Travelling with a parasite: the evolution of resistance and dispersal syndromes during experimental range expansion

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Rapid evolutionary change during range expansions can lead to diverging range core and front populations, with the emergence of dispersal syndromes (coupled responses in dispersal and life-history traits). Besides intraspecific effects, range expansions may be impacted by interspecific interactions such as parasitism. Yet, despite the potentially large impact of parasites imposing additional selective pressures on the host, their role on range expansions remains largely unexplored. Using microcosm populations of the ciliate Paramecium caudatum and its bacterial parasite Holospora undulata, we studied experimental range expansions under parasite presence or absence. We found that the interaction of range expansion and parasite treatments affected the evolution of host dispersal syndromes. Namely, front populations showed different associations of population growth parameters and swimming behaviours than core populations, indicating divergent evolution. Parasitism reshaped trait associations, with hosts evolved in the presence of the parasite exhibiting overall increased resistance and reduced dispersal. Nonetheless, when comparing infected range core and front populations, we found a positive association, suggesting joint evolution of resistance and dispersal at the front. We conclude that host–parasite interactions during range expansions can change evolutionary trajectories; this in turn may feedback on the ecological dynamics of the range expansion and parasite epidemics.

1. Introduction

Species range expansions are increasing in frequency in response to rapidly changing environments [1] and becoming crucial to reducing the risk of extinction [2]. While ecological and evolutionary processes have been well explored for single species, we are still lacking a good community perspective of range shifts [3,4]. Species might rarely ‘travel alone’, and it is conceivable that interactions with other organisms facilitate or slow down spatial spread, and add new selection pressures while a species is spreading.

Antagonistic species interactions have been suggested to be a major evolutionary factor defining species ranges [5,6]. Parasites, for example, are ubiquitous and exert strong demographic and evolutionary pressures on the host [7,8], which can limit geographical ranges as has been shown in some theoretical models [9–11]. Parasites may re-enforce range limits by reducing dispersal and/or by imposing additional mortality in already small populations at the range front. Such modifications of the ecological dynamics seem to limit the spread of Rhinella marina (the invasive cane toad) in Australia [12,13] and also play a role in other natural systems [14–16]. The evolution of counter-adaptation against parasites may remove such range limits, but also lead to more complex outcomes if parasite-mediated selection affects the evolution of other life-history traits known to be selected at range fronts. Novel trait combinations

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may then produce eco-evolutionary feedbacks influencing rates of spatial spread of both the host species and the parasite. This may cause knock-on effects on interactions with other species in the community [17,18] or increase the risk of spill-over of disease [19,20]. Thus, understanding how parasites shape evolutionary trajectories of phenotypic traits in a range expansion context is also of major interest to conservation [21], disease control [22–24] or invasive species management [25].

Range expansions often involve stochastic genetic change in small vanguard front populations (via drift or founder bias), but also the concerted evolution of dispersal, life-history, behaviour or physiology, often referred to as ‘dispersal syndrome’ [26,27]. Typically, range front populations may be populated by individuals with a colonizer phenotype, characterized, e.g. by high dispersal propensity and high reproductive rate. Kin selection and spatial assortative mating are then expected to reinforce such trait associations while the range front is expanding [28,29]. There is empirical and experimental evidence that such spatial selection is indeed a strong evolutionary force [4]. Dispersal is a heritable trait in various biological systems and covariation with other traits has a genetic basis [30], making it possible for selection to drive multi-trait divergence between core and front populations [31,32]. An example is the cane toad, where the evolution of dispersal and other life-history traits promoted the speed of spread at the invasion front [33–35]. Similar results were found for the invasive ladybird Harmonia axyridis [36] and in laboratory range expansions using experimental evolution approaches [37–39].

