Author Queries

Journal: Biology Letters

Manuscript: rsbl20100862

No Queries

biology letters
Animal behaviour

Biol. Lett. (2010) **00**, 1–3
doi:10.1098/rsbl.2010.0862
Published online 00 Month 0000

Parasitic infection reduces dispersal of ciliate host

Simon Fellous*,†, Elsa Quillery†, Alison B. Duncan and Oliver Kaltz

Institut des Sciences de l'Evolution UMR 5554, Université Montpellier, 2, Place Eugène Bataillon, 34095 Montpellier, France
**Author for correspondence (simonfellous@free.fr).*
†These authors contributed equally to the study.

Parasitic infection can modify host mobility and consequently their dispersal capacity. We experimentally investigated this idea using the ciliate *Paramecium caudatum* and its bacterial parasite *Holospora undulata*. We compared the short-distance dispersal of infected and uninfected populations in interconnected microcosms. Infection affected the proportion of hosts dispersing, with levels differing among host clones. Host populations with higher densities showed lower dispersal, possibly owing to social aggregation behaviour. Parasite isolates that depleted host populations most had the lowest impact on host dispersal. Parasite-induced modification of dispersal may have consequences for the spatial distribution of disease, host and parasite genetic population structure, and coevolution.

Keywords: dispersal; infection; microcosm

1. INTRODUCTION

Parasite infection affects life-history traits and behaviours that are important for host ecology [1]. Some of these effects involve alterations of host activity or mobility. Typically, infection may be expected to weaken the host and thereby reduce its locomotion ability, such as running speed or, flight velocity (e.g. [2,3]). Alternatively, infected hosts may become more active or have increased locomotion [4]. This suggests that parasites influence the dispersal of hosts (movement of individuals or propagules with potential consequences for gene flow across space [5]). However, since dispersal is also affected by decision-making and trajectory, variation in locomotion ability does not necessarily translate into variation in dispersal [6]. Direct evidence linking parasitic infection and dispersal in natural populations is rare [7,8], partly because dispersal is difficult to measure. Microbial model systems are convenient for investigating dispersal under controlled conditions in the laboratory [9,10], but have not been used to study the additional effect of infection.

We tested the effect of parasitic infection on host dispersal in experimental populations of the ciliate, *Paramecium caudatum*, and its bacterial parasite, *Holospora undulata*. Using different combinations of host and parasite genotypes, we compared infected and uninfected populations for their short-distance dispersal between interconnected experimental microcosms. This set-up is comparable to the patchy, ephemeral habitats that *Paramecium* occupies in the field (T. Berendonk, personal communication).

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2010.0862 or via http://rsbl.royalsocietypublishing.org.

2. MATERIAL AND METHODS

(a) *Biological system*

The protozoan *P. caudatum* inhabits stagnant freshwater bodies in the Northern Hemisphere [11]. The same mitochondrial haplotypes can be found thousands of kilometres apart, but also coexist in the same pond [12]. Our laboratory cultures are maintained at 23°C, in a medium prepared from dried organic lettuce supplemented with the food bacterium *Serratia marcescens*. Under permissive conditions, *P. caudatum* divides 1–2 times 24 h^{-1}.

The obligate parasite *H. undulata* (α-proteobacteria) develops in the host micronucleus [13]. Infectious forms are released upon host division or after host death. Horizontal transmission follows ingestion of infectious forms during food uptake, and vertical transmission host division. Infection reduces host division rate and survival [14].

(b) *Experimental set-up*

We used four genetically distinct host clones with different geographical origins (electronic supplementary material, table S1). Host clones were infected with five parasite isolates, which had evolved on different host clones for several months prior to this experiment. For each combination of host clone and parasite isolate, we isolated single infected individuals, in order to seed clonal replicate populations (prevalence $\approx$ 100%). Each replicate population was kept in different tubes for two months prior to the experiment. Here, we used 28 infected replicate populations from 10 host clone $\times$ parasite isolate combinations, and 22 uninfected control populations from each of the four host clones (electronic supplementary material, figure S1). The experiment was not designed to test for host clone $\times$ and parasite isolate interactions.

We measured dispersal in a system of three 5 ml plastic tubes, connected by 5 cm-long rubber tubing, where connections could be closed with clamps (electronic supplementary material, figure S1). This apparatus was previously used to study short-distance dispersal in another ciliate [10]. A given replicate population at carrying capacity (3 ml, 200–500 paramecia) was placed in the central tube. After 48 h of acclimation, fresh medium was added to the two lateral tubes and the clamps were removed, allowing the paramecia to move freely between tubes. After 3 h, the number of paramecia remaining in the central tube (=non-dispersers) was estimated from a 250 µl sample, and all *Paramecia* in lateral tubes (=dispersers) counted (see electronic supplementary material). Treatment order was randomized and tube identity unknown to the experimenter.

