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A symbiont's dispersal strategy: condition-dependent dispersal underlies predictable variation in direct transmission among hosts

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Direct horizontal transmission of pathogenic and mutualistic symbionts has profound consequences for host and symbiont fitness alike. While the importance of contact rates for transmission is widely recognized, the processes that underlie variation in transmission during contact are rarely considered. Here, we took a symbiont's perspective of transmission as a form of dispersal and adopted the concept of condition-dependent dispersal strategies from the study of free-living organisms to understand and predict variation in transmission in the cleaning symbiosis between crayfish and ectosymbiotic branchiobdellidan worms. Field study showed that symbiont reproductive success was correlated with host size and competition among worms for microhabitats. Laboratory experiments demonstrated high variability in transmission among host contacts. Moreover, symbionts were more likely to disperse when host size and competition for microhabitat created a fitness environment below a discrete minimum threshold. A predictive model based on a condition-dependent symbiont dispersal strategy correctly predicted transmission in 95% of experimental host encounters and the exact magnitude of transmission in 67%, both significantly better than predictions that assumed a fixed transmission rate. Our work provides a dispersal-based understanding of symbiont transmission and suggests adaptive symbiont dispersal strategies can explain variation in transmission dynamics and complex patterns of host infection.

1. Introduction

Understanding how symbiosis is maintained in natural populations requires, perhaps most importantly, an understanding of how symbionts are transmitted throughout host populations and the factors that influence individual transmission events [1,2]. Classic and contemporary models of transmission typically focus on the factors that operate at the level of the host population and influence the frequency of dispersal opportunities for symbionts such as host contact rates and symbiont prevalence. These models characteristically assume a fixed probability of transmission during host contact (i.e. a fixed dispersal strategy) [3–5]. While this assumption makes transmission models tractable, transmission processes are probably more complex and nuanced than is generally recognized [6]. For directly transmitted symbionts, transmission from one host to another is largely synonymous with the dispersal of free-living organisms from one patch to another, and during host contact each individual symbiont makes a decision to disperse, or to stay with their current host. Because dispersal presents risks as well as rewards, indiscriminate dispersal may not be the best strategy for symbiont individuals.

From a symbiont's perspective, a population or community of potential hosts is a heterogeneous and patchy landscape. Hosts often vary in quality across species [7–9] and across individuals within a species [10]. Even at the within-host level, within-host microhabitats or tissues may vary with respect to the resources they

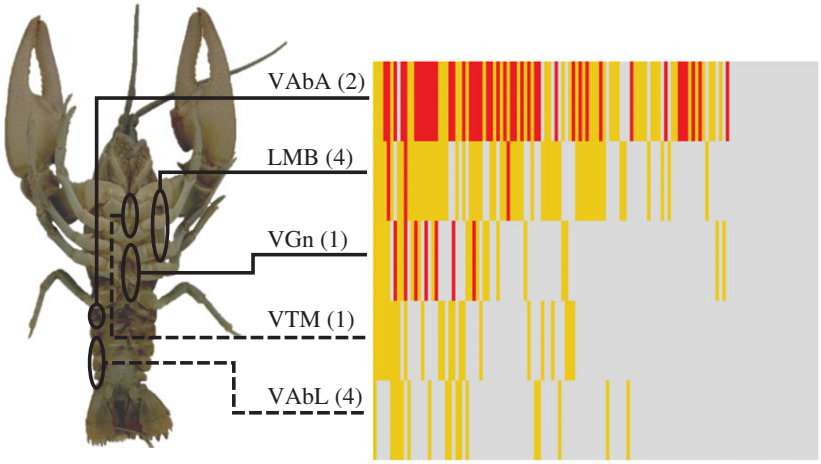

Figure 1. Field observations of microhabitat occupancy and reproductive success of *C. ingens* from 130 crayfish in Sinking Creek, Newport, VA, USA. Open circles delineate the five most commonly occupied microhabitats observed during field study (described in the electronic supplementary material, table S1), numbers in parentheses indicate the maximum number of worms typically found at each microhabitat. Bars show a nested pattern of microhabitat occupancy (rows). Columns represent individual crayfish organized left to right by decreasing total symbiont abundance. Grey bars indicate the absence of worms; gold bars indicate the presence of non-reproducing worms (no cocoons) and red bars indicate reproducing worms (with cocoons). Microhabitats with observed worm reproduction were typically occupied before other microhabitats. Photo credit: Spencer Bell.

offer or the risk of mortality to symbionts [11–13]. Moreover, each host and each microhabitat offers limited resources which are partitioned among symbionts creating the possibility of strong inter- and intraspecific competition among symbionts sharing a host [14–16]. Because hosts are variable in qualities that can directly influence symbiont fitness, it is probable that natural selection favours symbionts that move among hosts discriminately, just as free-living organisms display evolved condition-dependent dispersal strategies [17–19]. We hypothesized that variation in transmission dynamics that occur during pair-wise host encounters can be explained and even predicted by qualities of the host and interactions among symbionts that influence the fitness of individual symbionts, i.e. 'condition-dependent transmission'.