We currently know relatively little about parasite-mediated selection at range fronts and its consequences for dispersal syndrome evolution [3,4]. While a considerable bulk of literature exists on resistance evolution in host–parasite metapopulations [40,41], there is no theory in the context of range expansion. Intuitively, we may expect that virulent parasites select for increased resistance and that this removes a parasite-imposed range speed limit. Such a case of resistance evolution has been found for populations of the cane toad infected by lungworms at the range margin [42,43]. Theory further suggests that natural enemies select for changes in host dispersal. For example, parasite-induced fluctuations in host population dynamics can modify environmental predictability and fitness expectations and consequently favour increased host dispersal [44,45]. Parasites may also select for plastic responses in dispersal, with sometimes counterintuitive consequences for the spread of infection [46].

However, few if any studies have considered the joint action of parasite-mediated selection and spatial selection, and hence the interplay between the evolution of resistance and dispersal-related traits. Indeed, selection for resistance can be rapid and strong, but also modify genetic correlations with other life-history traits [47,48], often involving trade-offs [49,50] that constrain evolutionary trajectories. Hence, if selection for resistance is uncorrelated with dispersal syndrome traits (or even positively correlated), counter-adaptation against the natural enemy may simply remove the brake from range expansion. However, if resistance trades off with expansion-relevant traits, such as dispersal or growth, the additional parasite-mediated selection may shift populations to novel positions in multi-trait space, which finally may or may not impede spatial spread.

Using experimental evolution, we investigated the effect of a parasite on the emergence of dispersal syndromes. In a long-term experiment, we mimicked range expansions in interconnected microcosms of the freshwater protist Paramecium caudatum and its bacterial parasite Holospora undulata. In a range front treatment, the paramecia were constantly selected to disperse into a new microcosm, whereas in the range core treatment, only the non-dispersing fraction of the population was maintained. Range and core treatments were established for infected and uninfected populations. Evolved hosts were assayed for six traits, including dispersal, resistance, population growth characteristics and swimming behaviour. We found substantial divergence in multiple phenotypes in the trait space, attributable to the combined effect of our experimental treatments. A dispersal syndrome emerged, with higher population equilibrium density and movement tortuosity in the front treatment, and higher population growth rate and swimming speed in the core treatment. Evolution with the parasite affected some of these trait relationships and additionally left signatures in the observed levels of resistance and dispersal. Populations evolving with the parasite were more resistant, in particular those in the front range treatment. By contrast, Paramecium originating from parasitized populations generally dispersed less than their parasite-free counterparts. The parasite-driven evolution of such novel multi-trait associations may influence the speed of range expansions, but also the risk of spreading epidemics.

2. Material and methods

(a) Study system

Paramecium caudatum is a freshwater filter-feeding ciliate with a worldwide distribution [51]. Ciliates typically exhibit a nuclear dimorphism: the ‘germ-line’ micronucleus is active during the sexual stage, while the highly polyploid ‘somatic’ macronucleus regulates gene expression during the asexual stage, when replication occurs through mitotic division. In this experiment, clonal populations were maintained asexually (max. 1–2 population doublings per day at constant 23°C) in 50 ml tubes, using a sterilized lettuce medium (1 g dry weight of organic lettuce per 1.5 l of Volvic™ mineral water), supplemented ad libitum with the bacterium Serratia marcescens as a food resource (referred to as ‘bacterised medium’ or ‘medium’, hereafter). The gram-negative bacterium B. undulata is an obligate parasite, infecting the micronucleus of P. caudatum [52]. The infection life cycle comprises both horizontal and vertical transmission. Paramecium ingest infectious forms from the aquatic environment, which subsequently colonize the macronucleus and differentiate into multiplying reproductive forms; these reproductive forms are vertically transmitted to the daughter cells of mitotically dividing hosts. The infection life cycle is completed when reproductive forms differentiate into infectious forms (at 7 days post-infection), which are then released during host cell division or upon host death. With accumulating parasite loads, infection reduces cell division and survival of the Paramecium, as well as dispersal [53–55]. Experimental evolution of resistance to this parasite was demonstrated in previous long-term experiments and can come at reproductive costs [56,57].