(c) *Statistical analyses*

We used analyses of covariance (JMP package v.6.0.3, SAS Institute 2006) to analyse the proportion of paramecia that dispersed to the two lateral tubes. A first model contained the $\log_{10}$ total population density, infection status (fixed factors) and host clone (random factor). A second model, using infected replicate populations only, tested for an effect of parasite isolate (random factor), after controlling for host clone and population density. Fully factorial models were simplified by backward elimination of non-significant terms ($p > 0.1$). Homoscedasticity and normality of the residuals complied with standard model assumptions. We tested whether infection impact on host density and on dispersal was correlated across parasite isolates. We controlled for host clone effects by extracting residuals from models explaining dispersal or density by host clone; a Spearman rank correlation (ρ) test was performed on the residual means for each parasite isolate ($n = 5$).

3. RESULTS

Infection decreased the proportion of paramecia dispersing from central to lateral tubes from 52 to 39%, on average ($F_{1,44} = 9.72$, $p = 0.003$; figure 1). This negative effect of infection was consistent over the different host clones, indicated by the non-significant clone $\times$ infection status interaction ($\chi^2_1 = 1.04$, $p > 0.3$).

There was a significant negative relationship between population density and the proportion of dispersing paramecia ($F_{1,7} = 8.44$, $p = 0.024$): dispersal was highest at low densities (figure 1). To some extent, the slope of this relationship varied with

Received 17 September 2010
Accepted 27 September 2010

rsbl20100862—1/10/10—10:02–Copy Edited by: L. Shobana

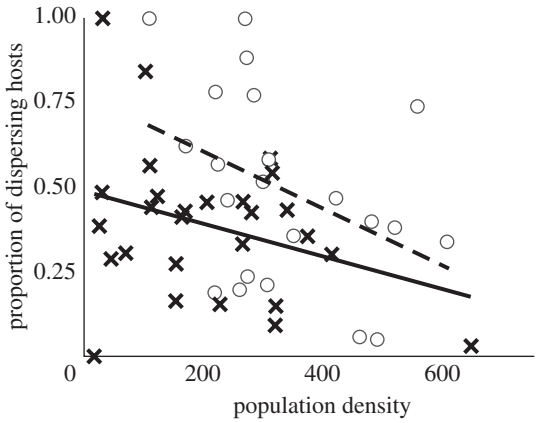

Figure 1. Effect of infection and population density on the proportion of dispersing hosts. Regression lines illustrate density-dispersal relationship for infected and uninfected populations. Open circles, uninfected and crosses, infected.

infection status and paramecium clone, as indicated by significant interactions of these two factors with population density ($F_{1,38} = 3.93$, $p = 0.054$ and $\chi^2_1 = 5.11$, $p = 0.023$, respectively; figure 1 and electronic supplementary material, figure S1). There also was a significant main effect of host clone ($\chi^2_1 = 9.53$, $p = 0.002$), with mean proportion of dispersing paramecia ranging from 29 to 62%.

We obtained a marginally non-significant effect of parasite isolate on the proportion of dispersing hosts ($\chi^2 = 2.78$, d.f. $= 1$, $p = 0.095$). Parasite isolates that reduced density the most also had the least effect on dispersal (Spearman rank correlation: $\rho = -0.9$, $p = 0.037$; figure 2).

4. DISCUSSION

(a) *Infection reduced dispersal*

We found that infection reduced dispersal. This is the first demonstration of such an effect in experimental populations; a similar negative relationship between ectoparasite infestation and dispersal was described for a natural bird population [7]. In several species, infection impairs host locomotion ability (e.g. [2]), presumably because of exploitation of host resources. This is also conceivable here, given the deleterious effects of infection by *Holospora* sp. [13]. From this it would follow that more harmful infections (i.e. those that decrease population density the most) have a more negative effect on dispersal. However, the across-isolate correlation (figure 2) provided no evidence for such a relationship. Interestingly, infection by the sister species *H. obtusa* increased rather than decreased the swimming speed of *P. caudatum* [15]. Although not measured here, this observation suggests that movement or activity does not necessarily equate to dispersal. Indeed, dispersal is determined by direction and various responses to environmental cues as well as locomotion [6]. For example, infection may increase attraction between individuals thus reducing dispersal. Alteration of social behaviour by parasites can reflect parasite-induced manipulation to increase transmission [16]. Thus, reduced dispersal may be another manifestation of virulence (reduction in host fitness), may be indirectly mediated by effects on host population density (see below).

