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The interplay between movement, morphology and dispersal in *Tetrahymena* ciliates

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Abstract

Understanding how and why individual movement translates into dispersal between populations is a long-term goal in ecology. Movement is broadly defined as "any change in the spatial location of an individual", whereas dispersal is more narrowly defined as a movement that may lead to gene flow. Because the former may create the condition for the latter, behavioural decisions that lead to dispersal may be detectable in underlying movement behaviour. In addition, dispersing individuals also have specific sets of morphological and behavioural traits that help them coping with the costs of movement and dispersal, and traits that mitigate costs should be under selection and evolve if they have a genetic basis.

Here we experimentally study the relationships between movement behaviour, morphology and dispersal across 44 genotypes of the actively dispersing unicellular, aquatic model organism *Tetrahymena thermophila*. We used two-patch populations to quantify individual movement trajectories, as well as activity, morphology and dispersal rate. First, we studied variation in movement behaviour among and within genotypes (i.e. between dispersers and residents) and tested whether this variation can be explained by morphology. Then, we address how much the dispersal rate is driven by differences in the underlying movement behaviour.

Genotypes revealed clear differences in terms of movement speed and linearity. We also detected marked movement differences between resident and dispersing individuals, mediated by the genotype. Movement variation was partly explained by morphological properties such as cell size and shape, with larger cells consistently showing higher movement speed and higher linearity. Genetic differences in activity and diffusion rates were positively related to the observed dispersal and jointly explained 47% of the variation in dispersal rate. Our study shows that a detailed understanding of the interplay between morphology, movement and dispersal may have potential to improve dispersal predictions over broader spatio-temporal scales.

Introduction

Individual movement is a universal feature of life with broad implications for the ecology and evolution of species (Turchin 1998). As most environments are spatially structured, understanding how individuals move across increasingly fragmented landscapes is of crucial importance (Baguette & Van Dyck 2007). Individual movement can be defined as "any change in the spatial location of an individual in time" (Nathan *et al.* 2008). Dispersal movements are more specifically defined as the result of a specific movement type, i.e. movement that can potentially (but does not necessarily) lead to gene flow (Baguette *et al.* 2014) and are vital for the persistence of spatially-structured populations. Although dispersal implies a change in spatial position, it goes beyond mere movement: it is a central life history trait (Bonte & Dahirel 2017), which can be conceptualized as a three stage process where decisions are taken during emigration, transition and immigration (Clobert *et al.* 2009). Movement patterns may hence vary according to the costs of dispersal (Bonte *et al.* 2012), for instance due to the type of habitat that is encountered (Schtickzelle *et al.* 2007). Few studies try to integrate drivers of small-scale individual movements with dispersal, although previous work has shown the potential of movement to predict large scale spatial dynamics from short spatio-temporal scales, if variation in movement is properly accounted for (Morales & Ellner 2002). This is important because dispersal has wide implications for population dynamics and the spatial distribution of genetic diversity (Bowler & Benton 2005; Ronce 2007; Clobert *et al.* 2012; Jacob *et al.* 2015a).

Variation in movement and dispersal, and covariation with traits such as morphology and behaviour, is the raw material for selection in spatially structured environments and can lead to dispersal syndromes, i.e. consistent co-variation among traits (Ronce & Clobert 2012; Stevens *et al.* 2012). Variation in both movement and dispersal has been reported within and among many different organisms (Austin *et al.* 2004; Mancinelli 2010; Chapperon & Seuront

2011; Ducatez *et al.* 2012; Debeffe *et al.* 2014; Dahirel *et al.* 2015). Some of this variation can be due to environmental causes (e.g. different resource availability, Fronhofer *et al.* 2018), but there is also evidence for genetic effects (Haag *et al.* 2005; Edelsparre *et al.* 2014). As only the latter can lead to the evolution of dispersal and movement strategies, it is important to understand when dispersal and movement variation is genetically or environmentally based. The development of new technology has recently given us a better grasp on how individual variation in movement is related to dispersal. Individual tracking of roe deer showed that exploratory movements were mainly performed by individuals that would later disperse (Debeffe *et al.* 2013, 2014), and butterflies show links between movement ability and dispersal (Stevens *et al.* 2010). Currently, effects of proxies like body condition are very species and context-specific. However, movement traits have potential to more generally predict which individuals are most likely to disperse.

Besides movement, differences in morphology, physiology and behaviour have been found when comparing dispersers and residents (Niitepõld *et al.* 2009; Edelsparre *et al.* 2014). For instance, body condition and morphology have been found to influence individual dispersal decisions in mole rats, ciliates, lizards and butterflies and many other organisms (O'Riain *et al.* 1996; Fjerdingstad *et al.* 2007; Clobert *et al.* 2009; Stevens *et al.* 2012; Turlure *et al.* 2016). Body size is another important predictor of movement, and has been shown to directly influence the speed with which animals can move (Hirt *et al.* 2017a, b). In general, larger animals can move faster, however, the relationship is non-linear with an optimum, suggesting that the largest species are not necessarily the fastest.

