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

33

## 34 **Introduction**

35

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

52

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

63

64 Both donor infection load and infection integral are often positively correlated with transmission  
65 speed [10-12], and load [12, 14-16], although the shapes and generality of these relationships  
66 remain unclear [1]. Intuitively, the more parasites a host has, the larger the number that can  
67 potentially transmit to a new host. However, in many systems this relationship may be more  
68 nuanced, for example because parasite dispersal rates may depend on individuals balancing the  
69 costs of density-dependent resource competition with the benefit of increased mating opportunities  
70 [22-24]. Similarly, donor infection integral (our measure of 'resistance') may often be positively  
71 correlated with transmission load, but can also be seen as a measure of a host's quality from the  
72 parasite's perspective. Parasites may be less likely to transmit from a less resistant host that  
73 provides the quantity or quality of resources necessary to sustain high parasite growth rates [23,  
74 25][Forbes et al., this issue], but such a relationship is likely only detectable while controlling for a  
75 donor's instantaneous infection load.

76

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

79 heavy infection loads could be infected with and therefore transmit faster growing parasite strains  
80 [7, 12, 17], or they may transmit less fit parasites due to increased resource competition [26, 27].  
81 Resistant donors may transmit slower growing parasites: those that were directly damaged by the  
82 host's immune response [13], or parasite genotypes that have reduced fitness associated with the  
83 cost of avoiding damage from that immune response [17].

84  
85 Other, largely neglected, sources of donor heterogeneity may contribute to the variation in  
86 transmission parameters. One such is the timing of the transmission event during the donor's  
87 infection (e.g. early or late stage of infection) which, for many infections, encompasses variation in  
88 the strength of the donor's immune response, infection load, symptoms and behaviour, as well as  
89 the demography of the infecting parasites [10, 13, 17, 28-31]. This potentially important source of  
90 donor heterogeneity remains poorly studied, but does appear to affect transmission: the time  
91 between trypanosome infection of donor bumblebees and transmission to the recipient affects  
92 parasite establishment success on the recipient [13]. Similarly, entomopathogenic nematodes  
93 extracted from caterpillars early in infection are larger and better able to establish infection in new  
94 hosts than those extracted late in infection [27]. Additionally, experience of transmitting an  
95 infection ('transmission experience') may contribute to donor heterogeneity by changing the  
96 interaction between the donor and its parasites, and the behaviour of both organisms in ways that  
97 alter the speed, number, or fitness of the parasites transmitting during subsequent transmission  
98 events. The number of transmission events experienced by an individual will depend on the rate at  
99 which it contacts others, which is highly variable in natural populations [3, 5, 32-35]. Highly  
100 connected individuals, simply by virtue of these connections, may give rise to superspreading  
101 events that accelerate epidemics [4, 5, 7, 36]: superspreaders do not necessarily differ from the rest  
102 of the population in their infection characteristics [36] (although this is common [7]). Despite the  
103 obvious importance of these superspreaders, the present study is, to our knowledge, the first to  
104 quantify how multiple transmission parameters are affected by donor experience; previous studies

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

107

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

124

## 125 **Materials and Methods**

126

### 127 *General experimental design*

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

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

146

#### 147 *Fish origin and maintenance*

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

153

#### 154 *Donor infection and parasite screening*

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

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

170

### 171 *Experimental procedure*

172 Building upon natural variation in resistance among the 65 experimental donors, we incorporated  
173 donor infection load at time of transmission, stage of infection and donor experience into the  
174 experimental design as follows. We divided the donors into two groups. One group transmitted  
175 parasites to recipients only at the 'late' stage of their infection, while the other group transmitted to  
176 recipients at both the 'early' and 'late' stages of their infection. Two time points were selected as  
177 representative of these infection stages: Day 5 and Day 12. On Day 5 in this system the parasite is  
178 established but infection loads tend to be low and relatively uniform, whereas by Day 12 infection  
179 loads are highly variable among hosts (e.g. [42]). For 'double donors' (n = 48), a naïve recipient  
180 fish was added to the tank on Day 5 and Day 12, whereas for 'single donors' (n = 17), a naïve  
181 recipient fish was added to the tank on Day 12 only (figure 1). At Day 5 (n = 48) and Day 12 (n =  
182 57; all donors minus double donors that had lost their infection by Day 12 [n=3], or were



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

195

#### 196 *Data Analysis*

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

206

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

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

221

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

232

233 **Results**

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

243

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

250

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

260 decrease in size, but those from inexperienced donors almost exclusively increased over the first 12  
261 days on the recipient (figure 4b). We found no evidence that the size of the recipient, or the  
262 difference between donor and recipient size affected any of our transmission parameters (all  $p >$   
263 0.05).

