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DISPERSAL PROPENSITY IN *TETRAHYMENA THERMOPHILA* CILIATES—A REACTION NORM PERSPECTIVE

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Dispersal and phenotypic plasticity are two main ways for species to deal with rapid changes of their environments. Understanding how genotypes (G), environments (E), and their interaction (genotype and environment; G × E) each affects dispersal propensity is therefore instrumental for predicting the ecological and evolutionary responses of species under global change. Here we used an actively dispersing ciliate to quantify the contributions of G, E, and G × E on dispersal propensity, exposing 44 different genotypes to three different environmental contexts (densities in isogenotype populations). Moreover, we assessed the condition dependence of dispersal, that is, whether dispersal is related to morphological, physiological, or behavioral traits. We found that genotypes showed marked differences in dispersal propensity and that dispersal is plastically adjusted to density, with the overall trend for genotypes to exhibit negative density-dependent dispersal. A small, but significant G × E interaction indicates genetic variability in plasticity and therefore some potential for dispersal plasticity to evolve. We also show evidence consistent with condition-dependent dispersal suggesting that genotypes also vary in how individual condition is linked to dispersal under different environmental contexts thereby generating complex dispersal behavior due to only three variables (genes, environment, and individual condition).

KEY WORDS: **Condition-dependent dispersal, context-dependent dispersal, density dependence, genotype × environment, phenotypic plasticity.**

Dispersal is a key trait for the ecological and evolutionary dynamics of a given species. It is broadly defined as the exchange of individuals between the natal and one or more reproductive sites (Matthysen 2012). Dispersal is crucially important for the spatial functioning of (meta)populations and (meta)communities, and can have profound impacts on both the ecological and evolutionary dynamics of populations and species (Hanski and Gaggiotti 2004; Leibold et al. 2004; Holyoak et al. 2005). Dispersal can compensate for local extinctions by recolonization of vacant habitats (Hanski and Gaggiotti 2004) or simply augment or "rescue" small populations (Brown and Kodric-Brown 1977). The exchange of individuals between populations influences gene flow and hence has implications for local adaptation, drift, genetic diversity, and population divergence of species (Ronce 2007). Understanding the factors that shape dispersal strategies and their genetic underpinnings is of great importance as dispersal is a crucially important means for species to mitigate global change both through direct movement and the subsequent gene flow that may facilitate local adaptation to new conditions (Berg et al. 2010; Chevin et al. 2010; Chaine and Clobert 2012).

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To understand the evolution of dispersal strategies and predict their impact on the ecology and evolution of a species, it is important to disentangle the different sources of variation in dispersal behavior, that is, the contributions of genetic (G) and environmental (E) effects and their interaction (G × E). The appropriate framework to quantify the relative contributions of G, E, and G × E are dispersal reaction norms, which "characterize the array of dispersal behaviors and dispersal types of a given genotype along an environmental gradient" (Clobert et al. 2009, p. 201). Dispersal reaction norms may be used to understand the underlying genetic architecture and evolutionary potential of dispersal decisions (Ronce 2007) and are therefore increasingly applied in dispersal studies (Massot and Clobert 2000; Donohue et al. 2005; Bonte et al. 2007, 2008a,b; Chaine et al. 2013). These studies show that G, E, and G × E have significant effects, but currently no study to our knowledge quantifies the relative contributions of G, E, and G × E on dispersal propensity in terms of explained dispersal variation.