(b) Experimental protocols

(i) Long-term range expansion experiment

Dispersal in two-patch systems. We used two-patch systems for this experiment (electronic supplementary material, figure S1). The systems were built from two 14 ml plastic tubes (‘core patch’ and ‘front patch’) interconnected by 5 cm silicon tubing (0.6 mm inner
diameter) serving as a corridor through which the *Paramecium* can actively swim. We define dispersal as the active displacement of *P. caudatum* from the core patch to the front patch. In the long-term experiment, short episodes of dispersal (3 h) alternated with periods of population growth and maintenance (one week). For a dispersal episode, we filled the two-patch system with 9.5 ml of fresh bacterized medium and then blocked the corridor with a clamp. The core patch was filled with 8 ml of culture containing *Paramecium* (approx. 2000 individuals), whereas the front patch was only topped up with non-bacterized medium and was thus ‘empty’. After the removal of the clamp, *Paramecium* could freely disperse to the front patch or to stay in the core. After 3 h, we blocked the corridor and estimated the cell density in the core and front patch, by sampling up to 1 ml from each tube and counting the number of individuals under a dissecting microscope. The dispersal rate is thus the number of dispersers divided by the total number of individuals in the two-patch systems, divided by 3 h.

**Range expansion treatments.** Two ‘range expansion’ treatments were imposed. In the range front treatment, only *Paramecium* that dispersed into the front patch were maintained and allowed to grow for one week until the next episode of dispersal. Conversely, in the range core treatment, only the non-dispersing *Paramecium* were maintained and allowed to regrow. These contrasting protocols were continued for a total of 26 cycles. The front treatment mimics the leading front of range expansion or a biological invasion, with populations continuously dispersing into a new microcosm. Populations from the range core treatment stay in place and continuously lose emigrants. Each new growth cycle was started by placing on average 200 paramecia from front and core treatments in 20 ml of fresh bacterized medium, and equilibrium density was then reached within the following 3–4 days. The experiment was conducted with a single host line [54]. This line (63D) had undergone 3 years of parasite-free core selection prior to the present experiment; initially started from a mix of strains, it has become fixed for a single cytochrome oxidase 1 haplotype [58].

**Parasite treatment.** Range core and front treatments were established for both infected and uninfected populations. The parasites were taken from an experiment [53] that had already been imposing core and front treatment on infected 63D populations for about 8 months (30 cycles). Using standard protocols (see the electronic supplementary material, S2), we extracted infectious forms of the parasite from five core lines and from five front lines, which were then used to inoculate our new, naive 63D hosts. In other words, we continued range core and front treatments for the parasite, but replaced the previous hosts by new unselected hosts. In addition to these 10 infected lines, we established three uninfected front lines and three uninfected core lines as controls, giving a total of 16 lines. After this initial inoculation of the experimental lines, we did not interfere with epidemiological dynamics and all transmission occurred naturally over the course of the 26 new cycles of the long-term experiment. We routinely measured dispersal and population density (electronic supplementary material, figure S5) and verified the presence of infection in the parasite treatment.

(ii) **Phenotypic trait assays**

At the end of the long-term experiment, phenotypic trait assays for *Paramecium* from all 16 lines were performed under common-garden conditions. Using a micropipette, we arbitrarily picked four uninfected paramecia from each line and placed them individually in single 1.5 ml Eppendorf tubes filled with bacterized medium, where they were allowed to grow for two weeks until small monoclonal lines had established (ca 7–8 asexual generations). Each monoclonal line was then split into three technical replicates and grown for a second common-garden period of 10 days in 50 ml tubes to obtain mass cultures for the phenotype assays (16 lines × 4 monoclonal lines × 3 technical replicates = 64 monoclonal lines and 192 replicates, electronic supplementary material, figure S5). Of the 64 monoclonal lines, one monoclonal line from the core treatment with parasite did not grow and was lost, leaving us with 63 lines available to measure six phenotypic traits.

