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

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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 expressed different movements in terms of speed and path tortuosity. 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 lower tortuosity. Genetic differences in activity and diffusion rates were positively related to the observed dispersal and jointly explained 45% 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.

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 expressed different movements in terms of speed and path tortuosity. 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 lower tortuosity. Genetic differences in activity and diffusion rates were positively related to the observed dispersal and jointly explained 45% 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 and 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). Although dispersal implies a change in spatial position, it goes beyond mere movement: it is a central life history trait (Bonte and 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 and Ellner 2002). This is important because dispersal has wide implications for population dynamics and the spatial distribution of genetic diversity (Bowler and Benton 2005, Ronce 2007, Clobert et al. 2012, Jacob et al. 2015b).

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 and 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 and 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), 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). Movement traits hence have potential to 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

95 movement behaviour with adequate resolution and sample size is technically challenging, leading
96 to the use of indirect methods (Flaherty et al. 2010). Alternatively, relationships between dispersal
97 and movement ability have been studied across taxonomic groups in a comparative fashion
98 (Dahirel et al. 2015). One noteworthy exception using a direct approach is a study that investigated
99 and supported links between phenotypic and genotypic differences in larval food foraging and
100 dispersal as adults in *Drosophila melanogaster* (Edelsparre et al. 2014). "Rover" larvae tend to
101 move longer distances and may leave food patches when foraging, whereas "sitters" tend to move
102 less and rest within their food patch (Osborne et al. 1997). In dispersal assays the "rover" genotype
103 also moved greater distances as adult flies, highlighting genetic links between larval mobility and
104 adult dispersal (Edelsparre et al. 2014).

105 Experimental approaches with microscopic organisms are a convenient way to measure movement
106 and dispersal simultaneously and hence allow us to study pattern and process at a relevant spatial
107 scale (Menden-Deuer 2010, Kuefler et al. 2012). Moreover, controlled experiments can partition
108 how much variation in movement is due to genetic and non-genetic sources and therefore advance
109 our understanding of the mechanistic underpinnings of movement strategies and their evolution.
110 In this study, we used the microbial *Tetrahymena thermophila* experimental system to characterize
111 small-scale individual movement (i.e. cell trajectories) and predict dispersal (i.e. emigration rate)
112 in two-patch systems. Extensive variation in dispersal has previously been observed among
113 genotypes of this actively moving ciliate, which could be partly explained by morphological
114 differences (body size and shape) among genotypes (Fjerdingstad et al. 2007, Pennekamp et al.
115 2014). Previous work has revealed that cells modify their dispersal decisions according to
116 cooperative strategies (Chaine et al. 2010, Jacob et al. 2016), conspecific density and density
117 proxies (Pennekamp et al. 2014, Fronhofer et al. 2015a), social information from conspecifics

(Jacob et al. 2015a) as well as competition (Fronhofer et al. 2015b), and perform adaptive habitat choice according to thermal preferences (Jacob et al. 2017). These studies provide compelling evidence that dispersal in this organism is not solely a diffusive process, but depends on individual decisions triggered by environmental cues.

Here, we characterized the movement behaviour of 44 genotypes in terms of activity (number of actively moving cells) and quantitative movement behaviour (movement speed, tortuosity and diffusion rate) via video-based cell tracking (Pennekamp et al. 2015). In addition, we measured morphological properties of each genotype, as well as its dispersal rate across the two-patch system. With this data, we addressed the following questions:

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

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 and 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 strains) and the dispersal status (dispersers vs residents). We used the same standardized two-patch 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), filled with medium (see supplementary material, Fig. 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. Each culture sample was loaded in a chamber of a counting slide and dark field images and videos were taken using a camera mounted on a microscope; 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 and 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.

To describe movement trajectories, we applied the procedure described by Turchin (1998): successive positions of a cell were linked as straight-line movements, i.e. steps, each separated by a turning angle. 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 other studies to extract movement characteristics from video sequences (Banerji et al. 2015, Fronhofer et al. 2015a). The position of each cell was followed over the all 1000 frames (40 s long video with 25 frames per second). 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). Every trajectory classified as "moving" was then simplified to remove spurious autocorrelation in step lengths and turning angles resulting from very frequent and regular sampling (Turchin 1998), i.e. oversampling positions, by dropping uninformative positions using the Douglas-Peucker algorithm. Movement **speed** was computed for each trajectory by dividing the total distance covered (sum of step lengths) by the trajectory