To test the hypothesis of condition-dependent transmission, we examined transmission during pairwise host encounters in the cleaning symbiosis between a freshwater crayfish (*Cambarus sciotensis*) and an ectosymbiotic annelid worm (*Cambarincola ingens*). We conducted a field survey of symbiont reproductive success to estimate the effects of host size and intraspecific symbiont competition for microhabitat on symbiont fitness. We then used estimates of fitness to make specific predictions about the frequency and magnitude of transmission events during pairwise host encounters and then tested our predictions in a laboratory experiment. We compared our predictions of fitness-based symbiont dispersal to a null model that assumed a constant rate of dispersal (i.e. 'fixed dispersal') and incorporated natural variability in the frequency of dispersal during host contacts. Our results refute the common assumption of a fixed symbiont dispersal strategy, and provide a first demonstration of an adaptive condition-dependent symbiont dispersal strategy that can be used to accurately predict variability in transmission dynamics during host contact.

2. Material and methods

(a) Study system

Crayfish throughout North America, Europe and parts of Asia are host to assemblages of obligate ectosymbiotic annelid worms called branchiobdellidans [20]. Several species of these worms provide a beneficial cleaning service to their hosts by feeding on potentially harmful epibiotic accumulations, which has been shown to increase host growth and survival in multiple field and laboratory experiments [21–24]. However, some branchiobdellidan species may be parasitic or commensal symbionts [25–28]; reviewed by Skelton *et al.* [29]. Branchiobdellidans attach to the exoskeletons of their hosts by the way of posterior and anterior duo-gland adhesive organs [30]. These adhesive organs allow branchiobdellidans to tightly attach to their host, but also easily release their grip and move freely across their host's body. Branchiobdellidans have a simple life history with no free-living stage, and available evidence indicates that branchiobdellidans are obligate symbionts with no free-living forms because they require a host to reproduce and require host–host contact for transmission [29,31,32].

All organisms included in this study were collected from Sinking Creek in Newport VA, USA, a mid-order tributary of the New River (37°18′07.0″ N 80°29′14.9″ W; lat/long in DDD). The crayfish fauna at this site was dominated by *Cambarus sciotensis*, and these crayfish support populations of at least five species of branchiobdellidans [33]. In this study, we focused on the crayfish *C. sciotensis* and the branchiobdellidan *C. ingens*. We focused on *C. ingens*, because it is a relatively large and conspicuous branchiobdellidan, which allows for easy and accurate non-destructive detection and experimental manipulation of worm abundance under field and laboratory conditions [21,22,29,34]. Field and laboratory studies were restricted to adult crayfish and sub-adult crayfish, because previous work has shown that smaller juvenile crayfish will often remove branchiobdellidans from their bodies, preventing colonization of worms on young hosts, whereas this response occurs at a much lower frequency in older crayfish [11].

(b) Field study and correlates of symbiont fitness

We collected 130 crayfish in late November 2013 to identify factors that influence symbiont fitness. Collections were limited to an intensive two-week period to avoid known confounding effects of seasonal variability on symbiont reproduction and abundance [35]. Crayfish were collected using seines, placed individually in plastic bags, and transported to the laboratory. Under a stereoscopic microscope, we determined the abundance and microhabitat use of all *C. ingens* on each crayfish (microhabitats illustrated in figure 1

and defined in the electronic supplementary material, table S1), and the number of cocoons containing viable eggs associated with each worm. Branchiobdellidans are simultaneous hermaphrodites that produce egg-containing cocoons which are attached to the host near the symbiont. *Cambarincola ingens* are also cannibalistic and not typically found in close proximity to one another (J.S., R.P.C. and B.L.B. 2014, personal observation). Therefore, cocoons could be reasonably assumed to be produced by adjacent individuals.

We examined three models of individual reproductive success of *C. ingens*: reproduction as a function of host size (model 1; M1), microhabitat (M2) and host size plus microhabitat (M3). Because the number of cocoons observed near each worm may be influenced by other factors such as variation in hatching times, loss of cocoons due to physical disturbance/predation and occupancy time of individual symbionts at the observed location, we used the binary response variable of cocoon presence/absence to assess individual reproductive success. Reproductive success was modelled using binomial general linear models (GLM), with host size as a continuous predictor and attachment site a categorical factor. Observations of worms occupying sites for which reproduction was not observed during the field study were excluded from analysis to avoid zero-inflation of the response variable [36]. Analyses were conducted using the glm function in the R-base package v. 2.15.1 [37].