**Resistance.** To measure resistance, the *Paramecium* were confronted with parasites from range core and front treatments. To this end, we prepared inocula by extracting infectious forms from mixes of the five infected core and the five infected front lines (for details of the protocol, see the electronic supplementary material, S2). For inoculation, ca 5000 paramecia were placed in a volume of 25 ml in a 50 ml tube, to which we added 4.5 × 10⁵ infectious forms (core-parasite or front-parasite inoculum). In this way, we set up 4–8 inoculated tubes per host line, balanced between the two parasite inocula (16 lines × 2–4 monoclonal lines × 2 technical replicates = 120 inoculated tubes). Four days post-inoculation, we fixed 20 individuals from each inoculated replicate with lacto-aceto-orcein [52] and inspected them for absence or presence of infection using a phase-contrast microscope (1000 x magnification). We define resistance as the proportion of uninfected individuals in the sample. Preliminary analysis showed that *Paramecium* from the four different treatments did not differ in their resistance to the mixes of front or core parasites (F₃,₁₂ = 0.44, n.s.); we therefore combined the two inoculum sources into a single ‘infected’ category for the main analysis. Note, however, that this does not rule out the possibility of specific responses to range or core parasites in other host traits.

**Dispersal rate.** Dispersal was measured in linear three-patch systems (50 ml tubes; electronic supplementary material, figure S5), where uninfected *Paramecium* dispersed from the middle tube into the two outer tubes (see the electronic supplementary material for detailed protocol). This system configuration allowed us to use bigger volumes of culture and thus obtain higher numbers of dispersers than in two-patch systems. Connections were opened for 3 h, dispersal rates were then estimated by counting the *Paramecium* in samples from the central tube (500 µl) and from the combined two outer tubes (3 ml). We employed technical replicates that had not been used for the resistance assay and were kept in 30 ml of fresh medium for several days prior to the dispersal test. One replicate per monoclonal line was tested (63 dispersal tests).

**Population growth rate and equilibrium density.** For the population growth assay, we placed groups of five arbitrarily picked *Paramecium* in 15 ml tubes filled with 10 ml of bacterized medium. Over 9 days, we tracked densities in 24 h intervals, estimated from the number of individuals present in 200 µl samples. We set up 6–12 tubes per host line (three technical replicates per monoclonal line), with a total of 180 replicates. Only uninfected *Paramecium* were tested. For each tube, estimates of intrinsic population growth rate (rₐ) were obtained by fitting a Beverton–Holt population growth model to each density time series, using a Bayesian approach [59]. For certain tubes, we obtained unsatisfactory fits of equilibrium density; we therefore decided to use the mean density over the second half of the assay (days 5–9) as a proxy for equilibrium density (N). A small fraction of 19 tubes (corresponding to nine monoclonal lines from lines from range front and parasitism treatment) failed to grow and remained at very low density for unknown reasons; it was not possible to fit our population growth model to these data, and these replicates were therefore excluded from analysis.

**Swimming speed and tortuosity.** At the end of the population growth assay, we assessed swimming behaviour, using an established pipeline of computer vision and automated video analysis to collect this data [60,61]. From a given tube, one sample of 119 µl was imaged under a Perfix Pro 10 stereomicroscope, using a Perfex SC8800 camera (15 frames s⁻¹; duration: 10 s; total magnification: 10 x). Videos were analysed using the benvi R-package [61], which provided individual-based data on swimming speed and the tortuosity of swimming trajectories.
(s.d. of the turning angle distribution). Swimming speed and tortuosity were averaged over all individuals in a sample prior to analysis. A total of 63 samples (one per monoclonal line) was used for analysis, giving 2-4 observations per host line. Three monoclonal lines (front parasite-exposed) went extinct prior to video recording.

(c) Statistical analysis

All statistical analyses were performed with R v.4.2.0 [62], using Bayesian models with the ‘rstan’ (version 2.21.5), ‘brms’ (version 2.17.0) and ‘rethinking’ (version 2.21) packages [63–65]. Focusing on the analysis of trait associations, we constructed a data matrix with the measurements of the six traits for 63 monoclonal lines (for resistance, the mean over the two technical replicates was calculated). To impute 24 missing observations (nine for N and r0, three for swimming speed and tortuosity), we used the ‘mice’ package version 3.14.0 [66] and took the mean of 1000 multiple imputations (24/378 imputed values). Trait distributions were standardized by using the ‘standardize’ function of the ‘rethinking’ package [65].