264

## 265 **Discussion**

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

278

279 Transmission speed increased with donor infection load, but the relationship was not linear. This  
280 nonlinearity indicates that the increase in infectiousness was not simply a result of there being more  
281 parasites and thus a higher probability of some transmitting. Instead, it appears that the host-parasite  
282 interaction changes, encouraging parasites to transmit, once a certain infection load is reached. In  
283 our data, there appeared to be a threshold of *ca.* 40 parasites, above which transmission rarely took  
284 longer than one day. Hendrichsen et al [50] found a similar pattern among Atlantic salmon infected  
285 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

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

320

321 While it seems likely that heavily infected donors transmit more parasites because more parasites  
322 leave these hosts, as described in other systems [9, 12, 13, 51], we cannot rule out an alternative  
323 explanation. We were unable to quantify the number of parasites lost by the donor during  
324 transmission, so our results may reflect a difference in the quality of these parasites: donors with  
325 fewer parasites, or higher infection integrals, may release poorer quality parasites that are less likely  
326 to attach to the recipient, and that therefore go unrecorded. Data collected by Scott and Anderson  
327 [30] provide partial support for this idea, but further empirical work is needed to rigorously test this  
328 hypothesis. Our experiment therefore subsumes the effects of variation in exposure to parasites in  
329 our measure of transmission load, but we acknowledge that a recipient's infection load after  
330 exposure to a given number of infectious particles is complex, and depends in part on its geno- and  
331 phenotype [58, 59]. More generally, considering exposure and susceptibility as separate aspects of  
332 disease transmission has been shown to improve the performance of transmission models [60].