Linking individual movement to dispersal requires us to characterize and understand the underlying sources of variation in both, which has so far mostly be done on insects (Niitepõld *et al.* 2009; Edelsparre *et al.* 2014). Assessing dispersal and movement simultaneously is

difficult because dispersal events (especially long-distance) are difficult to track in the field, and recording movement behaviour with adequate resolution and sample size is technically challenging, leading to the use of indirect methods (Flaherty *et al.* 2010). Alternatively, relationships between dispersal and movement ability have been studied across taxonomic groups in a comparative fashion (Dahirel *et al.* 2015). One noteworthy exception using a direct approach is a study that investigated and supported links between phenotypic and genotypic differences in larval food foraging and dispersal as adults in *Drosophila melanogaster* (Edelsparre *et al.* 2014). "Rover" larvae tend to move longer distances and may leave food patches when foraging, whereas "sitters" tend to move less and rest within their food patch (Osborne *et al.* 1997). In dispersal assays the "rover" genotype also moved greater distances as adult flies, highlighting genetic links between larval mobility and adult dispersal (Edelsparre *et al.* 2014). Experiments with microscopic organisms are ideal to study the connections between dispersal and movement experimentally, because they allow tight control of the genetic and environmental context and hence allow these to be disentangled.

Experimental approaches with microscopic organisms are a convenient way to measure movement and dispersal simultaneously and hence allow us to study pattern and process at a relevant spatial scale (Menden-Deuer 2010; Kuefler *et al.* 2012). Moreover, controlled experiments can partition how much variation in movement is due to genetic and non-genetic sources and therefore advance our understanding of the mechanistic underpinnings of movement strategies and their evolution. In this study, we used the microbial *Tetrahymena thermophila* experimental system.

There is compelling evidence that dispersal in this organism is not solely a diffusive process, but depends on individual decisions triggered by environmental cues. Previous work has revealed that cells modify their dispersal decisions according to cooperative strategies (Chaine

124 *et al.* 2010; Jacob *et al.* 2016), conspecific density and density proxies (Pennekamp *et al.* 2014;
125 Fronhofer *et al.* 2015b), social information from conspecifics (Jacob *et al.* 2015b) as well as
126 competition (Fronhofer *et al.* 2015a), and perform adaptive habitat choice according to thermal
127 preferences (Jacob *et al.* 2017, 2018). Extensive variation in dispersal has previously been
128 observed among genotypes of this actively moving ciliate, however, the underlying movement
129 processes have remained elusive.

130 Previous work has revealed extensive variation in life history traits among genotypes, including
131 trade-offs in general growth performance (including high dispersal ability) and formation of
132 specialized dispersal morphs (Fjerdingstad *et al.* 2007). Later work also revealed dispersal
133 plasticity regarding conspecific density, which could be partly explained by morphological
134 differences (body size and shape) among genotypes (Pennekamp *et al.* 2014)

135 In this study, we investigate the relationships between small-scale individual movement (i.e.
136 cell trajectories), dispersal (i.e. emigration rate) and morphological features (i.e. body size and
137 shape) across 44 genotypes of Tetrahymena thermophila. We characterized the movement
138 behaviour of in terms of activity (number of actively moving cells) and quantitative movement
139 behaviour (speed and the characteristic scale of autocorrelation) via video-based cell tracking
140 (Pennekamp *et al.* 2015). In addition, we measured morphological properties of each genotype,
141 as well as its dispersal rate across the two-patch system. With this data, we addressed the
142 following questions:

143 1) Is there variation in movement behaviour within genotypes (between dispersers and
144 residents) and among genotypes?

145 2) Can this movement variation be explained by morphology (cell size and shape)?

3) How much is the dispersal rate driven by differences in the underlying movement behaviour (activity and movement differences among genotypes)?

Materials and Methods

Model organism

Tetrahymena thermophila is a 30-50 µm unicellular, ciliated protozoan inhabiting freshwater ponds and streams in the eastern part of North America, where it naturally feeds on patches of bacteria and dissolved nutrients (Doerder & Brunk 2012). We used a set of 44 genetically distinct genotypes (clonally reproducing as isolated lines) differing in several life history traits (Fjerdingstad *et al.* 2007; Schtickzelle *et al.* 2009; Chaine *et al.* 2010; Pennekamp *et al.* 2014). All genotypes are stored in suspended animation (frozen in liquid nitrogen) and can be ordered from the Tetrahymena Stock Center (https://tetrahymena.vet.cornell.edu/). Genotypes were kept as isolated monocultures in "common garden" conditions over a large number of generations (> 100) after defrosting, under axenic conditions in Proteose peptone medium enriched with yeast extract, at constant 27°C in a light controlled incubator with a 14:10 h light/dark cycle both prior and during the experiment. Refer to the supplementary material (section 1) for additional information on these genotypes and details of culture conditions.

Experimental quantification of dispersal and movement parameters

We quantified dispersal rate and movement parameters of *T. thermophila* cells using a fully factorial experimental design implying two factors of interest: the genotype (44 genotypes) and the dispersal status (dispersers vs residents). We used the same standardized two-patch system (subsequently referred to as dispersal system) developed in previous work (Fjerdingstad *et al.* 2007; Schtickzelle *et al.* 2009; Chaine *et al.* 2010; Pennekamp *et al.* 2014), consisting of two 1.5 mL microtubes connected by a silicon pipe (internal diameter 4mm, tube length 17mm), filled with medium (see supplementary material, Figure S1). To start the experiment, cells of a

single genotype were pipetted into the "start" tube to obtain a density of 300000 cells/mL, an intermediate cell density commonly observed under our culturing conditions. After mixing the medium to distribute cells evenly in the start tube and 30 minutes of acclimation, the connecting pipe was opened, and cells could freely disperse. At the end of the experiment after six hours, the pipe was closed by a clamp and five independent samples were taken from both the start and the target tubes of each dispersal system. Cells found in the "start" or "target" are subsequently referred to "residents" or "dispersers", respectively, the two modalities possible for the dispersal status variable. Five dark field images (one for each chamber; resolution: 5616 x 3744 pixels) and one 40 s long video (of a randomly chosen chamber; HD resolution: 1920 x 1080 pixels; 25 frames per second) were then taken using a Canon EOS 5D Mark II mounted on a Nikon Eclipse 50i microscope with a 4x lens; the real size of the imaged area is about 6.3 x 4.5 mm and was not bounded by external borders, hence cells could swim in and out the viewing field. Supplementary material (section 2) gives additional information about the experimental protocol and material used.