The importance of both the G and E contributions to dispersal are well supported by empirical studies (Zera and Brisson 2012), but few have studied them simultaneously to describe their interaction. Heritable genetic variation in dispersal propensity is the raw material for natural selection and therefore provides an important means to adapt to prevailing environmental conditions. Several studies on insects (planthoppers, aphids) have shown that genetically based population variation in dispersal propensity was linked to habitat stability (Groeters 1989; Denno et al. 1996), providing evidence for local adaptation. Features of the environment also affect dispersal and the role of conspecific density on dispersal in particular has attracted both theoretical and empirical interest. Intuitively, intraspecific competition will increase with density; hence dispersal is a means of escaping unfavorable environmental conditions due to crowding (Bowler and Benton 2005) and can mitigate competitive and cooperative interactions between kin (Hamilton and May 1977). Although dispersal is commonly predicted to increase with density (Gandon and Michalakis 1999; Poethke et al. 2007), Allee effects or benefits of group living can exceed the costs of increased competition leading to negative density-dependent dispersal (Kim et al. 2009). Indeed, empirical studies report higher (Bengtsson et al. 1994; Hauzy et al. 2007; Bitume et al. 2013) or lower dispersal (Westerberg et al. 2008; Baguette et al. 2011; Fellous et al. 2012) when exposed to increased conspecific densities among species. Both positive and negative density-dependent dispersal are theoretically possible within a population and thus form an interesting focus for studies of genetic variation in dispersal reaction norms. For example, both positive and negative density-dependent dispersal has been shown in a colonial bird species (Kim et al. 2009), but such examples are rare and have received very limited attention in the dispersal literature so far.

Empirical studies have shown that a number of different dimensions of the environment can strongly influence dispersal decisions leading to the concepts of *context-* and *condition-dependent dispersal* (Ims and Hjermann 2001; Bowler and Benton 2005; Clobert et al. 2009). *Context-dependent dispersal* refers to the triggering effect of the abiotic and biotic environment, such as resource availability, predation risk, or density as described above. *Condition dependence* refers to the internal state or condition, such as stress level or nutrient reserves, of the potential disperser. In some cases, natural selection will favor dispersal by individuals that are best equipped to withstand the hardships encountered during the dispersal period. Indeed, consistent morphological, physiological, and behavioral differences have been found between resident and disperser individuals indicating that dispersal decisions depend on the *condition* of an individual (Benard and McCauley 2008; Clobert et al. 2009). Theoretical work has shown that models including conditional dispersal strategies often outperform unconditional strategies (e.g., the evolution of density-dependent dispersal; Travis et al. 1999), providing theoretical support for the occurrence of adaptive dispersal plasticity in nature (Ims and Hjermann 2001; Matthysen 2005). Because costs and benefits of dispersal depend on both the properties of the individual such as its internal state (*condition*) as well as the prevailing external *context* (e.g., environmental and/or social), there should be individual variation in response to environmental cues (Matthysen 2012). Thus the empirical framework for studying these effects should examine the interaction between genes and both external (E_E) and internal (E_I) dimensions of the environment. Accordingly, context-dependent dispersal is the significant effect of E_E on dispersal, while an effect of G × E_E indicates genetic variation in context-dependent dispersal. On the other hand, the significant effect of E_I indicates condition-dependent dispersal, whereas the effect of G × E_I indicates genetic variation in condition-dependent dispersal. Plasticity of dispersal is increasingly looked at but few studies investigate *context-* and *condition-dependent dispersal* simultaneously, although interactions between *context* and *condition* may explain inconsistencies in observed dispersal decisions (Clobert et al. 2009).

Genetic variation for phenotypic plasticity in dispersal is an important prerequisite for the adaptation and evolution of dispersal reaction norms and their role in ameliorating mismatches between phenotypes and environments (DeWitt and Scheiner 2004; Ghalambor et al. 2007). Plasticity in behavior is expected to result in a faster reaction to rapid environmental change than morphological or physiological changes and therefore may help species cope with global change (Charmantier et al. 2008; Vedder et al. 2013). Bonte et al. (2008b) analyzed genotype-by-environment interactions regarding thermal conditions during juvenile development and found that dispersal decisions of spiders to either perform long-distance (by ballooning) or short-distance dispersal

(by rappelling) differed according to the temperature conditions experienced during the juvenile stage and that this conferred fitness benefits. These results demonstrate that phenotypic plasticity in dispersal may provide an adaptive advantage (DeWitt and Scheiner 2004) and in this specific case also when compared to a fixed dispersal strategy (Bonte et al. 2008b). To gain a better understanding of how dispersal reaction norms evolve, we need to know about the extent of genetic variability for plasticity and the shape of the reaction norm (David et al. 2004). However, the numbers of genotypes tested and number of environments used are generally low in existing experimental studies, limiting conclusions about the shape of reaction norms and variation between genotypes. Of the above-mentioned studies examining the reaction norm of dispersal, only one (Bonte et al. 2008b) used a gradient of environments (five different temperatures), while all other studies contrasted only two environments of a given factor (e.g., high vs. low density, high vs. low wind velocity, herbivory vs. control, etc.).