We used microhabitat occupancy data from the field study to assess competition for preferred microhabitats. *Cambarincola ingens* are known to have strong preference for particular microhabitats [21] and the number of worms that can occupy a given microhabitat and the number of preferred microhabitats is likely limiting because of physical constraints of space, and strong negative intraspecific interactions such as cannibalism. From these premises, we predicted that only the best microhabitats, which represent a subset of all microhabitats, will be occupied on crayfish with few worms. Further, the remaining and less preferable microhabitats will only be occupied when the number of worms present is greater than the number of worms that can be supported by preferred microhabitats. To test this prediction, we constructed a symbiont presence/absence matrix of attachment sites by individual hosts and looked for a pattern of nestedness in symbiont occupancy of microhabitat. Nestedness was quantified as matrix temperature using the *nestedtemp* function in the R-vegan package 2.0–4. The significance of the observed nestedness metric was assessed by comparing observed matrix temperature to 10 000 randomized matrices with constrained row and column sums using the *oecosim* function in the R-vegan package 2.0–4. This type of null simulation provides the most conservative assessment of significance, because it accounts for variation in observed frequency of attachment site use (row sums) and the distribution of symbiont abundance (column sums; [38,39]).

(c) Transmission experiment

We experimentally observed transmission dynamics in 24 pairs of host crayfish. We collected 48 crayfish and removed all of their worms by 5 min immersion in 10% $MgCl_2$ hexahydrate solution [21]. These crayfish were not part of the field survey. We then ablated the dactyls of the first and second perieopods of all crayfish to limit symbiont removal by the host. This measure was taken because crayfish sometimes remove newly colonizing branchiobdellidans [11] which could have caused us to underestimate the frequency of transmission events. We also used elastic bands to bind the chelipeds closed to prevent crayfish from killing or dismembering each other. We then inoculated 24 'donor' crayfish, each with six adult *C. ingens*. We chose six worms per donor because it is well within the naturally observed density of *C. ingens* in the field [9], yet it is high enough to make intraspecific competition for preferred attachment sites likely. We chose to keep the number of worms applied to donors constant across all experimental units to minimize variability in transmission due to variation in worm density and six *C. ingens* per host is typical for adult *Cambarus* hosts in the New River watershed [9,22]. Each donor was then paired with an un-inoculated 'receiver' crayfish for 16 days in 40 l aquaria. Because our goal was to assess the factors that cause symbionts to disperse when given the chance, we used small enclosures to encourage contact between donors and receivers, and eliminate confounding effects of variable contact rates on transmission dynamics. Similarly, 16 days was chosen for experimental duration because it provided ample time for worms to disperse based on our previous experience (J.S., B.L.B. and R.P.C.), but was brief enough to preclude worm reproduction that would could have obfuscated our measurements of transmission. We used a stratified design to pair donors and receivers. Both donors and receivers were classified as either small (less than 35 mm CL) or large (more than 35 mm CL). Our sampling strata consisted of all four pairwise combinations of size classes. Therefore, we had six experimental units from each donor–receiver-size combination. Aquaria contained natural stream substrate, two artificial refugia and were aerated. They were maintained at ambient room temperature (approx. 16°C) and received natural sunlight from nearby windows. Crayfish were fed three to five shrimp pellets twice during the experiment. We recorded the number of *C. ingens* present at each microhabitat on all crayfish on days 1, 3, 6 and 16. The presence of *C. ingens* on the receiver signified successful transmission on day 16, and the magnitude of transmission was taken as the number of worms on the receiver.

We used generalized linear mixed models fit by maximum likelihood (glmer function, family = 'binomial', logit link, R-lme4 package v. 1.1–7; [40]) to identify the factors that best explained the proportion of worms that were transmitted during experimental encounters. Our initial model included donor size, receiver size, time and all interaction terms as fixed effects, and individual crayfish as a random effect. Chi square significance tests were used to sequentially remove model terms that did not improve model fit (drop1 function in the R-base package v. 2.15.3; [37]).