Images were treated using an objective and automated image analysis workflow to count individual cells and record morphology descriptors (**cell size** and **cell shape**); this workflow is based on ImageJ (Schneider *et al.* 2012) and was carefully validated and extensively optimized to produce accurate and repeatable results (Pennekamp & Schtickzelle 2013). **Dispersal rate** of a genotype was estimated as the ratio of density in the target tube to the overall density (start + target), i.e. the proportion of cells in the target.

Individual cell trajectories were obtained from the digital videos in a standardized and automated fashion with a workflow that was later transformed into the R package BEMOVI (Pennekamp *et al.* 2015) and was successfully used in previous studies extracting movement characteristics from video sequences (Banerji *et al.* 2015; Fronhofer *et al.* 2015b; Griffiths *et al.* 2018). The position of each cell was followed over all the 1000 frames (40 s long video

with 25 frames per second; Figure S2). First, the **activity** level of cells was computed from videos as the ratio of cells that moved (trajectory duration > 1 s and minimum displacement > 50 µm, i.e. one body length) divided by the total number of trajectories (moving and non-moving).

Then, trajectories were analysed with continuous time movement models (Fleming *et al.* 2014; Gurarie *et al.* 2017) to compute movement speed and linearity. Continuous time movement models are a natural choice for high-frequency sampling of video microscopy because they can deal with autocorrelation in the movement speed and positions. We used the smoove package in R (Gurarie *et al.* 2017) to fit a hierarchical family of correlated velocity model, basically continuous-time equivalents of the widely applied correlated random walk, with biologically intuitive parameters such as movement speed and characteristic time scale (a measure of the decay in directional persistence). For each genotype, we randomly subsampled 23 trajectories per replicate and tube resulting in a total of 6072 trajectories. The subsampling was necessary because analysis with continuous time movement models is computationally demanding due to the model selection procedure involved. Subsampling also ensured the same number of data points per genotype. For each trajectory, we fitted four models: an unbiased correlated velocity model (UCVM), an advective correlated velocity model (ACVM), a rotational correlated velocity model (RCVM) or a rotational advective correlated velocity model (RACVM). The best fitting model for a given trajectory was selected via a model selection procedure based on the Akaike information criterion (AIC), and parameters of the model estimated. For each trajectory, we extracted two parameters for further analysis: the random **movement speed** (r.m.s) and the characteristic time scale of autocorrelation (parameter tau), essentially a measure of **movement linearity**. When tau tends towards zero, the movement approaches random Brownian motion, while tau tending towards infinity indicates perfect linear motion (Gurarie *et al.* 2017). Before further analysis, we performed an outlier exclusion based on the

Median Absolute Deviation (MAD) with a threshold of 3 (Leys *et al.* 2013) for the two parameters estimated. The supplementary material (section 3) gives additional details concerning trajectory reconstruction from video, cleaning and estimation of movement metrics.

In summary, each dispersal system produced measures for six response variables: two morphology descriptors (cell **size** and **shape**, extracted from images), three movement descriptors (**activity**, **speed**, and **linearity** extracted from videos), and **dispersal rate** (computed from cell densities extracted from images). For all statistical analyses, these response variables were aggregated to produce two values per dispersal system, one for the start tube (residents) and another for the target tube (dispersers); indeed, the true level of replication in this experiment was the dispersal system (genotype x dispersal status combination) and not the individual trajectory. With 3 dispersal systems (replicates) per genotype, sample size was 264 (44 genotypes * 3 replicates * 2 dispersal status); note that one dispersal system (genotype 32_I) was discarded due to a technical failure of the dispersal system, meaning n=262. Cell size and shape were averaged over all cells found on the five images recorded per tube; activity was directly measured at the video level (1 measure per tube) and hence already "pre-aggregated" at the correct level; speed and linearity were averaged over the 23 trajectories analysed by continuous time movement models on each video; and dispersal rate was computed from densities averaged over the five images recorded per tube.

Statistical analyses

To address our first question, activity and movement metrics (speed and linearity) were compared among genotypes and among dispersal status (disperser vs resident cells) using a three-way ANOVA, with genotype and dispersal status as crossed and fixed effects, and replicate as random effect nested in genotype but crossed with dispersal status. Genotype was considered as a fixed effect, despite its common consideration as a random effect (e.g. Crawley

2007). This is because the set of genotypes cannot be considered as a random sample of the genetic variation exhibited by the species in the wild (some genotypes were selected due to previous results or based on their phenotypic characteristics, some others were created by inbreeding in the laboratory). Dispersal status was crossed with replicate because the data for the two statuses (disperser and resident, i.e. target and start tubes respectively) were paired for each dispersal two-patch system. Speed and linearity (tau) were ln-transformed to improve normality.