duration. **Tortuosity** was quantified for each trajectory by the fractal dimension D in the Fractal 5.20 software (Nams 1996); Fractal D is bounded between 1 and 2 with values close to 1 meaning straight trajectories, whereas values close to 2 would be so tortuous that the trajectory covers the entire plane). We transformed the fractal dimension in the regression analyses (i.e. log of FractalD – 1) to meet the assumptions of linearity and homoscedasticity. The **diffusion** coefficient is a population-level measure integrating speed, step length and turning angle distributions (Turchin 1998). We extracted step length, turning angle and net displacement (i.e. the distance between the start and the end position of the trajectory) from each trajectory. Then we used non-linear least squares estimation (nlsList function from nlme package in R) to estimate the diffusion coefficient D for each replicate (pooling the two dispersal statuses, i.e. dispersers and residents from the same system) as the linear slope of mean squared displacement MSD over time according to the following formula: MSD = 4 * D * time (Giometto et al. 2014, Fronhofer et al. 2015a). Because cells can leave the viewing field, we observed a saturation in MSD over time. The diffusion coefficient was therefore estimated over the initial 10s of the video. The supplementary material (section 3) gives additional details concerning trajectory reconstruction from video, cleaning, simplification and estimation of movement metrics.

In summary, each dispersal system produced measures for seven response variables: two morphology descriptors (cell **size** and **shape**, extracted from images), four movements descriptors (**activity**, **speed**, **tortuosity** and **diffusion** rate, 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; speed, tortuosity and diffusion were averaged over all trajectories recorded on each video; activity was directly measured at the video level (1 measure per tube) and hence already "pre-aggregated" at the correct level; 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, tortuosity, diffusion) 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.

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 investigate this condition-dependence of movement, we built ANCOVA models that related movement speed and tortuosity 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 also investigated the relationship between movement and morphology aggregating at the genotype level (mean response), and controlling for the genotype and dispersal status effect by only looking how differences in morphology affect differences in movement.

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, diffusion rate, or both together. We used the R^2 of a linear regression to quantify the match between dispersal and movement, and compared the three models with the Akaike Information criterion (AIC). For this analysis, movement metrics (activity and diffusion rates) were averaged at the genotype level, i.e. over dispersers and residents.

Results

Q1: variation in movement behaviour within and among genotypes

Genotypes differed in activity (39% to 70% of total cell population moving) and movement descriptors: mean movement speed (gross speed 27 to 226 µm/s) and mean trajectory tortuosity (Fractal D: 1.04 to 1.35), combining into differences of diffusion rate (0.0028 to 0.0268 µm²/s) (Table 1). Additionally, a highly significant difference was shown between dispersal status: compared to residents, dispersers were characterized by a higher activity (0.62 +/- 0.008 vs. 0.57 +/- 0.009) and faster and less tortuous movements (mean speed +/- SE: 107 +/- 4.4 µm/s vs. 67 +/- 4.3; Fractal D: 1.14 +/- 0.005 vs. 1.18 +/- 0.006) all combining into a higher diffusion rate (0.0124 vs 0.070 µm²/s). While in most genotypes the dispersers moved faster and less tortuously, in some

cases the opposite pattern was observed (significant genotype x dispersal status interaction for both movement metrics; Table 1, Figure 1). Across genotypes the speed and tortuosity strongly negatively co-varied ($b = -0.08469$, $t = -7.756$, $p < 0.001$), meaning faster cells also swim straighter, the two combining into higher diffusion rate. Both intercept and slope did not differ between residents and dispersers (Figure 2).

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. S3). Regarding path tortuosity, it was found that larger cells moved less tortuous. The slope of this relationship did not differ among dispersal status, however, dispersers moved less tortuous on average (Tab. S4). The relationship between shape and tortuosity again was dependent on the dispersal status: whereas higher elongation led to less tortuous movement for dispersers, residents showed the opposite pattern of more tortuous movements with more elongation (Tab. S5).

To disentangle the contribution of among and within genotype variation, we further looked at the morphology - movement relationships, first aggregating across genotypes and second when accounting for morphology differences between dispersal status and genotypes: among genotypes, only size positively co-varied with movement speed and the average speed differed among dispersal status (Figure S3, Table S6-S9). Within genotypes, positive relative size increases led to positive relative movement speed increases, whereas a positive relative shape increase resulted in a decrease in relative speed (Figure S4, Tab. S10-S13).

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 diffusion coefficient explained an even larger percentage of the dispersal variation (34%, AIC = -61.06), showing that the specifics of the movement behaviour cannot be fully captured by the activity. Finally, including both the activity and diffusion term explained the highest amount of variation (45%, AIC = -67.10). This result indicates that both activity and movement features influence the dispersal rate exhibited by a genotype and provide complementary information about dispersal (Figure 5).