We then fitted five multi-variate multilevel models, from the intercept to the full interaction model, using a normal error structure (chain length: warmup = 1000 iterations, chain = 20,000 iterations). The explanatory factors of the full model were range expansion treatment (core versus front lines), parasite treatment (infected versus uninoculated control lines) and their interaction; line identity was included as a random term. Models were fitted with default vague informative priors of the ‘brms’ package. We compared and ranked the five models using the Watanabe–Akaike information criterion (WAIC) [67], a generalized version of the Akaike information criterion [68]. We used a conservative approach based on WAIC weights and not on the best-ranked model. We averaged the posterior predictions of the models and calculated the relative importance (RI) of the explanatory variables. The RI corresponds to the sum of the respective WAIC model weights in which the explanatory variable was present. From this same dataset and following the same procedure, we also performed univariate analyses for each of the six traits. In addition, for a complementary graphical inspection of the results, we used the above data matrix to carry out a principal component analysis (PCA).

3. Results

Multi-variate analysis revealed signatures of selection history, with strong effects of range expansion treatment (RI = 1.00) and parasitism treatment (RI = 0.77) on the observed phenotypic variation and covariation (Table 1). After model selection, the interaction between range expansion and parasitism (RI = 0.55) was retained in the best model fit (lowest WAIC; model 5 in Table 1), indicating that the effect of spatial selection acted jointly with the presence of the parasite. In additional univariate analyses, all six traits showed signals of the two experimental treatments in combination or alone (Table 2; details for all traits in the electronic supplementary material, tables S3–S8).

PCA visualizes the (co)variation in multi-variate trait space (figure 1) and the relative contribution of the six measured traits to the observed patterns of divergence among monoclonal lines (individual points in figure 1) and combinations of treatments. As shown by the arrows in figure 1, the six traits have similar weight but different orientation (electronic supplementary material, table S2; PCA loadings), with some pointing in opposite directions, indicative of trade-offs. The details of the PCA are provided in the electronic supplementary material, tables S1 and S2.

In long-term treatments without parasites, we observed a main pattern of divergence between range core and front treatments along the horizontal PC1 axis (red versus blue open points, figure 1). The direction and length of the different arrows in figure 1 show that this was mainly driven by opposing trends in traits related to demography and movement (r0/N and speed/tortuosity have highest PC 1 loadings; electronic supplementary material, table S2). Accordingly, univariate analyses further revealed high levels of RI (RI > 0.58) of the range expansion for these traits (electronic supplementary material, tables S3–S8). Thus, Paramecium from the range front populations generally had a 31% lower population growth rate (figure 2c) and 54% higher equilibrium density (figure 2d) than Paramecium from the range core.

Table 1. Statistical models and parameters included in the main multi-variate trait analysis and model averaging. (Rows are the different models; the best model is highlighted in italics. Columns are the explanatory variables included with the corresponding WAIC, s.e. of the WAIC and WAIC weights for each model. The RI row indicates the relative importance of the explanatory variables.)

| model | range expansion | parasitism | range parasitism | WAIC | s.e. | WAIC weight |
|-------|-----------------|------------|------------------|------|------|-------------|
| 1     | X               | X          | X                | 951.2| 40.2 | 0.00        |
| 2     | X               |            |                  | 952.3| 39.5 | 0.00        |
| 3     | X               |            |                  | 942.1| 40.0 | 0.23        |
| 4     | X               | X          |                  | 942.2| 39.5 | 0.22        |
| 5     | X               | X          |                  | 940.4| 39.1 | 0.55        |
| RI    | 1.00            | 0.77       | 0.55             |      |      |             |

Table 2. Best model and WAIC weights from model averaging of single-trait analysis. (The explanatory variables included in the best model are indicated with X. The complete list of the models for each trait is given in the electronic supplementary material.)

| trait | range expansion | parasitism | range parasitism | WAIC weight |
|-------|-----------------|------------|------------------|-------------|
|       | dispersal       | X          |                  | 0.28        |
|       | resistance      | X          | X                | 0.42        |
|       | r0              | X          | X                | 0.26        |
|       | N               | X          |                  | 0.23        |
|       | speed           | X          |                  | 0.62        |
|       | tortuosity      | X          |                  | 0.39        |
Figure 1. PCA for six traits measured for *P. caudatum* from four long-term treatment combinations, showing the first two principal component axes, PC1 and PC2. Each of the 63 points represents the average phenotypic value of a given monoclonal line in multi-variate space, with long-term treatment origins specified: range core (blue) versus range front (red) treatment; evolved in the presence (full circles) versus absence (empty circles) of the parasite *H. undulata*.