All cells belonging to the same genotype should have the same genetic make-up; however, environmental differences encountered during the cell life cycle may lead to different morphologies and cell states. Therefore, to answer our second question, we tested whether differences in movement behaviour between residents and dispersers may be explained by morphological differences such as cell size and shape. To see if there were differences between residents and dispersers, we built ANCOVA models that related movement speed and linearity to morphology properties (size and shape) across genotypes, accounting for differences due to dispersal status. As some of the observed variation may be due to variation across replicates, we investigated how within replicate differences in morphology affect differences in movement. We used the Akaike Information criterion (AIC) to determine the most parsimonious model, i.e. the simplest model (in terms of number of parameters) within 2 units (deltaAIC < 2) of the best model (i.e. with the lowest AIC).

To address our third question about the power of movement behaviour to predict dispersal rate, we assessed how much variation in dispersal rate was explained by genotype-specific activity, movement speed, movement linearity and all predictors together. We used the R^2 of a multiple regression and compared the three models with the Akaike Information criterion (AIC) to find the best fitting model. For this analysis, movement metrics (activity, movement speed and linearity) were averaged at the genotype level, i.e. over dispersers and residents.

Results

Q1: variation in movement behaviour within and among genotypes

Model selection across the four types of correlated velocity models revealed that the advective correlated velocity model (ACVM) was the most common across genotypes, indicating the genotypes show directed movement. The dispersal status did not change the overall pattern, but genotypes showed variation in the relative frequencies of movement models (Figure 1). Genotypes differed in activity (min. 39% to max. 70% of total cell population moving) and movement parameters extracted from the correlated velocity models: movement speed (min. 75 to max. 289 µm/s) and linearity (tau: min. 0.039 to max. 0.13). Additionally, a highly significant difference was shown between dispersal status: compared to residents, dispersers were characterized by a higher activity (0.62 +/- 0.05 vs. 0.57 +/- 0.08) and faster and more linear movements (speed +/- SD: 171 +/- 52.5µm/s vs. 139 +/- 52.0; tau: 0.0804 +/- 0.0271 vs. 0.0602 +/- 0.0244). For the majority of genotypes the dispersers moved faster and more linear, while for some genotypes the opposite was observed (significant genotype x dispersal status interaction for both movement metrics; Table 1, Figure 2). Across genotypes the speed and linearity strongly positively co-varied ($b = 0.000383$, $t = 10.961$, $p < 0.001$), meaning faster cells also swam straighter. Neither intercept nor slope differed between residents and dispersers (Figure S3).

Q2: link between movement behaviour and morphology

First, the influence of cell morphology on cell movement across genotypes and replicates was analysed (Figure 3). The most parsimonious model indicated a positive effect of size on movement speed in addition to the higher speed generally found in dispersers (Tab. S2). Speed was also affected by shape differences: more elongated disperser cells moved faster, whereas the opposite was observed for residents (Tab. S2). We also found that larger cells moved

straighter. The slope of this relationship did not differ among dispersal status, however, dispersers moved straighter on average (Tab. S3). The relationship between shape and linearity again was dependent on the dispersal status: whereas higher elongation led to more linear movement for dispersers, residents showed no pattern with higher elongation (Tab. S3). Within genotypes, larger relative size of dispersers compared to residents led to higher relative movement speed, whereas a larger relative elongation resulted in a decrease in relative speed (Figure S4, Tab. S4-S5).

Q3: predicting dispersal rate based on movement parameters

Consistent with previous experiments, we observed major differences among genotypes in dispersal rate in the two-patch experiment (Figure 4). The genotypes had significantly different dispersal rates over 6 h (one-way ANOVA: $F_{43,87} = 9.93$, $p < 0.001$), continuously distributed in the 7 - 71% range; the majority of genotypes had a dispersal rate lower than 50%. Variation among the 44 genotypes in activity and movement behaviour explained a substantial amount of the variation observed in their dispersal rates. Only considering activity explained 27% of the variation in dispersal rates among genotypes (AIC = -56.21). The genotype-specific movement linearity explained a lower amount of variation (24%, AIC = -54.55) while speed explained a larger percentage of the dispersal variation (37%, AIC = -62.86). Including activity, speed and linearity explained almost 50% of the variation in dispersal (47%, AIC = -66.79). This result indicates that activity and movement features jointly influence the dispersal rate exhibited by a genotype and provide complementary information about dispersal.

Discussion

We show that 44 genotypes of *Tetrahymena thermophila* kept in “common garden” conditions over many generations exhibit continuous variation in movement parameters (activity, movement speed and linearity). Activity, movement speed and linearity were found to be

317 genotype-dependent, and differed with dispersal status. Although cells within the same
318 genotype have the same genetic make-up, environmental differences encountered during the
319 cell life cycle may lead to different movement behaviours. We show that some of the movement
320 variation can indeed be explained by morphological differences among genotypes and this may
321 explain also within genotype variation. Finally, movement variation and cell activity was
322 highly predictive of dispersal, explaining 47% of the observed variation.