Discussion

We show that 44 genotypes of *Tetrahymena thermophila* kept in "common garden" conditions over many generations exhibit continuous variation in movement parameters (activity, swimming speed and trajectory tortuosity), and that this variation affects dispersal. Activity and movement differences were found to be genotype-dependent but in addition, differed within genotypes, with the differences between dispersal status being contingent on the genotype. Although cells within the same genotype have the same genetic make-up, environmental differences encountered during the cell life cycle may lead to different movement behaviours. We show that some of the movement variation can indeed be explained by morphological differences among genotypes and this may

299 explain also within genotype variation. Finally, movement variation integrated via cell activity and
300 diffusion coefficient was highly predictive of dispersal, explaining 45% of the observed variation.

301 Genotype-based movement behaviour differences

302 So far there are a limited number of model systems where the genetic basis of dispersal has been
303 studied in detail (summarized by Wheat 2012). In *Drosophila*, allelic variation in the candidate
304 gene *for* is known to influence the foraging behaviour of larvae; additionally recent research has
305 demonstrated that phenotypic and genotypic variation mainly due to the *for* gene also influences
306 adult dispersal distances (Edelsparre et al. 2014). Interestingly, the protein encoded by the *for* gene
307 in *Drosophila*, a cGMP-dependent protein kinase, responsible for the observed behavioural
308 variation in foraging, is also known to influence cilia-mediated chemotaxis in *T. thermophila*
309 *(Leick and Chen 2004)*. Another example is the nematode *Caenorhabditis elegans* where the *npr1*
310 gene is associated with both foraging strategy and dispersal behaviour (Gloria-Soria and Azevedo
311 2008). Finally, dispersal is heritable in the butterfly *Melitaea cinxia* on the Aland archipelago:
312 young and isolated populations have higher frequencies of dispersive female individuals carrying
313 the *PGI* genotype, a genotype associated with higher flight metabolic rate that increases the
314 probability to reach such habitats (Haag et al. 2005). These examples show that genetic links
315 between movement and dispersal exist and match our results, where movement over short spatio-
316 temporal scales correlates with dispersal over much larger spatio-temporal scales. This could
317 indicate that dispersal and foraging in *T. thermophila* may not have evolved completely
318 independently but could be, at least partly, due to other fitness influencing behaviours such as
319 foraging (Van Dyck and Baguette 2005). An ecological reason for this link may be that the quest
320 for foraging patches is also a quest to find conspecifics. Therefore, foraging and dispersing are
321 probably linked in non-territorial animals. The idea that dispersal is influenced by selection on

traits with other functions currently receives more attention (Burgess et al. 2016). *T. thermophila* may be a good model species for studying these questions using experimental evolution approaches. Promising directions for future research would be to understand how different selection pressures for movement (within patches) and dispersal (among patches) interact and affect eco-evolutionary dynamics in metapopulations (Van Petegem et al. 2015, Jacob et al. 2015b, 2017) and during range expansions (Fronhofer and Altermatt 2015), contributing to a broader understanding of spatial patterns in ecology.

Movement behaviour differences between dispersers and residents, and their relationship with morphology

We have found significant variation in movement within genotypes, which was modulated by the genotype (significant genotype by dispersal status interaction): disperser cells within the same genotype moved faster and straighter than residents, suggesting different movement strategies, which were realized to different degrees by different genotypes. These differences are partly explained by cell morphology co-varying with movement. This is expected, as the energetic costs of movement of microscopic organisms in aquatic environments are heavily influenced by their morphology such as cell elongation and size (Mitchell 2002, Young 2007). Indeed, we found that larger cells moved faster and less tortuously, regardless of their dispersal status. The shape of the cells also influenced speed and tortuosity: dispersing cells that were more elongated moved faster and less tortuously, whereas the opposite was true for resident cells. The differences in movement speed are likely due to different costs associated with motion in the liquid medium, with larger cells potentially having larger energy reserves and/or better movement machinery (Mitchell 2002). This is corroborated by the fact that size always favoured faster and less tortuous movement, even when accounting for the genotype effect. Our results therefore closely agree with recent findings

about a general allometric relationship between body size and movement speed (Hirt et al. 2017a, b).