Similarly, the range front treatment was associated with 12% lower swimming speed (figure 2e) and 23% more nonlinear (i.e. tortuous) movement trajectories (figure 2f), compared to the range core treatment.

In long-term treatments with the parasite, phenotypic differentiation between infected and parasite-free lines can be seen along the PC2 axis, and in part along the PC1 axis (figure 1, filled versus empty dots). To some degree, this parasite effect varied for range and core treatments, leading to overlap of the different treatment combinations in figure 1. We identify three patterns. First, resistance and dispersal are the key traits associated with the PC2 axis (figure 1), with trait trajectories indicating higher resistance for lines evolved with the parasite and higher dispersal for parasite-free lines. Univariate analyses are in line with these trends. For resistance, there was an interaction between range expansion and parasitism (table 2; electronic supplementary material, table S4; RI interaction = 0.42). Thus, the presence of parasites tended to increase resistance in the range front treatment (+15%), but less so in core treatment (figure 2b) compared to the unexposed evolved counterparts. Third, the long-term presence of the parasite had no obvious effect on swimming behaviour (figure 2c,f), leaving only the range treatment in the best model in the univariate analysis (RI > 0.93; electronic supplementary material, tables S7 and S8).

4. Discussion

Eco-evolutionary dynamics in expanding edge populations can favour specific adaptations in dispersal capacity and reproductive strategies (dispersal syndromes), but still little is known about how these processes are modulated by interactions with natural enemies or parasites. This study investigated the interplay between spatial selection (range front versus core treatment) and parasite-mediated selection (absence versus presence of parasite). We found substantial divergence between our experimental range core and front populations, involving traits commonly known to produce ‘dispersal syndromes’ (dispersal, population growth, movement behaviour). Exposure to the parasite in infected populations brought into a play an additional trait (resistance), but also affected divergence in the other traits. In particular, populations evolved with the parasite were more resistant, but dispersed less than their uninfected counterparts. Three pairs of traits acted as opposing vectors in multi-trait space defining the phenotypic divergence between treatments: dispersal–resistance, population growth rate (\( r_0 \))–equilibrium density (\( N \)) and swimming speed–tortuosity. Below we focus on the evolutionary forces driving these trait associations and discuss possible feedbacks on range expansion speed.

(a) Resistance and dispersal: trade-off or concerted evolution?

Dispersal and resistance are among the most prominent traits expected to be under selection in range expansion and host-
parasite coevolution contexts, respectively. In our study, we investigated their joint evolution. The PCA shows that the two traits have opposite signs, indicating a general negative relationship between dispersal and resistance. This negative association further scales up to the ‘parasite treatment’ level (figure 2a, b), suggesting that parasite-mediated selection for increased resistance is accompanied by a decrease in dispersal. This general pattern was most obvious for populations in the range front treatment (panels on the right in figure 2a, b), for which we also detected a clear negative quasi-genetic (i.e. across monoclonal lines) correlation between the two traits (electronic supplementary material, table S9).