323 Genotype-based movement behaviour differences

324 So far there are a limited number of model systems where the genetic basis of dispersal has
325 been studied in detail (summarized by Wheat 2012). In *Drosophila*, allelic variation in the
326 candidate gene *for* is known to influence the foraging behaviour of larvae; additionally recent
327 research has demonstrated that phenotypic and genotypic variation mainly due to the *for* gene
328 also influences adult dispersal distances (Edelsparre *et al.* 2014). Interestingly, the protein
329 encoded by the *for* gene in *Drosophila*, a cGMP-dependent protein kinase, responsible for the
330 observed behavioural variation in foraging, is also known to influence cilia-mediated
331 chemotaxis in *T. thermophila (Leick & Chen 2004)*. Another example is the nematode
332 *Caenorhabditis elegans* where the *npr1* gene is associated with both foraging strategy and
333 dispersal behaviour (Gloria-Soria & Azevedo 2008). Finally, dispersal is heritable in the
334 butterfly *Melitaea cinxia* on the Aland archipelago: young and isolated populations have higher
335 frequencies of dispersive female individuals carrying the *PGI* genotype, a genotype associated
336 with higher flight metabolic rate that increases the probability to reach such habitats (Haag *et*
337 *al.* 2005). These examples show that genetic links between movement and dispersal exist and
338 are consistent with our results, where movement over short spatio-temporal scales correlates
339 with dispersal over much larger spatio-temporal scales. *T. thermophila* may be a good model
340 species for studying these questions using experimental evolution approaches. Promising
341 directions for future research would be to understand how different selection pressures for

342 movement (within patches) and dispersal (among patches) interact and affect eco-evolutionary
343 dynamics in metapopulations (Jacob *et al.* 2015a, 2017, 2018; Van Petegem *et al.* 2015) and
344 during range expansions (Fronhofer & Altermatt 2015), contributing to a broader
345 understanding of spatial patterns in ecology.

346 Movement differences between dispersers and residents, and their relationship
347 with morphology

348 We have found significant variation in movement within genotypes, which was modulated by
349 the genotype (significant genotype by dispersal status interaction): disperser cells within the
350 same genotype moved faster and straighter than residents, suggesting different movement
351 strategies, which were realized to different degrees by different genotypes. These differences
352 are partly explained by cell morphology co-varying with movement. This is expected, as the
353 energetic costs of movement of microscopic organisms in aquatic environments are heavily
354 influenced by their morphology such as cell elongation and size (Mitchell 2002; Young 2007).
355 Indeed, we found that larger cells moved faster, regardless of their dispersal status. The shape
356 of the cells also influenced speed and linearity: dispersing cells that were more elongated
357 moved faster and more linear, whereas resident cells did not show such a relationship. The
358 differences in movement speed are likely due to different costs associated with motion in the
359 liquid medium, with larger cells potentially having larger energy reserves and/or stronger
360 movement machinery (Mitchell 2002). This is corroborated by the fact that size always
361 favoured faster movement, even when accounting for the genotype effect (see Figure S4). Our
362 results therefore closely agree with recent findings about a general allometric relationship
363 between body size and movement speed (Hirt *et al.* 2017a, b).

364 We have shown that movement variation can be partly explained by different cell sizes and
365 shapes. This is in line with previous findings on the condition dependence of dispersal that

366 indicated that cell size and shape have an influence on the dispersal propensity (Pennekamp *et*
367 *al.* 2014). However, in contrast to dispersal, larger and more elongated cells move faster and
368 straighter, whereas more elongated and smaller cells disperse more. This contrasting result
369 suggests that although larger cells may be superior in terms of movement ability, they may not
370 disperse as much as expected as other causes of dispersal may be more important; for instance,
371 dispersal decisions may be taken as a function of competitive ability rather than movement
372 ability *per se* (Fronhofer *et al.* 2015a). If cell size positively co-varies with competitive ability,
373 smaller cells may disperse to escape the local competition although they have relatively weaker
374 movement capabilities.

375 Aggregation behaviour of *T. thermophila* ciliates is another candidate for explaining movement
376 differences because aggregation affects the spatial cohesion of a population and is a proxy for
377 cooperative behaviour (Schtickzelle *et al.* 2009; Chaine *et al.* 2010; Jacob *et al.* 2015b). In a
378 previous study, genotypes characterized by different degrees of aggregation did not show any
379 relationship with dispersal (Schtickzelle *et al.* 2009). Instead aggregation co-varied with the
380 occurrence of specialized dispersal morphs, which only appear during prolonged periods of
381 starvation. Given the strong correlation we found between dispersal and movement,
382 aggregation seems less likely to be a causal driver of the observed differences in movement,
383 albeit information about cooperative strategies was found to influence dispersal decisions
384 (Jacob *et al.* 2015b).

385 ## Explaining dispersal rate with activity and movement variation

386 The amount of variation explained increased from 27% accounting only for genotype-specific
387 cell activity level, to 37% when considering only genotype-specific movement speed, and up
388 to 47% when considering genotype-specific activity and movement. Activity and movement
389 hence provide complementary information about dispersal. For instance, in certain genotypes,

individual cells may move faster and straighter, but their activity level may be lower, compared to a less mobile genotype were cells are generally more active. The increasing amount of variation explained in our study supports the claim of previous studies that behavioural differences are important for the correct prediction of large scale population distributions from small scale movement observations (Morales & Ellner 2002; Newlands *et al.* 2004). However, our results also indicate that other processes, including subtle behavioural differences among genotypes to enter narrow tubes, may contribute to the observed variation in dispersal. As the causes of movement and dispersal are not entirely known for each genotype in our study, both positive and negative influence on the genetic variation are plausible as one cause (e.g. density of conspecifics) may be more important for some genotypes than for others (Pennekamp *et al.* 2014).

What are the consequences of the geno- and phenotypic variation in movement behaviour observed in our study?