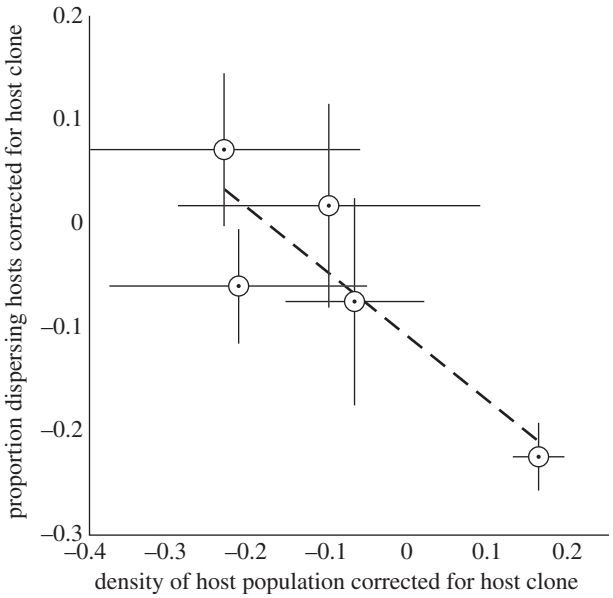

Figure 2. Relationship between parasite effect on host density and dispersal. Mean (±s.e.) residual density or dispersal calculated per parasite isolate, after statistically controlling for host clone effects.

(b) *Dispersal decreased with population density*

It is often assumed that higher population density increases dispersal because of increased competition for limited resources [17]. We found the opposite pattern, with generally lower dispersal at higher densities. Negative density-dependent dispersal may be owing to social factors, such as cooperation or facilitation [17]. Paramecia and other ciliates are social organisms, where swarming and dispersal behaviour depend on chemical signals from or direct physical contact with conspecifics [10,18]. Thus, population density may influence individual dispersal behaviour through signal concentration or contact frequency.

Our experimental populations varied in density according to their natural carrying capacities. Statistical analysis was corrected for this variation, and therefore the difference in dispersal between infected and uninfected populations does not simply reflect differences in density. Explicit experimental testing of effects of crowding on dispersal would require artificial manipulation of population density.

(c) *Genetic variation in dispersal*

How genetic and phenotypic inter-individual differences shape dispersal is currently debated [19]. In our study, the genetic component is represented by the differences in dispersal among *Paramecium* clones, confirming results for another ciliate [10]. Parasitic infection produced a form of phenotypic plasticity causing decreased dispersal. Host clones were similarly affected by infection, indicating the generality of this plastic effect.

We found that parasites producing more harmful infections tended to have a less negative effect on dispersal (figure 2), suggesting a negative relationship

between parasite ability to disperse and to exploit host resources. Optimal parasite strategies may thus depend on the balance between local transmission and the capacity to reach new habitats through dispersal [20].

(d) *Implications for epidemiology, population genetics and (co)evolution*

Dispersal is an essential determinant of population dynamics and persistence in metapopulations [6]. For parasites, dispersal of infected individuals may be key to the spread of infection in spatially structured populations. If infection reduces dispersal, disease may remain spatially clustered. As dispersal determines gene flow, restricted dispersal may increase spatial genetic differentiation and evolutionary divergence among parasite populations [21]. If infected host populations emit fewer migrants, parasitism may lead to asymmetry in gene-flow and impact population genetic structure.

Finally, differential dispersal probably affects coevolutionary outcomes. Host–parasite coevolution critically depends on the arrival of novel host or parasite alleles in populations. Reduced dispersal of infected hosts may translate into lower gene flow rates of the parasite, and this can reduce its evolutionary potential relative to that of the coevolving host [22,23].

5. CONCLUSIONS

Our experiment identified parasitism as a potentially important factor reducing host dispersal. In a metapopulation framework, this may have consequences for epidemiology, genetic population structure and (co)-evolution. Further work on the precise mechanisms underlying the observed variation in dispersal is necessary for generalization of our findings to other biological systems.

We thank Aurélie Coulon for discussion and two anonymous reviewers for comments. This work was supported by the French Agence National de Recherche (ANR-09-BLAN-0099 and ANR-09-PEXT-01102).