We have shown that movement variation can be partly explained by different cell sizes and shapes. This is in line with previous findings on the condition dependence of dispersal that indicated that cell size and shape have an influence on the dispersal propensity (Pennekamp et al. 2014). However, in contrast to dispersal, larger and more roundish cells are moving faster and straighter, whereas more elongated and smaller cells disperse more. This contrasting result suggests that although larger cells may be superior in terms of movement ability, they may not disperse as much as expected as other causes of dispersal may be more important; for instance, dispersal decisions may be taken as a function of competitive ability rather than movement ability per se (Fronhofer et al. 2015b). If cell size positively co-varies with competitive ability, smaller cells may disperse to escape the local competition although they are relatively weaker in terms of the movement ability.

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

Explaining dispersal rate with activity and movement variation

The amount of variation explained increased from 27% accounting only for genotype-specific cell activity level, to 34% when considering only genotype-specific movement, and up to 45% when considering both genotype-specific activity and genotype-specific movement. Activity and movement 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 increase 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 and 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 and 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

391 but poorly dispersing resident vs. poor growing and well dispersing establisher) and if the ratio
392 between genotypes in these parameters varied two- to ten-fold. Looking at the variation of our
393 genotypes (Figure 4), we see that the ratio in dispersal rate can be up to ten-fold depending on the
394 genotypes contrasted. This suggests that with a known variation in growth rate with a factor of
395 about two (Pennekamp 2014), accelerating invasions of *Tetrahymena* are possible, if natural
396 populations are more phenotypically diverse. Validating these predictions in experiments with
397 mixed populations and their link with local adaptation would be a fruitful avenue for future
398 research.

399 Conclusions

400 Our study showed a close link between movement and dispersal on multiple levels. Dispersal
401 predictions steadily improved when genotype differences in both activity level and movement
402 behaviour were considered. This highlights that predictions of dispersal will benefit from a detailed
403 understanding of the underlying movement behaviour, although other factors matter. To move
404 beyond short-term ecological predictions of dispersal dynamics, e.g. range expansions and range
405 shifts due to environmental change, we would need to further improve our understanding of how
406 movement is affected by environmental variation, such as temperature (Jacob et al. 2017).

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Figure 1(on next page)

Overview of among and within genotype variation in activity, movement metrics (speed and tortuosity) and diffusion.

Each line shows a genotype and its slope indicates differences in movement among status (disperser vs resident).

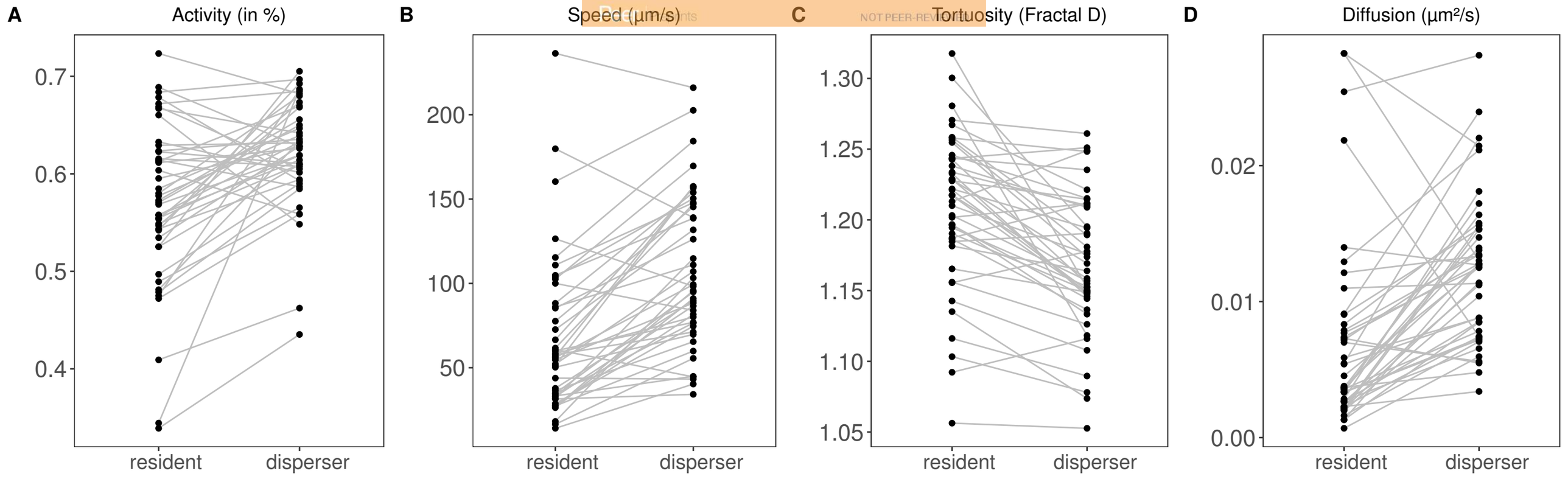

Figure 2(on next page)

A negative correlation between path tortuosity and movement speed was found across genotypes.

