Such effects could act within or across parasite generations, and be due to parasite plasticity [65] or genetic effects [66] (though the latter may be less likely here, given the highly inbred parasite strain we used).

In conclusion, our results indicate that heterogeneity in infection load, resistance and transmission make diverse and in some cases complex, interactive contributions to variation in the speed, number

and fitness of parasites transmitting. We found little support for an effect of the donor's stage of infection on transmission, suggesting that donor experience and infection load, which were both associated with stage of infection, explained most of the variation that would otherwise have been attributed to this factor. Our results support the common assumption that heavily infected donors contribute disproportionately to epidemics, but show that donor resistance and transmission experience can modulate this relationship substantially. Transmission load may be particularly important to the success of transmission in natural settings where transmission is risky for *Gyrodactylus*: about 60% of parasites leaving the donor fail to infect a recipient [30]. Although a single gyrodactylid parasite is sufficient to establish an infection, the more individuals that attempt to transmit, the higher the probability of one successfully establishing on a recipient host, similar to the 'infective dose' of single-celled pathogens [26, 58, 59]. Donor heterogeneity may continue to have an effect on epidemic progression even after successful establishment of the parasite on the recipient, however, as parasite fitness on the recipient depends on the previous host [27]. Parasite growth rate is often correlated with virulence (i.e. the damage inflicted on the host) [Leggett et al 'Growth rate…'], so this result implies that the host from whom an infection is acquired may affect the severity of the infection on the subsequent host. While the mechanisms behind these findings require elucidation, this study further validates recent calls for more holistic consideration of the effects of within-host processes on between-host transmission [McCallum et al, this issue][1, 2].

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Ethics

This work was conducted under UK Home Office license (PPL 30/2876) with approval by the Cardiff University Animal Ethics Committee.

Data Accessibility

The dataset supporting this article is provided as part of the Supplementary Material.

Authors' Contributions

JC and SEP designed the experiment; JC and JF collected the data; JFS and KAY conceived the study and with JJ analysed and interpreted the data; JFS wrote the manuscript with substantial input from KAY. All authors contributed to revisions, gave final approval for publication, and agreed to be accountable for all aspects of the work.

Competing Interests

We have no competing interests.

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596

Tables

Table 1

model	data	response variable	error family (link function)	explanatory variable	estimate	SE	test statistic	*p*-value
1	all transmission events, donor identity as random factor	transmission speed	Poisson (log)	log(donor load)	-0.19	0.07	-2.81 (*z*)	0.005
2		transmission load	Negative binomial (log)	stage of infection (late)	0.27	0.15	1.72 (*z*)	0.085
				donor load	0.03	0.004	7.18 (*z*)	<0.0001
				donor integral	0.27	0.23	1.19 (*z*)	0.236
				donor load: donor integral	-0.01	0.005	-2.63 (*z*)	0.009
3		initial parasite growth rate on the recipient	Gaussian (identity)	none remained after model simplification	-	-	-	-
4	late infection transmission events only (recipient added on day 12)	transmission speed	Poisson (log)	log(donor load)	-0.16	0.08	-2.01 (*z*)	0.044
5		transmission load	Negative binomial (square-root)	donor load	0.04	0.008	5.61 (*z*)	<0.0001
				donor integral	0.53	0.29	1.82 (*z*)	0.069
				donor experience (yes)	0.44	0.20	2.17 (*z*)	0.030
				donor load: donor integral	-0.03	0.008	-3.72 (*z*)	0.0002
6		initial parasite growth rate on the recipient	Gaussian (identity)	donor experience (yes)	-0.25	0.08	-3.11 (*t*)	0.003

Table 1. Results from the final, simplified models described in the main text (with further details of the full analyses in S1). ‘Stage of infection’ denotes which day of infection, 5 (early) or 12 (late), the recipient was added to the donor tank; ‘donor load’ is the number of parasites on the donor at transmission; ‘donor integral’ is the area under the curve of donor infection load over the course of its infection (or the 30 day observation period if this was shorter); ‘donor experience’ denotes whether or not the donor had previously transmitted infection to a recipient. ‘log(donor load)’ is the natural log of the number of parasites on the donor at transmissi