333

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

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

345

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

361

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

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

382

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386

### 387 **Ethics**

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



390

391 **Data Accessibility**

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

393

394 **Authors' Contributions**

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

399

400 **Competing Interests**

401 We have no competing interests.

402

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407

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596

597 **Tables**598 **Table 1**

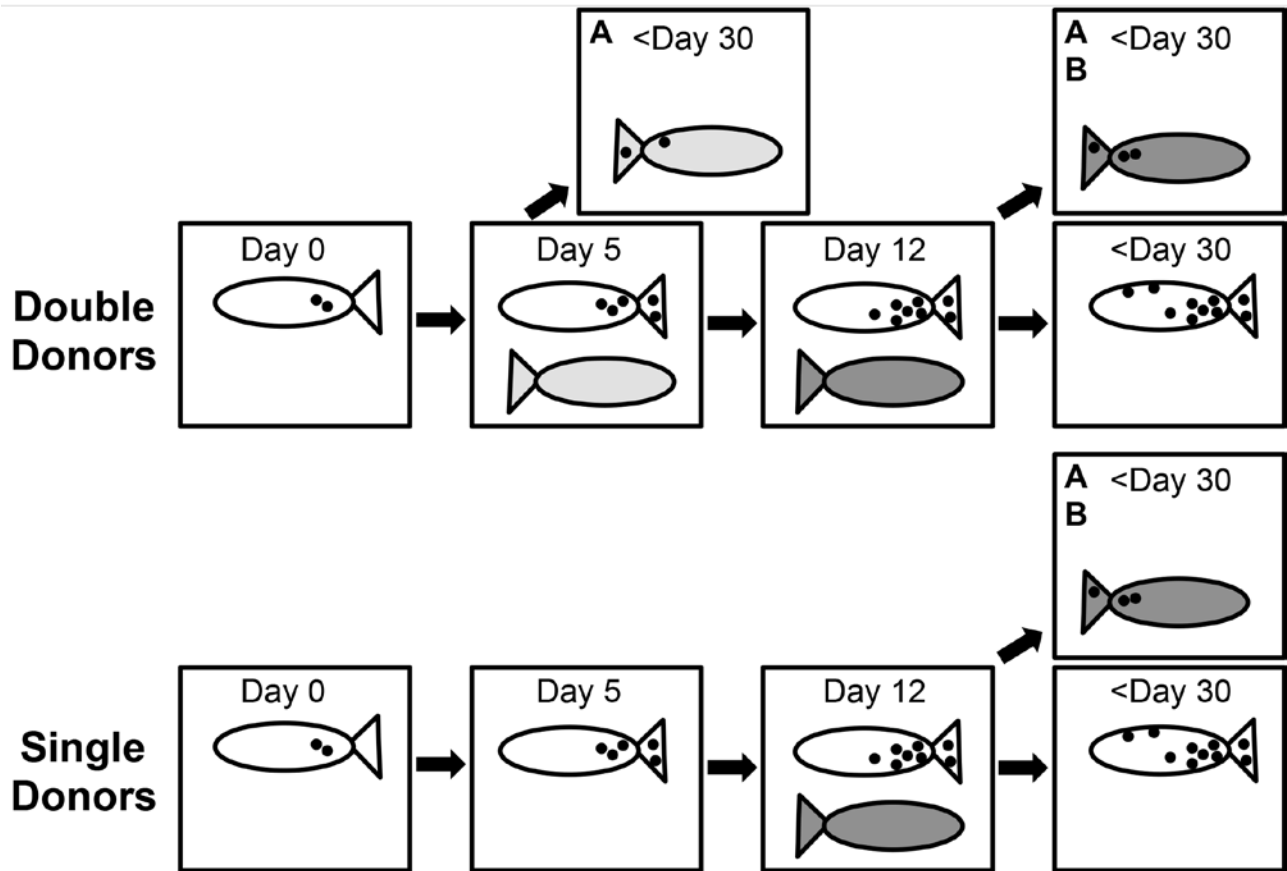
599

model	data	response variable	error family (link function)	explanatory variable	estimate	SE	test statistic	p-value
1	all transmission	transmission speed	Poisson (log)	log(donor load)	-0.19	0.07	-2.81 (z)	0.005
2	events, donor identity as random factor	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 speed	Poisson (log)	log(donor load)	-0.16	0.08	-2.01 (z)	0.044
5	transmission events only (recipient added on day 12)	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

600

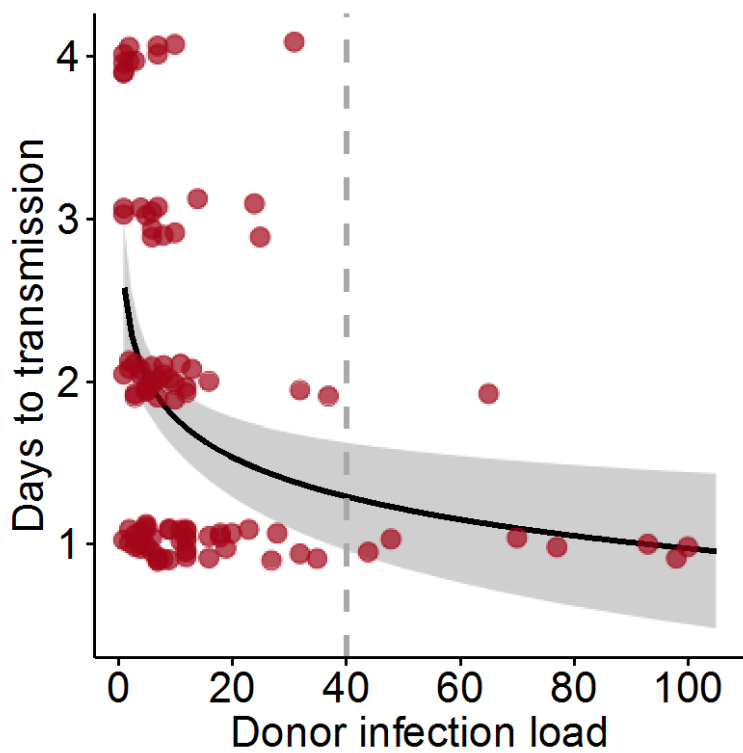
601

602 **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’  
603 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  
604 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  
605 this was shorter); ‘donor experience’ denotes whether or not the donor had previously transmitted infection to a recipient. ‘log(donor load)’ is the  
606 natural log of the number of parasites on the donor at transmissi