Faster genotype moved in a less tortuous fashion, the two combining into higher diffusion rate. The strength of the relationship did not differ regarding dispersal status.

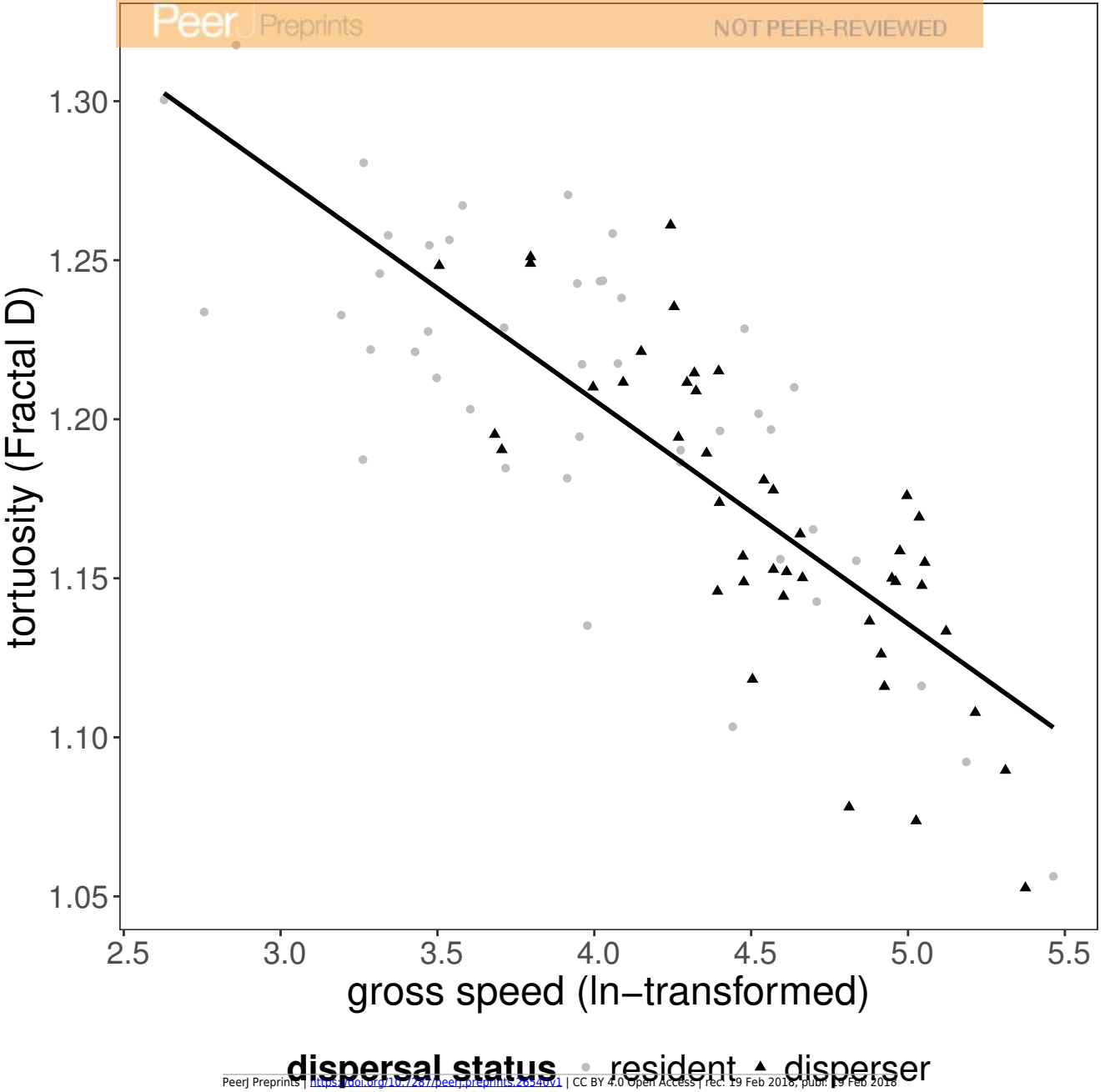

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Figure 3(on next page)

Relationships between movement metrics (speed and tortuosity) and cell morphology (N=262).