Natural populations of *Tetrahymena thermophila* ciliates are often constituted of multiple genotypes (Doerder *et al.* 1995), which may differ in movement behaviour as shown here. Modelling work has shown that communities/populations consisting of multiple phenotypes can actually show faster invasion speeds than that of the fastest monomorphic population alone (Elliott & Cornell 2012). This was, however, only the case if the two phenotypes, i.e. a resident and a dispersive type, showed co-variation between growth rate and dispersal ability (e.g. well growing but poorly dispersing resident vs. poor growing and well dispersing establisher) and if the ratio between genotypes in these parameters varied two- to ten-fold. Looking at the variation of our genotypes (Figure 4), we see that the ratio in dispersal rate can be up to ten-fold depending on the genotypes contrasted. This suggests that with a known variation in growth rate with a factor of about two (Pennekamp 2014), accelerating invasions of

Tetrahymena are possible, if natural populations are more phenotypically diverse. Validating these predictions in experiments with mixed populations and their link with local adaptation would be a fruitful avenue for future research.

Conclusions

Our study showed a close link between movement and dispersal on multiple levels. Dispersal predictions steadily improved when genotype differences in both activity level and movement behaviour were considered. This highlights that predictions of dispersal will benefit from a detailed understanding of the underlying movement behaviour. To move beyond short-term ecological predictions of dispersal dynamics, e.g. range expansions and range shifts due to environmental change, we would need to further improve our understanding of how movement is affected by environmental variation and the relative fitness prospects of cells if staying in their current habitat patch or emigrating to another patch, which can lead to habitat choice, which has been shown in the species linked to temperature (Jacob *et al.* 2017, 2018).

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Tables

Table 1: Three-way ANOVA to assess the effect of genotype and the dispersal status (i.e. difference between dispersers and residents) on three movement metrics: activity (proportion of moving cells), movement speed and linearity. Genotype and dispersal status were considered as crossed and fixed effects, and replicate as random effect nested in genotype but crossed with dispersal status because data from the two status were paired per replicate (i.e. the start and target tubes of one dispersal system). Arrows indicate the error term used to test for each effect, according to the ANOVA model; "-" denote the factors that cannot be tested because the error has no degrees of freedom in this model.

	Response variable		activity				speed: ln(speed)				linearity: ln(tau)			
Test	Factor	DF	SS	MS	F value	p	SS	MS	F value	p	SS	MS	F value	p
	genotype	43	0.872	0.020	2.88	< 0.0001	24.927	0.580	12.40	< 0.0001	24.666	0.574	7.50	< 0.0001
	dispersal status (disperser vs resident)	1	0.186	0.186	42.88	< 0.0001	3.193	3.193	149.28	< 0.0001	6.718	6.718	93.19	< 0.0001
	genotype * dispersal status	43	0.445	0.010	2.39	0.0003	3.977	0.092	4.32	< 0.0001	7.036	0.164	2.27	0.0006
	replicate (genotype)	87	0.612	0.007	-	-	4.067	0.047	-	-	6.653	0.076	-	-
	replicate * dispersal status (genotype)	87	0.377	0.004	-	-	1.862	0.021	-	-	6.272	0.072	-	-
	error	0	0	na			0	na			0	na		
	total	261	2.490				38.020				51.317			