608

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



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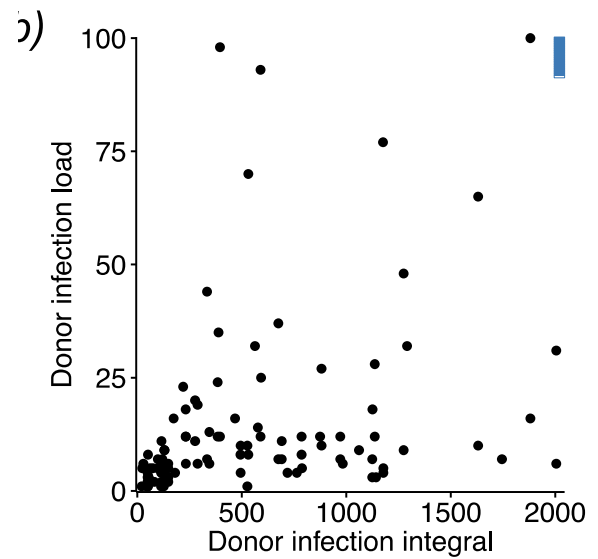
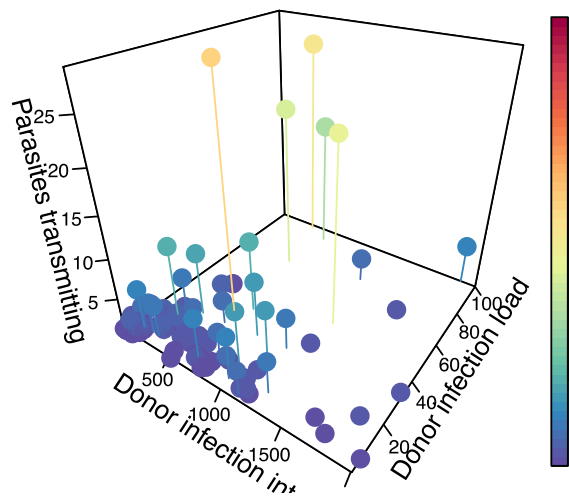
621 **Figure 2.** The speed of parasite transmission increased with the infection load of the donor. The  
622 solid line shows the values predicted by the final model, the shading around it the standard error.

623 The dashed line highlights an apparent threshold of 40 parasites (see main text for details).

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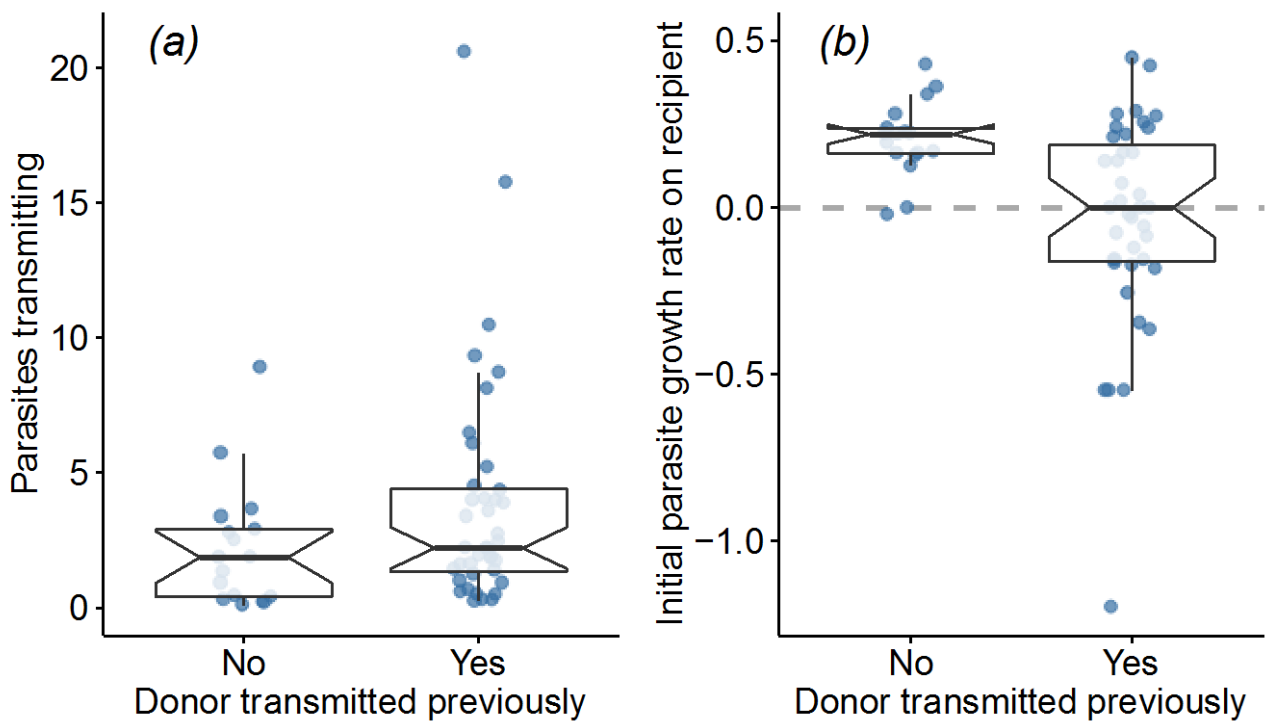
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629 **Figure 3.** The number of parasites a donor transmitted increased with its infection load (the number  
 630 of parasites it had at transmission), but the strength of this relationship depended on the donor's  
 631 resistance, or ability to 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.



638

639

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

649