Disentangling the contributions of G, E, and G × E on dispersal propensity is a challenging task that requires controlled breeding schemes, which are more easily achieved under laboratory conditions allowing tight control of both genotypes and environments. This is particularly important when considering density-dependent dispersal and dispersal phenotypes as field conditions often involve confounding factors (Matthysen 2005). Carefully controlled density manipulations allow separation of specific density effects from confounding factors and makes measuring the phenotypes of resident and disperser fractions of a population less difficult. Experimental dispersal systems have proven to be valuable research tools in ecological studies of dispersal (e.g., the common lizard, *Zootoca vivipara*, and the Glanville fritillary butterfly *Melitaea cinxia*) and similar systems could be used in evolutionary studies. Aquatic microbes are particularly convenient models for dispersal (Donahue et al. 2003; Fjerdingstad et al. 2007; Hauzy et al. 2007; Fellous et al. 2012) and a series of characteristics of *Tetrahymena thermophila* make the species an appropriate model for evolutionary studies that investigate the dispersal reaction norm: (i) cells disperse actively, so the dispersal response is measured directly and does not depend on external dispersal vectors such as wind or currents; (ii) reproduction is clonal in populations that contain only one of the seven mating types (Collins and Gorovsky 2005), hence dispersal can be measured on the population level as the population consists of cells with an identical genotype; and (iii) the small cell size facilitates manipulation and a high degree of control over the environment, while dispersal can be measured over relevant spatial scales.

Here we report the results of a fully factorial G × E experiment where the dispersal reaction norms of the aquatic ciliate *T. thermophila* were quantified in controlled laboratory conditions. Forty-four clonal cell lines (G) originating from single cells, hereafter called genetic lines or genotypes, were exposed to three different conspecific densities (E) and their dispersal rate measured in a two-patch system. This experiment aimed to provide answers to the following questions:

1. What is the relative contribution, and therefore, evolutionary importance of G, E, and G × E to the phenotypic variation in dispersal propensity in *T. thermophila*?
2. What is the extent of variation in reaction norms between different genotypes and what potential may there be for further adaptation of plastic responses?
3. Are there consistent phenotypic (morphological or behavioral) differences between the disperser and the resident cells that may indicate traits and trade-offs important for *condition-dependent dispersal* and is this specific to each particular genotype?

Materials and Methods

MODEL ORGANISM

Tetrahymena thermophila is a ca. 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). It has long been used as a model organism in molecular biology and genetics, which has led to well-established protocols of cell culture and maintenance in the laboratory (Collins 2012). Biological samples of different lines of *T. thermophila* were kindly provided by F. P. Doerder (University of Illinois) or directly ordered from the National *Tetrahymena* Stock Centre (https://tetrahymena.vet.cornell.edu), the American Type Center Collection (www.atcc.org), or the Culture Collection of Algae and Protozoa (www.ccap.ac.uk).

We used a set of 44 genetically distinct lines, which differ in geographic origin and time since extraction from the field (Table S1), but also in several life-history characteristics such as growth rate, maximum density, and survival under starvation conditions (Fjerdingstad et al. 2007; Pennekamp 2014). All 44 genetic lines were maintained under standard culture conditions with clonal reproduction before and during the described experiment: axenic liquid culture in a nutrient medium (broth consisting of 2% Proteose peptone and 0.2% yeast extract [Becton Dickinson], diluted in ultrapure water), kept at constant 27°C temperature in a light controlled incubator with a 14:10 h light/dark cycle. Culture stocks were renewed every 10 days by inoculating a 2 ml sample of fresh medium with 100 μl of culture and maintained in 2 ml multiwell plates (CELLSTAR® multiwell plates, Greiner BioOne, Belgium). All manipulations of axenic cultures were conducted under sterile conditions in a laminar flow hood (Ultrasafe 218 S, Faster, Italy).