Figures

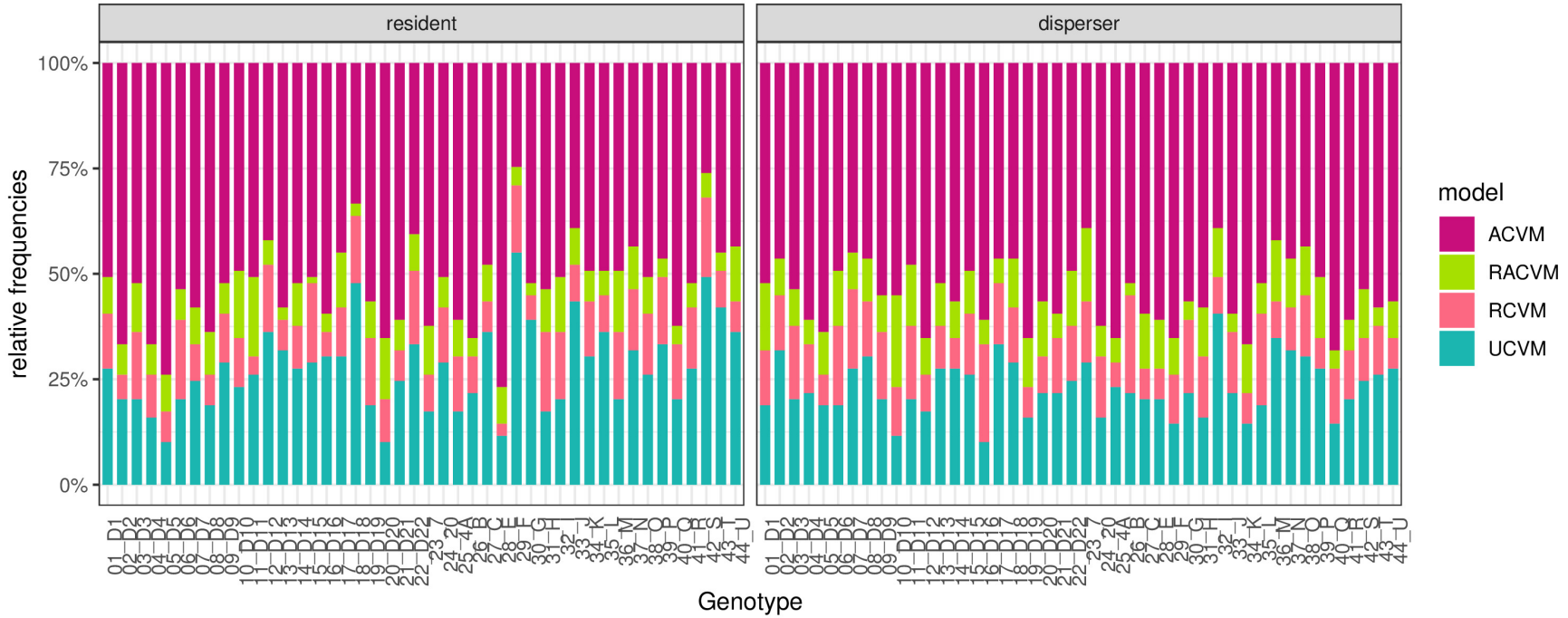


Figure 1: Model selection for the four types of continuous time movement models fitted to 23 randomly selected trajectories per combination of genotype and dispersal status (disperser vs resident). Relative frequencies of the most parsimonious model shown. The ACVM model is the most represented, followed by the UCVM. Some trajectories are best represented by rotational variants (RACVM and RCVM).

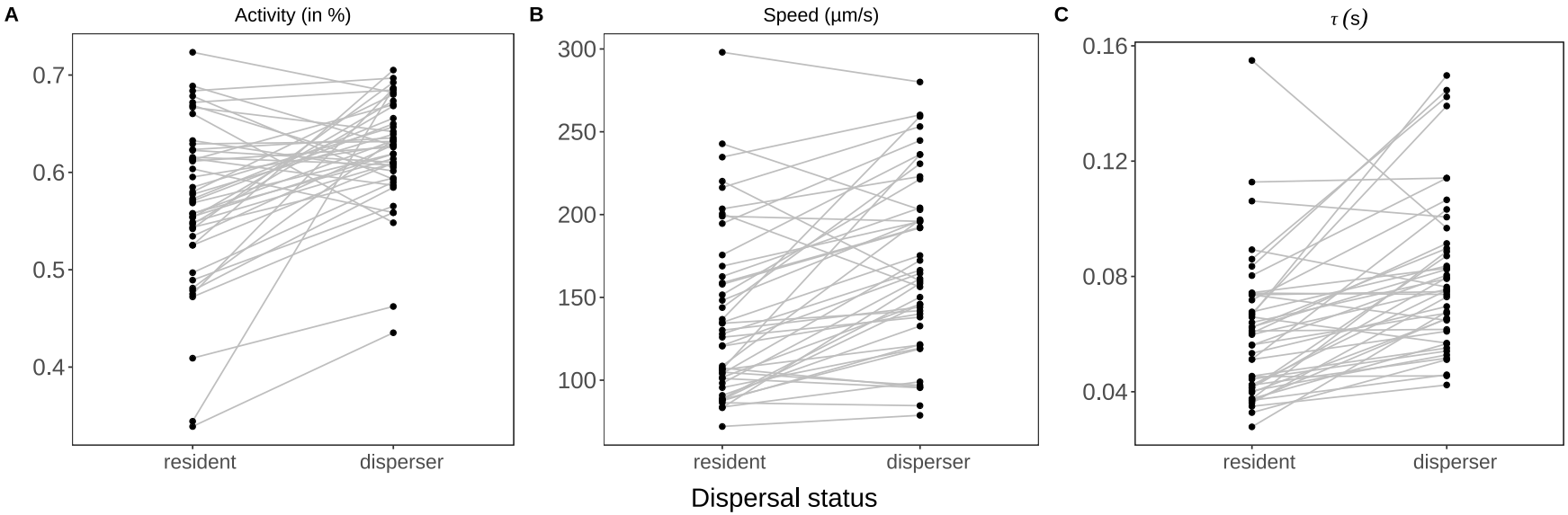


Figure 2: Overview of among and within genotype variation in activity and movement metrics (speed and tau, i.e. linearity). Each line shows a genotype and its slope indicates differences in movement among status (disperser vs resident).