(d) Transmission models and assessment

We used the GLMs of reproductive success in the field and a logit link to estimate the probability of reproductive success for each microhabitat on each donor crayfish. We then combined these estimates with observations of typical maximum occupancy for each attachment site to make a set of predictions of condition-dependent transmission for each model of symbiont fitness (abbreviated as P1, P2 and P3, corresponding to fitness models M1, M2 and M3). We made predictions based on each of the three GLMs, rather than just the best model because we did not know if both factors (host size and microhabitat) were perceivable to the symbiont and thus likely to influence dispersal behaviour. Consequently, the best model for predicting symbiont dispersal could have been a subset of the best model of reproductive success.

Each set of condition-dependent dispersal predictions was made following three rules: (i) symbionts will occupy attachment sites in order of their reproductive fitness values based on GLM estimates from field data, (ii) occupancy at each attachment site is limited to the typical maximum number of symbionts found at that attachment site during the field study, (iii) symbionts that are unable to procure an attachment site with an estimated probability of reproductive success greater than a minimum threshold value (minimum acceptable fitness, MAF) will disperse to the alternative host. Competition for preferable microhabitats was implicit in P2 and P3 because microhabitats were filled to maximum occupancy in order of their fitness value and therefore

Table 1. Summaries of three generalized linear models for the effects of host size and symbiont microhabitat on the probability of symbiont reproduction observed for 300 *C. ingens* from 130 crayfish during our field survey. All terms were highly significant within each model. The model containing both host size and symbiont microhabitat (M3) provided the strongest result based on Akaike information criterion (AIC). Bold values denote *p*-values below 0.05, and lowest AIC for competing models.

model	coefficient	estimate	*z*-value	*p*-value	AIC
M1	intercept	−6.552	−4.679	**<0.0001**	263.65
	host size	0.145	4.126	**<0.0001**	
M2	intercept	−3.1355	−5.318	**<0.0001**	244.09
	VAbA	2.848	4.630	**<0.0001**	
	VGn	2.037	2.777	**<0.01**	
M3	intercept	−10.624	−5.786	**<0.0001**	**221.01**
	VAbA	3.137	4.921	**<0.0001**	
	VGn	1.952	2.584	**<0.01**	
	host size	0.188	4.483	**<0.0001**	

some worms were predicted to experience reduced fitness as a result of conspecifics filling preferable microhabitats. No intra-symbiont competition was implied in P1 because fitness was predicted to be equal across microhabitats and therefore predictions of dispersal were based on host size alone and unrelated to the presence of conspecifics.

We did not have an *a priori* expectation for the exact value of MAF, so MAF was treated as a free parameter and optimized iteratively. The significance of each set of condition-dependent predictions was verified using a conservative null model. For each set of predictions (P1, P2 and P3), we assessed the accuracy of predictions for all possible values of MAF from 0.001 to 1, at increments of 0.001, by comparing predictions to observed transmission events. For each value of MAF, we calculated a 'goodness of fit index' (GFI) to quantify the degree to which experiment-wide predictions deviated from the observed transmission (equation (2.1)). For each experimental unit i, R_o and R_P are the observed and predicted number of worms observed on the receiver at the end of the experiment, and 'Total' is the sum of worms recovered from the donor and the receiver of each tank

$$\mathrm{GFI} = \sum_{i=1}^{n} \frac{(R_{o_i} - R_{p_i})^2}{\mathrm{Total}_i}. \tag{2.1}$$

Thus, GFI equals zero when experiment-wide predictions perfectly match observations, and increases with increasing discrepancy between predictions and observations. To test for significant improvements of the condition-dependent model over a null expectation of an experiment-wide fixed probability of dispersal, we used a one-tailed test of the observed GFI to a null distribution created by matrix permutations of the observed data. The null distribution was created by 10 000 randomized permutations of the observed transmission dynamics in which columns represented donors and receivers, and rows represented experimental units and each cell contained the observed number of worms at the conclusion of the experiment. To minimize the probability of type I error, row sums and column sums were conserved during permutations so that the experiment-wide proportion of worms that dispersed was held constant, as were the number of worms recovered within each experimental unit [38,39]. Because the total number of transmission events is conserved among permutations (column sums), this null model serves as the expectation under the assumption that transmission probabilities are fixed across all host contacts while incorporating the naturally observed variability in transmission events. Each permutation of the original dataset was then used as a null prediction to calculate a GFI score, and GFI scores from all permutations formed the null distribution that we used to assess the one-tailed significance of each set of predictions based on symbiont fitness.