Lines show the fit of the most parsimonious ANCOVA model relating cell morphology to movement metrics, considering variation due to the dispersal status. Larger cells moved faster and less tortuous and the effect was additive. In contrast, only in dispersing cells elongation resulted in faster and straighter movement, whereas the opposite was observed in resident cells.

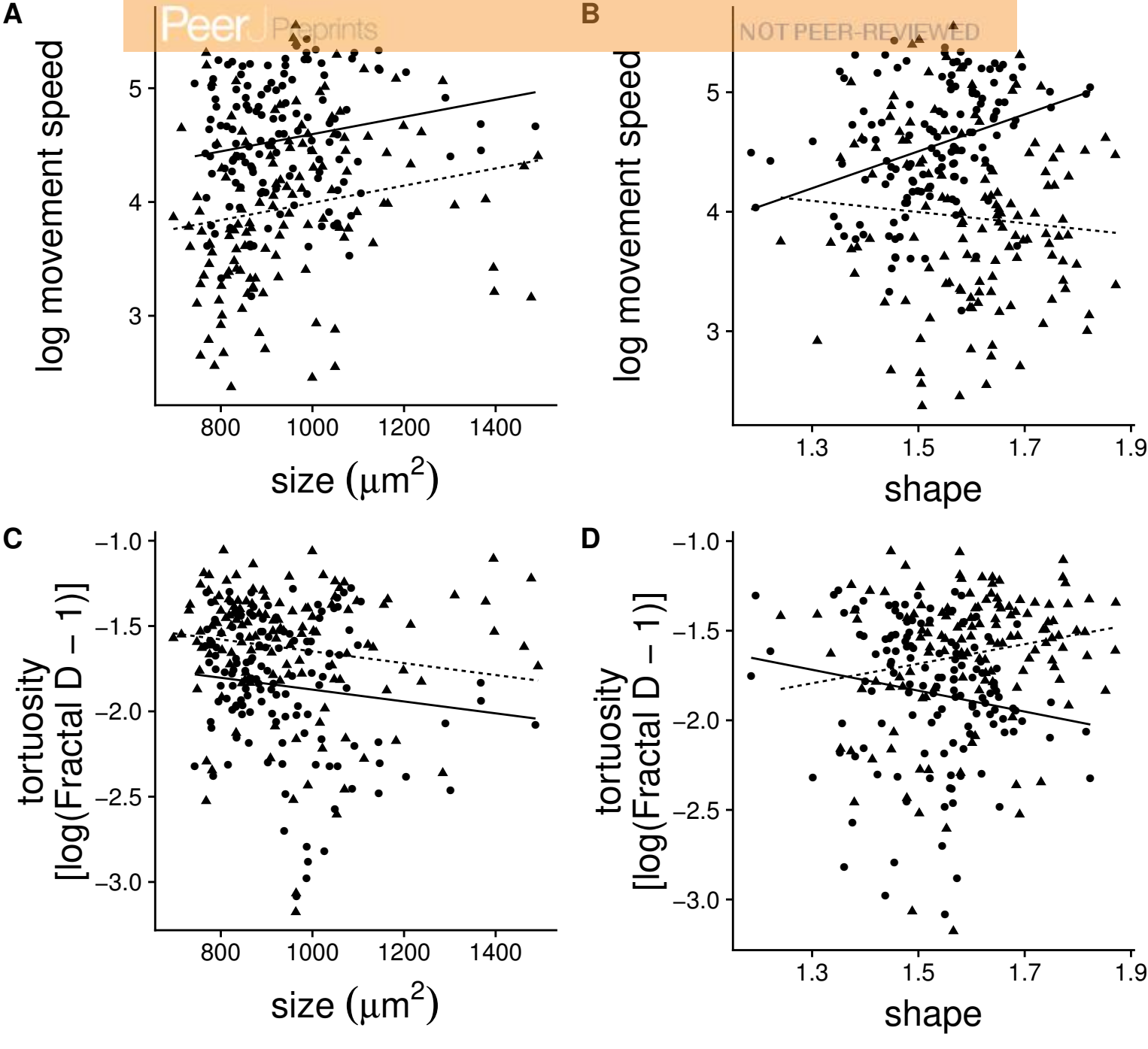

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Figure 4(on next page)

The 44 genotypes differed in their dispersal rate in the two-patch experimental system over a period of 6 h.

The point represents the mean dispersal and the error bars the standard error of the mean (n=3 per genotype). The dashed line indicates the 50% dispersal rate.