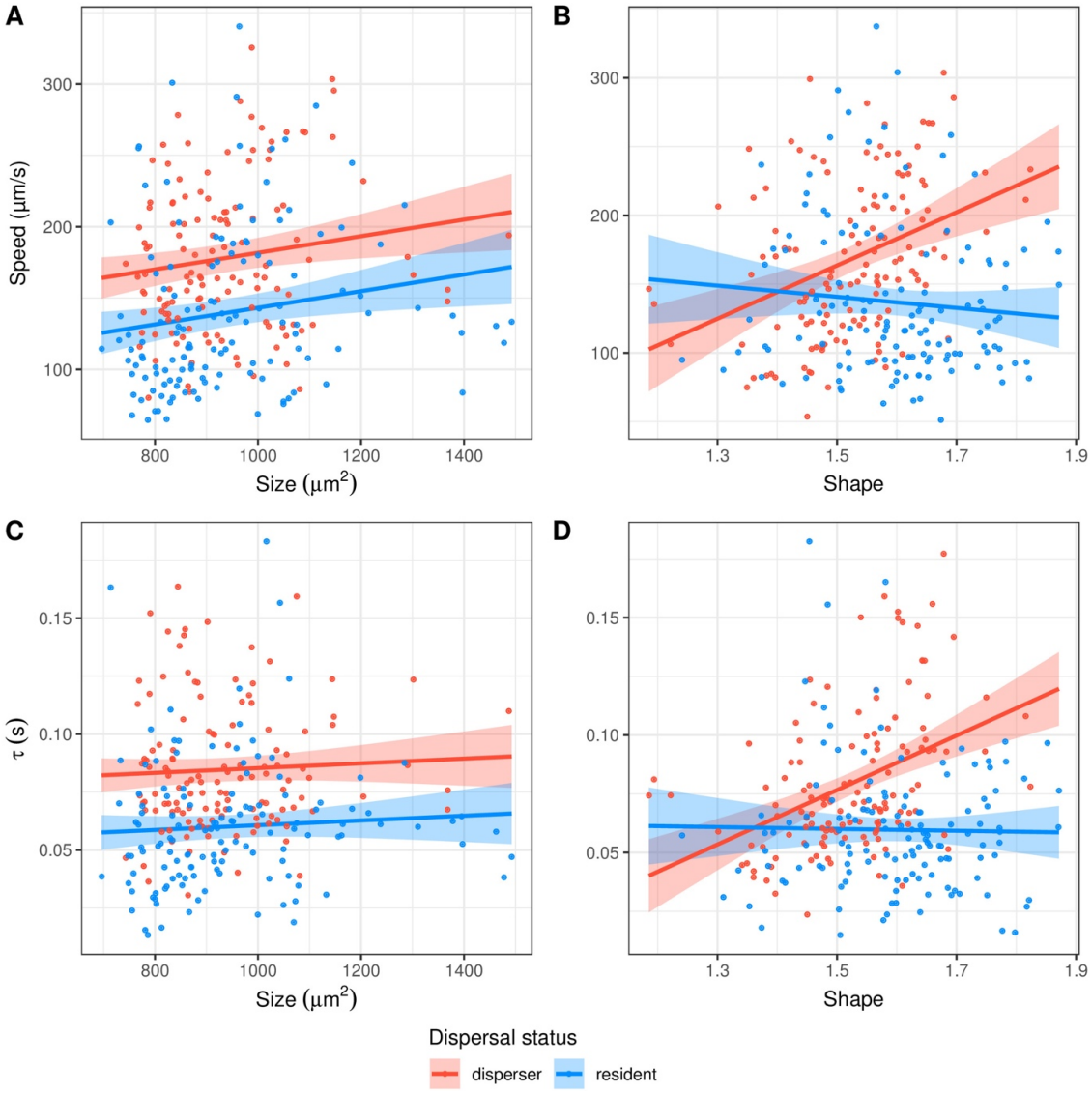
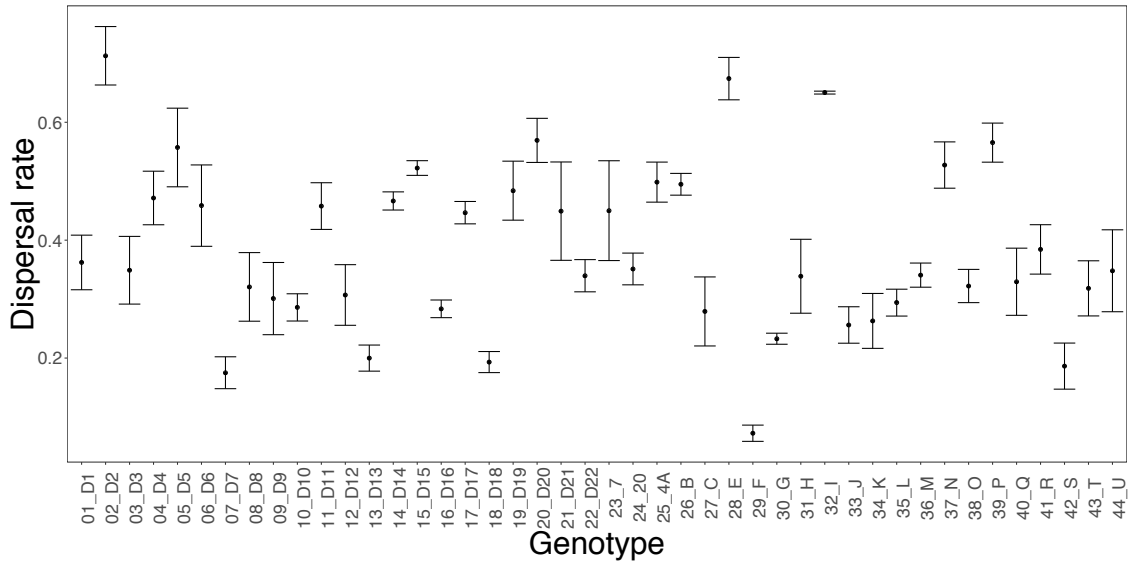


Figure 3: Relationships between movement metrics (speed and tau, i.e. linearity), dispersal status (red and blue) and cell morphology (size and shape). Lines and confidence intervals show the partial effects of size and shape of the most parsimonious ANCOVA model (n=262). Larger cells moved faster but not more linear, with an overall higher level in dispersing cells. In contrast, only in dispersing cells elongation resulted in faster and straighter movement, whereas the opposite was observed in resident cells.



620

621 **Figure 4:** The 44 genotypes differed in their dispersal rate in the two-patch experimental
622 system over a period of 6 h. The point represents the mean dispersal and the error bars the
623 standard error of the mean (n=3 per genotype).

624