3. Results

(a) Field study

We observed 300 *C. ingens* and 351 cocoons on 130 crayfish. Symbiont occupancy and reproduction varied across microhabitats (electronic supplementary material table S2; figure 1). The highest observed intensity was nine *C. ingens*. The most frequently occupied microhabitat was the most anterior portion of the ventral abdominal surface, abbreviated VAbA (69%; as per cent of crayfish with worms present at that microhabitat), and followed by the lateral margin of the carapace, abbreviated LMB (45.4%), and area around the genitals, VGn (22.3%). The microhabitats, VTM and VAbl, were infrequently occupied (17.7% and 13.8%, respectively). Worms attached at VAbA showed the highest frequency of reproductive success (46.7%; as per cent of worms present that had cocoons), followed by VGn (22.3%) and LMB (5.1%). Worms attached to VTM and VAbL had no observed reproduction in this study. We detected a strong nested pattern of microhabitat occupancy across hosts. Microhabitats in which reproduction had been observed were usually occupied by symbionts, and other sites were typically occupied only when sites with reproduction were also occupied, creating a significantly nested pattern of microhabitat occupancy (observed matrix temperature = 11.455, $z = -3.248$, $p = 0.0007$).

Host size and microhabitat were strong predictors of worm reproductive success, both alone (M1 and M2) and together (M3). All coefficients in all three generalized linear models were highly significant (table 1). AIC indicated that the model that included both microhabitat and host size (M3) was better than the reduced models M1 and M2. A direct comparison of M1 and M2 could not be made using AIC because neither is a nested subset of the other. Generally, the probability of reproductive success increased with host size (M1 and M3) and was highest at attachment site VAbA, followed by VGn and lastly LMB (M2 and M3).

(b) Transmission experiment

All crayfish (24/24) and half of their symbionts (77/144) survived the 16 day experiment. There was a considerable

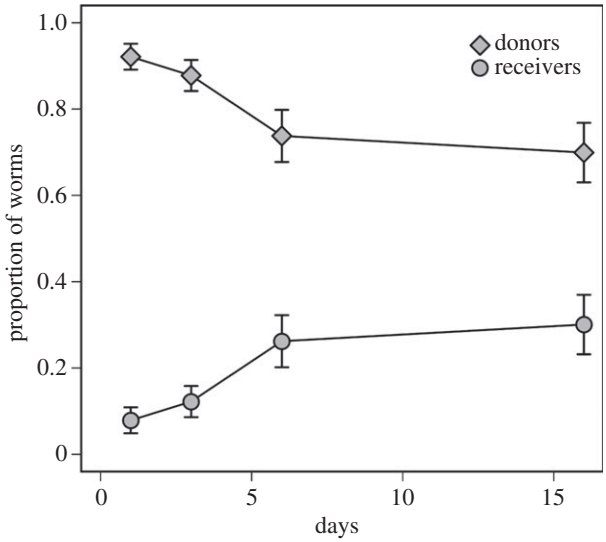

Figure 2. Transmission dynamics during a 16 day experiment. Symbols show the experiment-wide mean proportion of worms present on donors and receivers, error bars represent ± 1 s.e. The proportion of worms present on receivers increased for the first 6 days of the experiment and subsequently changed very little.

exchange of worms from donors to receivers during the first 6 days of the experiment, but little exchange from day 6 to 16 (figure 2). For both donors and receivers, microhabitat occupancy became increasingly similar to the nested pattern observed in the field over the course of the experiment. Although initially the majority of worms were present on the lateral margins of the donors' carapaces, they gradually moved from the donors' carapaces to the anteroventral portion of the donor's abdomen (VAbA), or dispersed to the receivers over the duration of our experiment (figure 3). Once on the receivers, the majority of dispersed worms also moved to the ventral portions of their new hosts (VAbA).

At the end of the experiment, transmission had occurred in 13/21 (61%) of remaining pair-wise encounters. Of the 77 recovered worms, 24 (31%) had transferred from the donor to the receiver. Three experimental units from which no worms were recovered were excluded from further analysis. Model selection recovered a model with significant main effects of donor size (estimate $= -0.138$, s.e. $= 0.053$, $z = -2.602$, $p = 0.009$) and time (estimate $= 0.092$, s.e. $= 0.027$, $z = 3.477$, $p < 0.001$) on the proportion of worms that were transmitted.

After optimization of MAF, predictions based on host size and competition for attachment sites (P3) were significantly better than null predictions based on fixed transmission rates. For this model, MAF was optimized at 0.061–0.068, yielding a significantly lower GFI than null predictions (GFI $= 4.33$, $p = 0.0019$; figure 4). The optimized model correctly predicted the occurrence of transmission in 20/21 (95%) of pairwise encounters and exactly predicted the magnitude of transmission (number of worms that switched hosts) in 14/21 (67%) of experimental encounters. Transmission predictions based on fitness models 1 and 2 failed to predict better than null predictions at $\alpha = 0.05$ for any value of MAF.