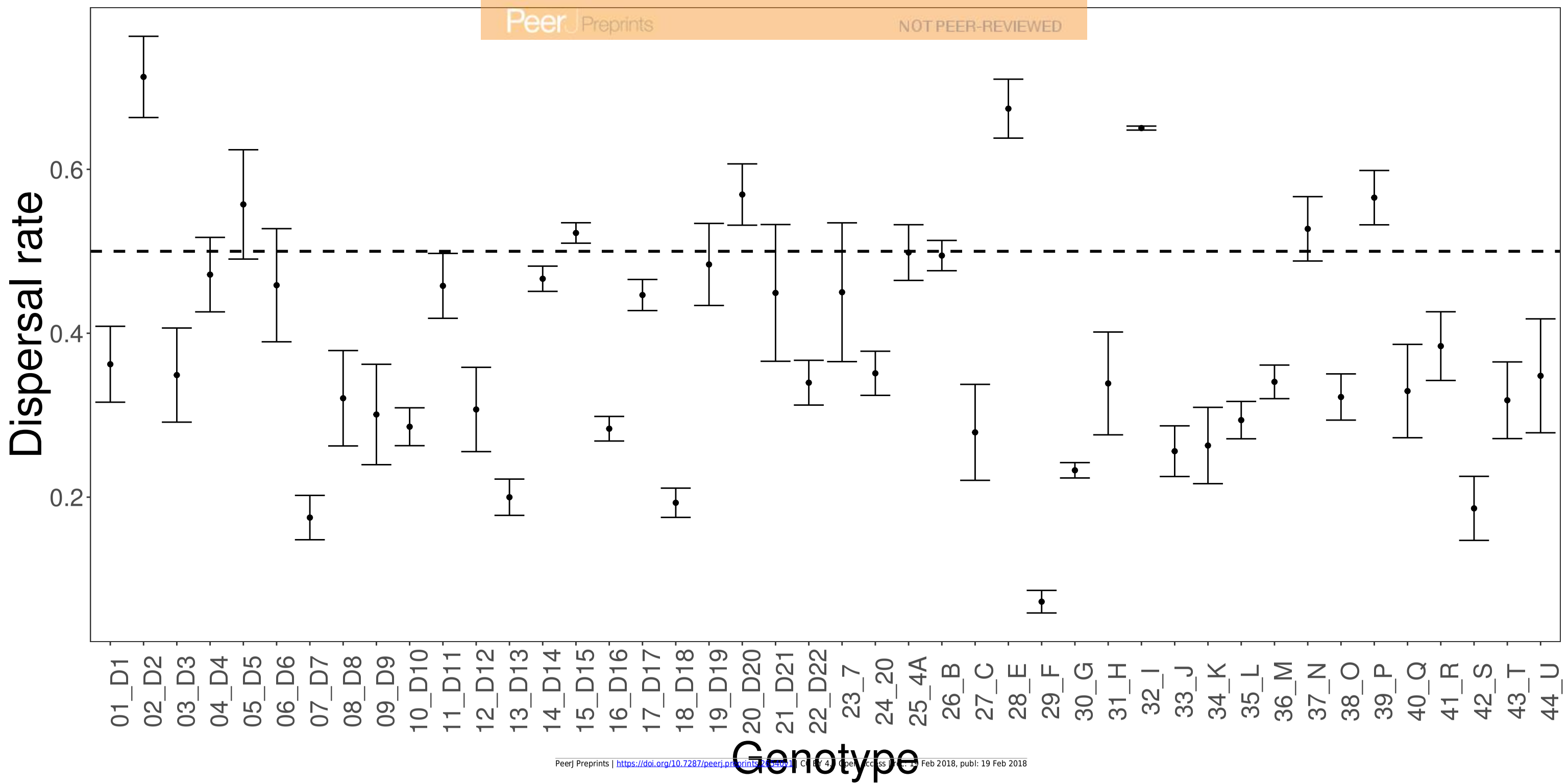

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Figure 5(on next page)

Response surface plot showing the dependency of dispersal rate on activity and diffusion rates.

Each point represents the mean of a genotype. 45% of the variation among genotypes in dispersal rate was explained by differences in their activity and movement behaviour (swimming speed and tortuosity, integrated as diffusion coefficient).