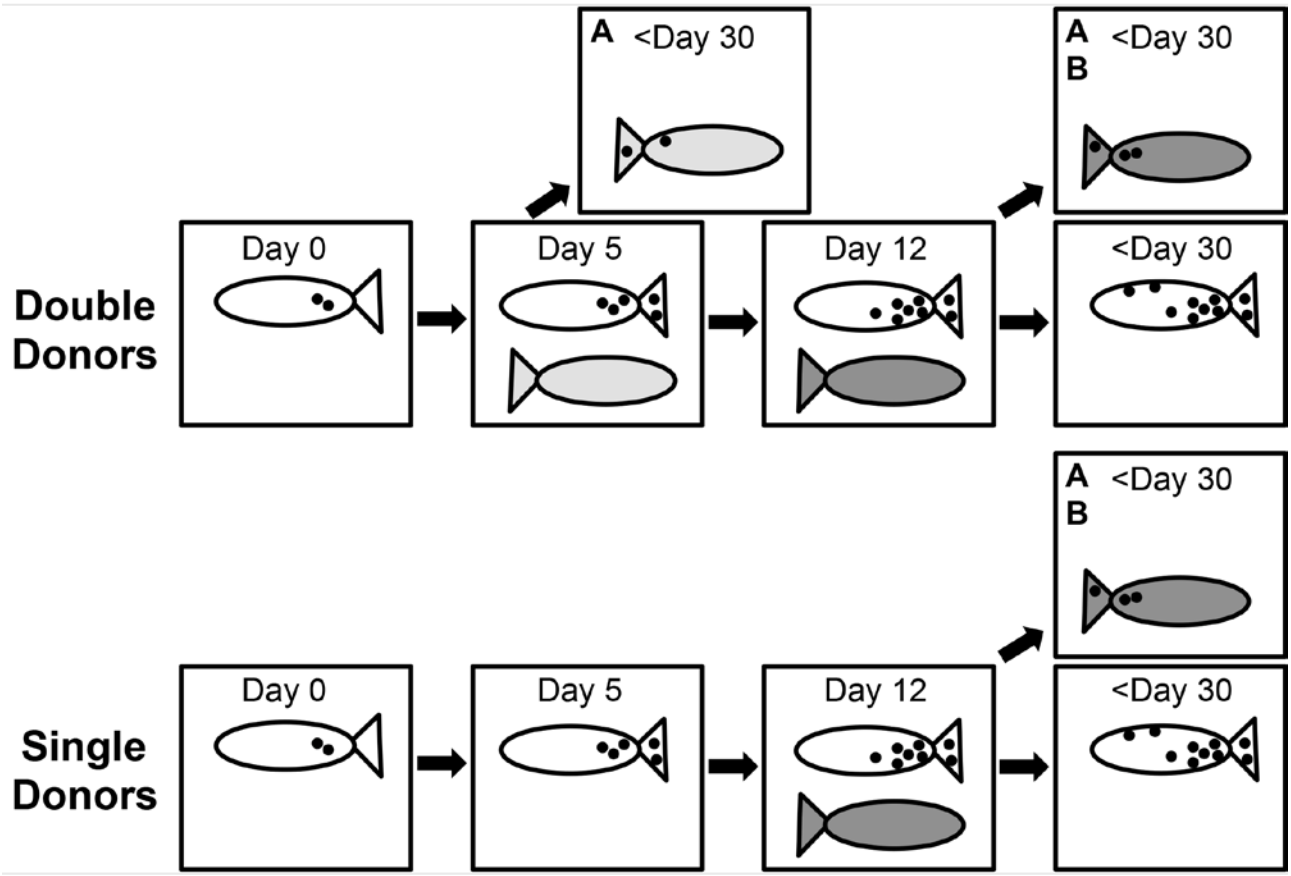


Figure 1. Diagram of the transmission experiment design. At Day 0, all donors (unshaded) were isolated and infected with two individual *Gyrodactylus turnbulli* (black dots). Their infection was monitored every other day for 30 days. At Days 5 (double donors only) and 12 (all donors), *G. turnbulli*-naïve recipients (light grey shading for Day 5, dark grey for Day 12) were added to the donor tanks. Both fish were screened for infection every 24 hours. Once a recipient had become infected, it was isolated and its infection monitored every other day for 30 days. **A:** Data from these recipients were used to test the role of donor heterogeneity in infection load, resistance and stage of infection on the speed, number and fitness of parasites transmitting to recipients (see table 1). **B:** Data from these recipients were used to test the hypothesis that a donor's previous experience of transmission affected the parameters of subsequent transmission events.