4. Discussion

Patch heterogeneity underlies the selective advantages of condition-dependent dispersal strategies [17]. Thus, our hypothesis of condition-dependent transmission is based on the premise that host populations represent a patchy and heterogeneous landscape with respect to factors relevant to individual symbiont fitness. The results of our field study confirmed that premise. Variation in symbiont reproductive success was coupled with variation in host size and intra-symbiont competition for high-quality microhabitats. Positive relationships between host size and symbiont density or biomass are well known, especially in parasite systems (e.g. [41–45]). Similarly, branchiobdellidan and cocoon abundance are frequently correlated with crayfish size [9,11,32]. Several previous studies of branchiobdellidans have speculated that this relationships is driven by factors that influence symbiont fitness and mortality including resource availability, moult frequency, host quality and age-specific host resistance [9,11,29,31,46–48]. Our results provide correlative evidence of a causal relationship between host size and symbiont fitness, and suggest that variation in host size and symbiont loads creates heterogeneity in host quality within a host population.

We also observed within-host heterogeneity in microhabitat quality and evidence of competition for high-quality microhabitats. Microhabitat affinities have been described for many internal and external symbionts [13,49–52], as well as several species of branchiobdellidans [21,29,53]. While interspecific competition and species-specific variation in microhabitat use have been used to explain species coexistence of symbiont taxa in other systems (e.g. [12,13]) our study suggests that intraspecific competition for microhabitat is also an important determinate of individual symbiont fitness. Microhabitats in which symbionts were reproductively successful were typically occupied whenever symbionts were present on a host (i.e. VAbA, VGn and LMB), whereas microhabitats where reproduction was not observed were typically only occupied when preferable sites were already occupied (i.e. VAbL and VTM), resulting in a nested pattern of microhabitat occupancy. Each microhabitat typically supported a limited number of individuals. We have frequently observed cannibalism in *C. ingens*, often involving worms of nearly equal size and strongly suspect this tendency as the reason for the apparent maximum occupancy of each microhabitat. Together, the results of the field survey suggest that a condition-dependent dispersal strategy could provide a selective advantage for branchiobdellidans increasing the probability of locating a high-quality host and avoiding interspecific competition for high-quality microhabitats on heavily infested hosts.

Experimental results showed that transmission among hosts is highly variable, even under controlled laboratory conditions. Because we were specifically interested in the factors that cause a symbiont to disperse, our experiment was explicitly designed to remove barriers to symbiont dispersal by providing long-term (16 days) host–host contact within a confined space (40 l aquaria). Throughout the experiment, we observed crayfish in all tanks frequently contacting each other while exploring and often resting in direct contact. We also limited variation in post-dispersal colonization success by ablating host dactyls and therefore greatly reducing the ability of all hosts to resist colonization [11]. Despite these measures, we observed tremendous variation in the transmission dynamics that occur during experimental host–host encounters. The proportion of symbionts within each host pairing that did disperse to the alternative host varied from 0 to 100%, with no detected transmission in a third of host encounters (7/21, 33%). Repeated observations of host infestation