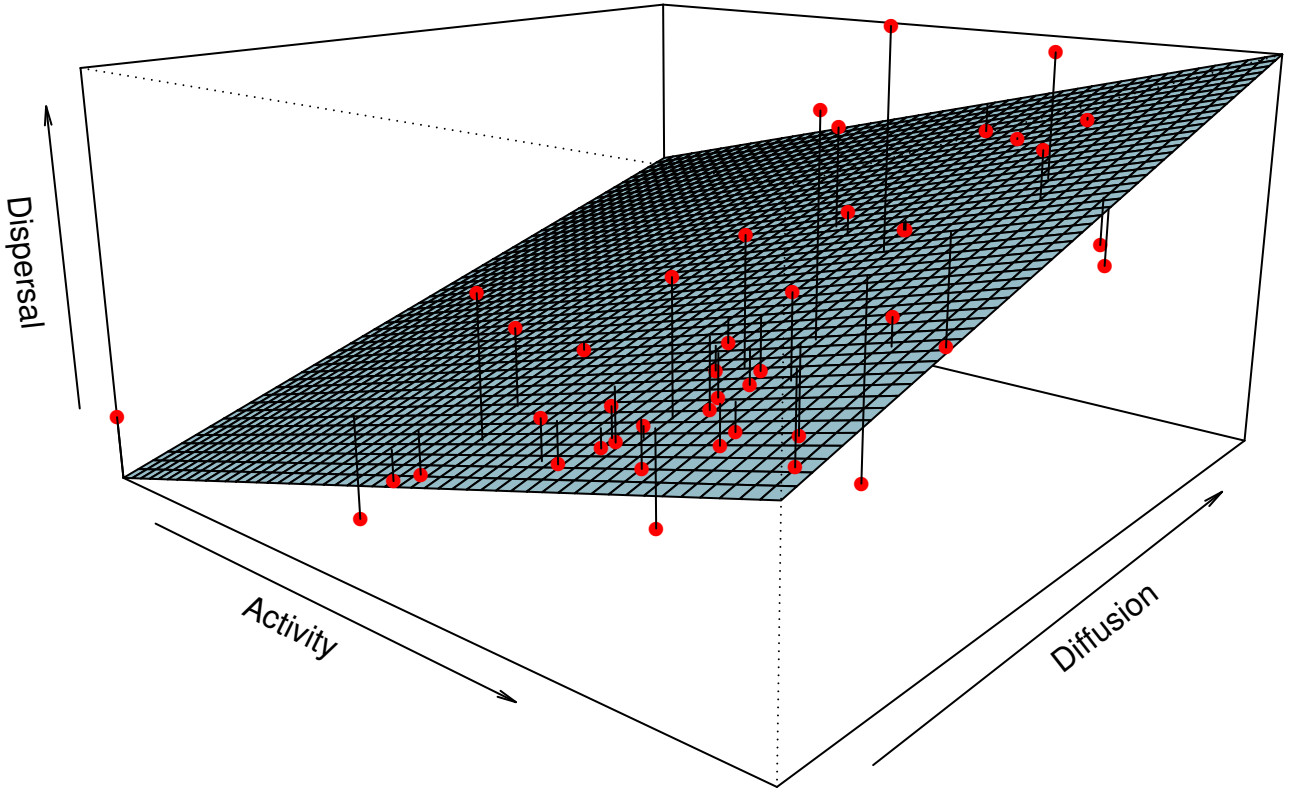

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Table 1(on next page)

Three-way ANOVA to assess the effect of genotype and the dispersal status (i.e. dispersers and residents) on three movement metrics.

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).

	activity					speed: ln(gross speed)				tortuosity: ln(Fractal D - 1)				diffusion rate			
	DF	SS	MS	F value	p	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	65.897	1.532	10.65	< 0.0001	26.309	0.612	9.05	< 0.0001	0.00712	0.00010	4.44	< 0.0001
dispersal status (disperser vs resident)	1	0.186	0.186	42.88	< 0.0001	23.282	23.282	287.66	< 0.0001	3.209	3.209	105.08	< 0.0001	0.00190	0.00190	69.36	< 0.0001
genotype * dispersal status	43	0.445	0.010	2.39	0.0003	15.099	0.351	4.34	< 0.0001	3.428	0.080	2.61	< 0.0001	0.00291	0.00007	2.47	0.0002
replicate (genotype)	87	0.612	0.007	- *	- *	12.520	0.144	- *	- *	5.879	0.068	- *	- *	0.00324	0.00004	- *	- *
replicate * dispersal status (genotype)	87	0.377	0.004	- *	- *	7.041	0.081	- *	- *	2.657	0.030	- *	- *	0.00239	0.00003	- *	- *
total	261	2.490	-	-	-	123.894	-	-	-	41.480	-	-	-	0.01760	-	-	-

- * : cannot be tested