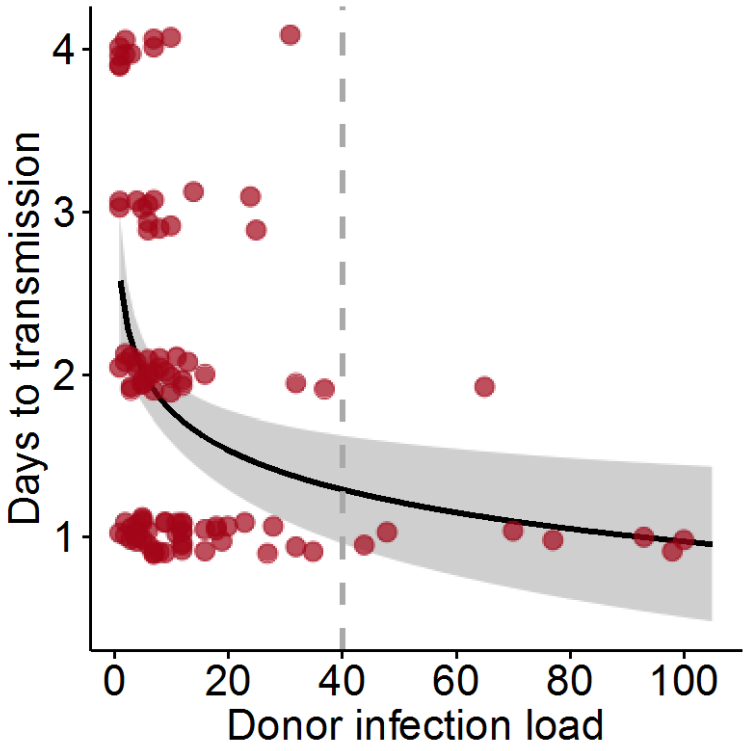
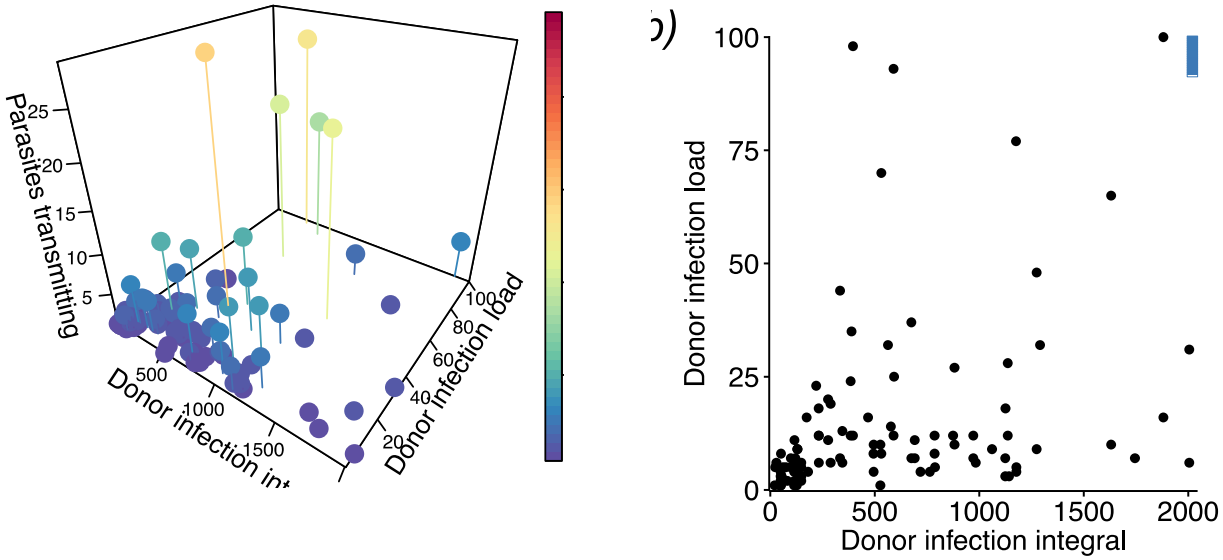


Figure 2. The speed of parasite transmission increased with the infection load of the donor. The solid line shows the values predicted by the final model, the shading around it the standard error. The dashed line highlights an apparent threshold of 40 parasites (see main text for details).