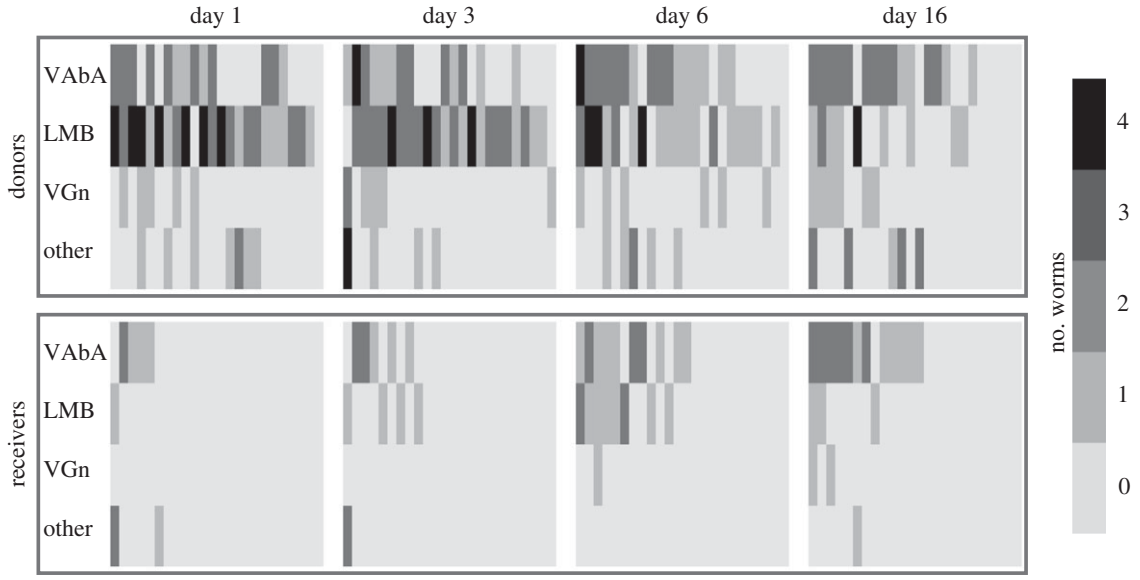

Figure 3. Microhabitat occupancy and transmission dynamics during transmission experiment. For each matrix, rows represent microhabitat and columns represent individual crayfish. Cell colour shows the number of worms present at each microhabitat on each donor (top row of matrices) and receivers (bottom) at four points in time (columns of matrices). The number of worms on the donor's carapace (LMB) decreased as worms either moved to the donor's abdomen (VAbA) or dispersed to the receiver. Worms colonizing receivers also moved from LMB to VAbA through time.

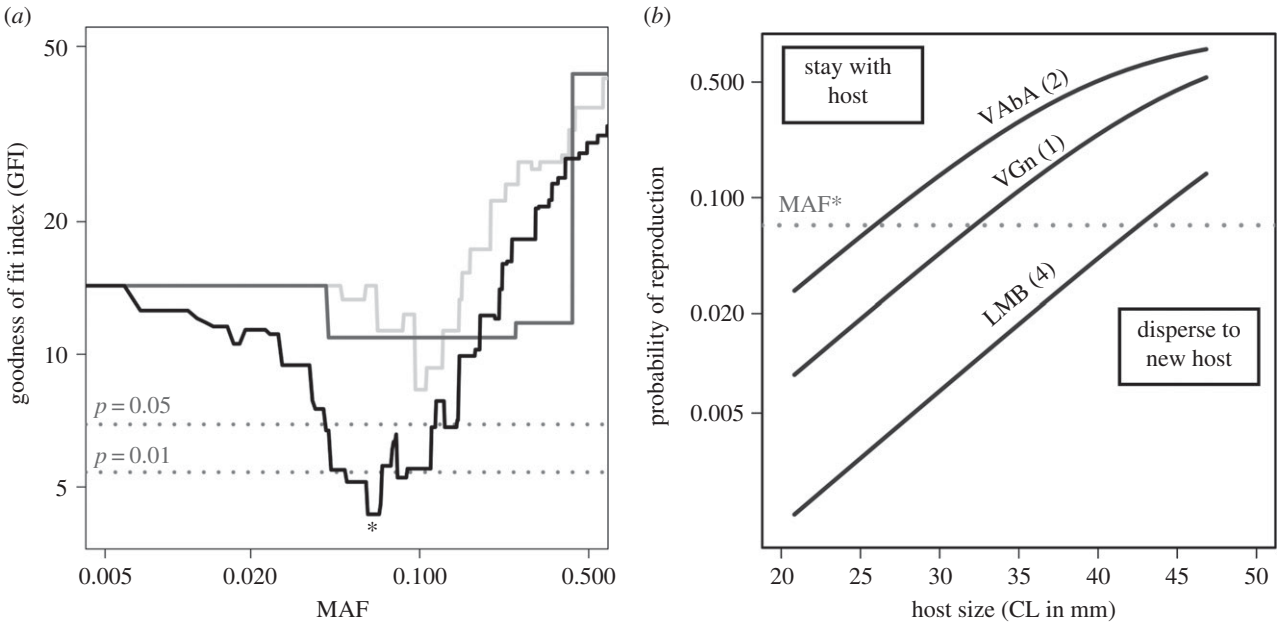

Figure 4. (*a*) Model performance for three competing sets of predictions: P1, light grey; P2, dark grey; P3, black. Horizontal dotted lines represent the lower bounds for one-tailed significance cut-offs for goodness of fit statistic determined by null model permutations ($\alpha = 0.05$ and 0.01). Asterisk denotes optimized value for the assumed minimum predicted probability of reproduction for which symbionts will not disperse (MAF = 0.061–0.068, where $p = 0.0019$). (*b*) Graphical representation of the best predictive model. Solid lines show predicted probability of symbiont reproduction. Numbers in parentheses represent the maximum occupancy of each microhabitat. Optimized value of MAF from the best transmission model is shown as horizontal grey dotted line, labelled as MAF*. Symbionts are predicted to fill microhabitats in the order VAbA, VGn and LMB. Symbionts that cannot obtain a microhabitat with an estimated probability of reproductive success that is greater than MAF* are predicted to disperse.

confirmed that 16 days was sufficient time for dispersing worms to find their new hosts. After an initial 6 days of increase, the number of worms present on the receivers did not vary significantly for the remainder of the experiment.