1 Thomas, F., Guégan, J.-F. & Renaud, F. 2007 *Ecology and evolution of parasitism*. Oxford, UK: Oxford University Press.
2 Bradley, C. A. & Altizer, S. 2005 Parasites hinder monarch butterfly flight: implications for disease spread in migratory hosts. *Ecol. Lett.* **8**, 290–300. (doi:10.1111/j.1461-0248.2005.00722.x)
3 Cameron, P. G., Semlitsch, R. D. & Bernasconi, M. V. 1993 Effects of body size and parasite infection on the locomotory performance of juvenile toads, *Bufo bufo*. *Oikos* **66**, 129–136.
4 Sorci, G., Massot, M. & Clobert, J. 1994 Maternal parasite load increases sprint speed and philopatry in female offspring of the common lizard. *Am. Nat.* **144**, 153–164. (doi:10.1086/285666)
5 Ronce, O. 2007 How does it feel to be like a rolling stone? Ten questions about dispersal evolution. *Ann. Rev. Ecol. Syst.* **38**, 231–253.
6 Clobert, J., Danchin, E., Dhondt, A. A. & Nichols, J. D. 2001 *Dispersal*. New York, NY: Oxford University Press.
7 Heeb, P., Werner, I., Mateman, A. C., Kolliker, M., Brinkhof, M. W. G., Lessells, C. M. & Richner, H. 1999 Ectoparasite infestation and sex-biased local recruitment of hosts. *Nature* **400**, 63–65. (doi:10.1038/21881)
8 Møller, A. P., Martin-Vivaldi, M. & Soler, J. J. 2004 Parasitism, host immune defence and dispersal. *J. Evol. Biol.* **17**, 603–612.
9 Dennehy, J. J., Friedenberg, N. A., Yang, Y. W. & Turner, P. E. 2006 Bacteriophage migration via nematode vectors: host–parasite–consumer interactions in laboratory microcosms. *Appl. Environ. Microbiol.* **72**, 1974–1979. (doi:10.1128/AEM.72.3.1974-1979.2006)
10 Fjerdingstad, E., Schtickzelle, N., Manhes, P., Gutierrez, A. & Clobert, J. 2007 Evolution of dispersal and life history strategies–*Tetrahymena* ciliates. *BMC Evol. Biol.* **7**, 133. (doi:10.1186/1471-2148-7-133)
11 Wichterman, R. 1986 *The biology of Paramecium*. New York, NY: Plenum Press.
12 Barth, D., Krenek, S., Fokin, S. I. & Berendonk, T. U. 2006 Intraspecific genetic variation in *Paramecium* revealed by mitochondrial cytochrome C oxidase I sequences. *J. Eukaryot. Microbiol.* **53**, 20–25. (doi:10.1111/j.1550-7408.2005.00068.x)
13 Fokin, S. I. 2004 Bacterial endocytobionts of ciliophora and their interactions with the host cell. *Int. Rev. Cytol.* **236**, 181–249. (doi:10.1016/S0074-7696(04)36005-5)
14 Nidelet, T., Koella, J. C. & Kaltz, O. 2009 Effects of shortened host life span on the evolution of parasite life history and virulence in a microbial host–parasite system. *BMC Evol. Biol.* **9**, 65. (doi:10.1186/1471-2148-9-65)
15 Fujishima, M., Kawai, M. & Yamamoto, R. 2005 *Paramecium caudatum* acquires heat-shock resistance in ciliary movement by infection with the endonuclear symbiotic bacterium *Holospora obtusa*. *FEMS Microbiol. Lett.* **243**, 101–105. (doi:10.1016/j.femsle.2004.11.053)
16 Moore, J. 2002 *Parasites and the behavior of animals*. Oxford, UK: Oxford University Press.
17 Bowler, D. E. & Benton, T. G. 2005 Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. *Biol. Rev. Camb. Philos. Soc.* **80**, 205–225.
18 Ogata, M., Hondou, T., Hayakawa, Y., Hayashi, Y. & Sugawara, K. 2008 Adaptation-induced collective dynamics of a single-cell protozoan. *Phys. Rev. E* **77**, 011917. (doi:10.1103/PhysRevE.77.011917)
19 Gibbs, M., Saastamoinen, M., Coulon, A. & Stevens, V. M. 2010 Organisms on the move: ecology and evolution of dispersal. *Biol. Lett.* **6**, 146–148. (doi:10.1098/rsbl.2009.0820)
20 Lion, S. & Boots, M. 2010 Are parasites 'prudent' in space? *Ecol. Lett.* **13**, 1245–1255. (doi:10.1111/j.1461-0248.2010.01516.x)
21 Prugnolle, F., Theron, A., Pointier, J. P., Jabbour-Zahab, R., Jarne, P., Durand, P. & De Meeus, T. 2005 Dispersal in a parasitic worm and its two hosts: consequence for local adaptation. *Evolution* **59**, 296–303. (doi:10.1554/04-522)
22 Kaltz, O., Gandon, S., Michalakis, Y. & Shykoff, J. A. 1999 Local maladaptation in the anther-smut fungus *Microbotryum violaceum* to its host plant *Silene latifolia*: evidence from a cross-inoculation experiment. *Evolution* **53**, 395–407. (doi:10.2307/2640776)
23 Morgan, A. D., Gandon, S. & Buckling, A. 2005 The effect of migration on local adaptation in a coevolving host–parasite system. *Nature* **437**, 253–256. (doi:10.1038/nature03913)

rsbl20100862—1/10/10—10:02–Copy Edited by: L. Shobana