Previous work in our study system provided evidence for ample naturally occurring genetic variation in both resistance and dispersal [55,69], and both traits readily evolve under experimental conditions [56,58]. The genetic basis of the two traits is unknown, but we speculate that a trade-off could result from energy constraints arising from mounting an effective (constitutive) defence against the parasite, and/or from concomitant behavioural change. For example, the

Figure 2. Standardized single-trait panels of *P. caudatum* evolved in range core and range front treatments in the presence or absence of parasite: (a) dispersal, (b) resistance, (c) growth rate ($r_0$), (d) equilibrium density ($N$), (e) swimming speed, and (f) swimming tortuosity. For each panel, blue and red represent core and front treatment, while the parasitism treatment is specified as follows: evolved in the presence of the parasite (filled dots and lines) versus parasite-free (empty dots and dashed lines). The points are the means for 63 monoclonal lines, isolated from a total of 16 long-term lines (for details, see text). The shaded areas and thick lines are the 95% compatibility interval and median of the averaged model of the posterior distributions.
filter-feeding paramecia become infected when they ingest infectious spores together with other food particles. Hence, contact rates with the parasite may be reduced via reduced feeding activity. Such behavioural aspects of resistance may also include altered swimming behaviour, which can, in turn, affect dispersal (see below).

Even though resistance [70] and dispersal [71] are usually considered costly traits, we do not necessarily always expect trade-offs to evolve. In certain bacteria, cell surface modification conferring resistance to bacteriophages leads to reduced motility [72,73]. Experiments also demonstrated that phage selects for increased bacterial dispersal [72,74]. However, the link between dispersal and resistance was not clear, implying that the two traits evolved independently. This suggests that, apart from mechanical or physiological constraints, the emergence of evolutionary trade-offs also depends on the underlying genetic architecture and relationships with fitness, or on the environmental conditions [46,55,75,76].

Finally, we highlight that trade-offs can be a matter of perspective and scale. A (genetic) trade-off with resistance indeed suggests that parasites constrain the evolution of dispersal syndromes at the range front. Yet, if we compare the two traits between range core and front treatments (rather than between parasite/no parasite treatments), their association is positive: infected front populations have both higher resistance and higher dispersal, when compared with the corresponding core populations (figure 2a,b, right panels). In fact, this pattern matches findings for natural populations of the invasive cane toad, where front populations show both higher resistance to lungworm parasites and larger dispersal-related morphological traits [33,42]. These observations suggest that resistance and dispersal both respond positively to selection at the invasion front. Of course, this positive association may not be a general rule. Depending on the biological system, parasite encounter rates may be more or less frequent at invasion fronts, generating higher or lower defence levels [77,78].

(b) Parasitism exacerbates $r_0 - N$ trade-off, but reduces range core-front divergence

On the demographic axis of the multi-trait space, we found opposing directions for equilibrium density ($N$) and population growth rate ($r_0$) along the first PC axis, indicating a trade-off typical of classic r-K selection theory (figure 1). This axis also separated populations from the range treatment front (high $N$, low $r_0$) and range core populations (low $N$, high $r_0$). This pattern seems to contradict common ideas about dispersal syndrome evolution [79], predicting competitive strategies in the core (high $K$ or $N$) and opportunistic strategies at range fronts (high $r_0$). However, in our system as well as in another protist, competitive ability is characterized by growth rate, rather than equilibrium density [58,60]. Thus, if food bacteria are a slowly regrowing resource, rapacious high-growth protists arefavoured in the core treatment and more slowly growing variants at the front [60].

As in the previous section, parasite effects on these demographic traits are a matter of perspective. On the one hand, the $r_0 - N$ trade-off is more pronounced for populations evolving with the parasite than for parasite-free populations. This trend appears relatively weak for overall treatment means (figure 2c,d), but is well supported by supplementary analysis of correlations across monoclone lines within treatments, showing consistent negative $r_0 - N$ correlations for the parasite treatment (electronic supplementary material, table S9). Possibly, this pattern reflects indirect responses to selection on resistance, via costs of resistance, acting on demographic traits [80]. On the other hand, the presence of the parasite decreased the trait differences between range core and front treatments, when compared to the parasite-free treatments (figure 2c,d). This equalizing tendency might mean that the parasite makes demographic conditions in core and front populations more similar, and/or that potential general indirect effects of parasite-mediated selection partly override spatial selection on growth traits.