Applying condition-dependent dispersal theory to symbiont transmission vastly improved our ability to predict highly variable transmission dynamics during host contact. Based on the premise that individual symbionts only emigrate when host size and competition for microhabitats on their current host create a fitness environment below a minimum threshold (MAF), we were able to predict independent transmission events much more accurately than predictions based on a fixed probability of transmission across all host encounters. The best predictions were based on the symbiont fitness model that included both host size and microhabitat (P3), and by model design, implicitly incorporated intra-symbiont competition for preferred microhabitats. Once we optimized the free parameter for MAF, this model correctly predicted the occurrence of transmission in all but one of the experimental host encounters (95%) and

correctly predicted the exact number of dispersing symbionts in most encounters (67%). Competing models based on host size alone, or competition for microhabitat alone, did not predict transmission better than null predictions based on a fixed probability of dispersal for all individuals. This result shows that dispersal of branchiobdellidan symbionts is condition-dependent and related to host size and intraspecific competition with other symbionts for limited host resources.

Recent experimental work on a similar symbiotic system also supports the hypothesis of condition-dependent transmission; the symbiosis between snails (*Helisoma*) and another ectosymbiotic annelid, *Chaetogaster limnaei* [54]. Much like branchiobdellidans, *Chaetogaster* are obligate and potentially beneficial ectosymbionts. *Chaetogaster* can be beneficial because they consume detrimental trematode parasites [55], but they may also have negative effects on their host, especially at high densities [56]. Also like branchiobdellidans, *Chaetogaster* largely depend on direct host–host contact for transmission [54]. A recent experimental study showed that *Chaetogaster* were more likely to disperse, even in the absence of host contact, when their host dies [54]. Furthermore, *Chaetogaster* dispersal rates were dependent on host size and trematode infection status, though the observed effects were opposite to the researchers' predictions. Rather than dispersing to larger hosts, which presumably provide more resources for *Chaeotagaster*, the symbiont tended to disperse to smaller hosts. Thus, the specific underlying selective advantages for condition-dependent dispersal in the *Helisoma*/*Chaetogaster* symbiosis remain to be discovered [54]. Nonetheless, our results and results emerging from other systems such as *Helisoma*/*Chaetogaster* suggest that an understanding of symbiont transmission dynamics will be vastly improved by considering individual symbiont fitness and the context of transmission opportunities.

5. Conclusion

We have shown that the symbiont *C. ingens* is likely to stay with its host when it is profitable to do so, and leave when it is not. This seemingly simple realization has potentially profound consequences for how we understand transmission dynamics and the evolution of dispersal strategies in natural symbiont populations. Our understanding of transmission dynamics in host populations has been largely guided by canonical epidemiological models (e.g. [4,57]). There has been considerable investigation of the factors that cause variation in contact among hosts such as host density [4], spatial properties of the local environment and host population [1], and host interactions such as interference competition among foraging hosts [58]. However, the probability of transmission during a contact between a susceptible and infected host is often assumed to be constant within a host population [5]. Conversely, our work shows that symbiont transmission is highly variable even when the opportunity to disperse and the likelihood of successful colonization post-dispersal are held constant. More importantly, we show that this variation can be explained in terms of fitness outcomes for individual symbionts and knowledge of the factors that influence symbiont fitness can therefore be used to accurately predict individual transmission events during host encounters.

Our results are particularly timely as many researchers that study symbioses are moving towards conceptual frameworks that embrace within-host ecological interactions and dispersal processes to understand patterns of symbiont prevalence and diversity (e.g. [59–61]). Recent synthetic work has made it clear that understanding the evolutionary forces that shape dispersal strategies is a difficult, but necessary, step towards improved metapopulation and metacommunity frameworks [17,62]. The work presented here demonstrates that adaptive dispersal strategies may be equally as important to symbionts as they are to free-living organisms. We urge ecologists who study parasite and other symbiotic systems to consider the factors that contribute to individual symbiont fitness and how those factors guide dispersal to better understand symbiont transmission, ecology and evolution.

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material.

Authors' contributions. J.S., B.L.B. and R.P.C. contributed equally to conceiving and planning the study. J.S. and B.L.B. collected data. J.S. analysed data and prepared initial manuscript, and B.L.B. and R.P.C. contributed to manuscript revisions.

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