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628

629 **Figure 3.** The number of parasites a donor transmitted increased with its infection load (the number
630 of parasites it had at transmission), but the strength of this relationship depended on the donor's
631 resistance, or ability to limit the growth of the parasite population. The less resistant the donor, the
632 higher its infection integral (the area under the curve when infection load is plotted over the time
633 course of the infection, or the 30 day observation period if this was shorter), and the fewer parasites
634 it transmitted to the recipient for a given infection load. Panel (*a*) shows the raw data, with points
635 coloured according to the number of parasites transmitted, as shown by the scale bar; panel (*b*)
636 shows the raw data (black points) laid over the number of parasites transmitted predicted by the
637 final model, again shown by the scale bar.

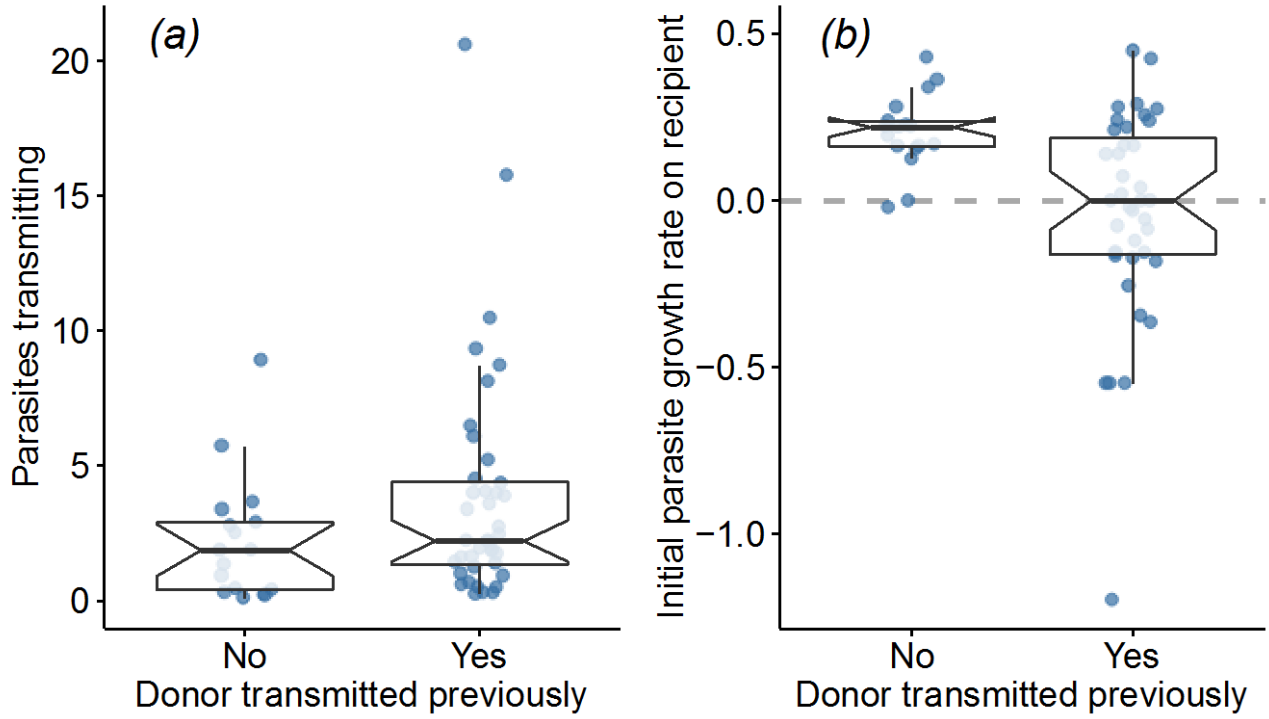

Figure 4. Donors that had transmitted parasites to a recipient earlier in their infection transmitted more parasites than those without transmission experience (*a*), but these were less fit, i.e. exhibited lower population growth rates over the first 12 days on the recipient (*b*) than parasites transmitting from inexperienced donors. Panel (*a*) shows the partial residuals of the donor experience term in model 5 in table 1, and thus the effect of donor experience on the number of parasites transmitting independent of the other terms in the model. The dashed line on (*b*) marks a growth rate of 0, and highlights that while parasite populations transmitted by experienced donors were equally likely to increase or decrease in size, those from inexperienced donors almost exclusively increased over the first 12 days on the recipient.