(c) Range expansion, but not parasitism influences swimming behaviour

Our data show that *Paramecium* can (evolve to) swim either fast and fast or more slowly and take more turns (figure 1). *Paramecium* from the range front treatment were clearly more of the slow type, consistent with previous observation [58]. This contradicts results in a very similar experiment with another ciliate [60] and observations in other biological systems, where populations at the range front show enhanced movement ability [35,38]. In our case, speed reduction was associated with increased swimming tortuosity. This latter tendency to change directions while swimming could represent an exploratory behaviour that might influence the probability of finding the dispersal corridors in our connected microcosms. While our range selection treatment had a strong impact on movement behaviour, there was no discernible effect of the parasite treatment. Thus, unlike for the other trait pairs, parasite-mediated selection does not seem to play a role in shaping the evolution of these traits. As stand-alone traits, speed and tortuosity are only weakly correlated with dispersal (electronic supplementary material, table S9), suggesting that the effect of parasite treatment on dispersal must operate through other aspects of movement.

(d) From eco to evo and back: is the evolution with a parasite slowing down range expansions?

The evolution of dispersal syndromes typically produces ecological feedbacks that speed up range expansions [4]. Parasites, by contrast, may keep in check host populations and thereby limit or slow down range fronts, as generically shown for interspecific (antagonistic) interactions [5,11,81]. Parasites may indeed modify ecological dynamics by affecting host demography and dispersal during a range expansion, and consequently alter the strength or sign selection on these traits. Moreover, parasite resistance is yet another trait under biological feedbacks that speed up range expansions [4].

The open question is how these evolutionary changes in turn feedback on the ecological dynamics of a range expansion, regarding the demography, dispersal or expansion speed. One of our main findings is that the presence of the parasite in the range front treatment favours higher resistance, but also leads to lower dispersal, when compared to the parasite-free treatment (figure 2a,b). Thus, an intuitive prediction is that evolution with the parasite slows down range expansion speed, owing to this resistance-dispersal trade-off. To apply this prediction to natural systems is difficult because these might rarely be replicated with and without parasites.
However, as discussed above for the cane toad example, there are cases where resistance evolution does occur at the range expansion front [42]. Even though it is unknown how the resistance evolution in the toad is correlated with dispersal traits, it seems obvious that, in the face of a detrimental parasite (a lung worm), having the resistance will benefit the range expansion more than not having it [12], at least as long as parasites equally infect high- and low-dispersal individuals, preventing escape into enemy-free space.

Beyond these simple considerations, however, answers may be more complex [82], if we add a multi-trait perspective. As we have shown here, parasite-mediated selection may affect some dispersal syndrome components, but not others, or even modify the correlation between these components. Moreover, a full picture would require measurements of growth, dispersal and movement in the presence of infection, thereby integrating aspects or virulence/tolerance or context-dependent dispersal [54,55]. In a next step towards a more comprehensive understanding of eco-evo feedbacks, future experiments can use multi-microcosm landscapes to actually measure range expansion speeds for the different types of evolved hosts, with and without parasites. Ideally, this would be accompanied by theory exploring the simultaneous evolution of dispersal and interaction traits [83] or accounting for additional factors, such as parasite evolution [54], dispersal plasticity [46,55] or the general upregulation of immune responses, known to occur during range expansion [77,78].

5. Conclusion

Our selection experiment illustrates the possible impact of biotic interactions on evolutionary trajectories during range expansions. Parasite-mediated selection changed the structure of dispersal syndromes, shaping the phenotypic divergence between front and core populations for multiple traits, including dispersal, life-history and resistance. Here we focused on the single-sided host evolution, but dispersal syndromes also evolved in the parasite [54]. It could mean that our observed host changes may have an additional coevolutionary component. This may further complicate the interplay between spatial selection and biotic (co-)evolution and make it even more difficult to predict the accompanying ecological feedbacks on range expansion dynamics or disease spread. Replicated range expansions under laboratory conditions represent one tool to disentangle these processes. Although being simplified abstractions of the real world, such experiments may also provide baseline information for applied issues of bioccontrol or the monitoring of emerging diseases.

Data accessibility. The data from this study are available at https://doi.org/10.5281/zenodo.7123494 [84].

The data are provided in the electronic supplementary material [85].

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