DISPERSAL QUANTIFICATION SYSTEM

We used a standardized protocol to measure dispersal behavior of *T. thermophila* cells following protocols outlined in our previous work (Fjerdingstad et al. 2007; Schtickzelle et al. 2009; Chaine et al. 2010). The way we measured dispersal encompasses several phases of the dispersal process such as emigration and transience and therefore cannot clearly be attributed to one of the three dispersal phases (Bowler and Benton 2005); accordingly, we will refer to our dispersal measure as "dispersal propensity."

A dispersal system was constructed from two standard 1.5 ml Eppendorf® microtubes connected by a 17-mm-long silicon pipe (internal diameter 4 mm; VWR, Belgium). The dispersal system was filled prior to the experiments with 3 ml of the standard medium through one tube to ensure fluid transition between the tubes. The system was then closed by placing a clamp in the middle of the connecting pipe. To start the experiment, a specific density of cells from a given genetic line was placed into the "start" tube of the system and the tube content was homogenized to encourage cells to move freely throughout the start tube. After 30 min of acclimation to the new medium, the clamp closing the connecting pipe was removed and cells could freely disperse between the two tubes for six hours.

At the end of the experiment, the system was closed and a series of measures extracted for the "start" and "target" tubes using a digital image analysis procedure, which allows estimation of cell densities and several phenotypic traits in an objective and automated manner (Fjerdingstad et al. 2007; Pennekamp and Schtickzelle 2013). To do so, five independent samples were taken from each tube after culture homogenization, and each loaded in a chamber of a counting slide (Kima, Italy); for each chamber, a series of three dark field images (1 sec time lapse) were taken using a Canon EOS 5D Mark II mounted on a Nikon Eclipse 50i microscope. Images were then treated and segmented using ImageJ software (version 1.47, Schneider et al. 2012) to identify individual cells and extract measures. This workflow, containing several data cleaning steps to eliminate artifacts, has been carefully validated and extensively optimized to produce accurate and repeatable results (see details and measures of error rates in Pennekamp and Schtickzelle 2013).

Each dispersal system produced five basic measures: (1) one measure of density per tube (start and target), computed as the average of the densities measured on the first image taken on each of the five chambers; (2) one measure of dispersal propensity per system, computed as the final proportion of cells found in the target tube, that is, $\text{density}_{\text{target}}/(\text{density}_{\text{start}} + \text{density}_{\text{target}})$; (3) one measure of average cell size; (4) average cell shape (i.e., the ratio between the length and the width of the cell); and (5) proportion of active cells (hereafter called activity) per tube. These last three measures were obtained by aggregating data extracted at the individual cell level for all cells present on the first image taken on each of the five counting chambers. A cell was classified as active if its displacement over the three sequential pictures of a given chamber was higher than 7.055 μm (i.e., 5 pixels); images 2 and 3 in a given time series for a specific chamber were used only to assess cell activity, and no other measures were extracted from them. The displacement threshold value was manually validated to distinguish between slightly moving and immobile cells.

EXPERIMENTAL DESIGN

We used a fully factorial genotype × environment (G × E) experimental design: dispersal was quantified using systems described above for all 44 genetic lines at three different initial cell densities (100,000, 200,000, and 300,000 cells/ml), corresponding to a range of cell densities commonly observed under our culturing conditions. Our experiments used single genotypes and thus densities reflect the density of kin. Cell lines differ in how they react to nonkin and the characteristics of those other lines, thus mixed genotype populations that would represent competition among nonkin would greatly complicate the interpretation of our results and require a detailed understanding of how specific cell lines interact with other lines, which we currently lack.

In addition to the strict control of all our *T. thermophila* culture conditions, two standardization steps were performed prior to the experiment. First, a preculture of each genetic line was started from the stock by transferring 100 μl of culture into 2 ml of fresh medium on a 24-well plate and allowed to grow exponentially for 4 days to synchronize populations to the logarithmic phase of population growth (Collins 2012). Second, at the end of this synchronization phase, cell density was estimated for each genetic line and new cultures, to be used for the experiment, were launched at an equal starting density of 10,000 cells/ml, in culture flasks (CELLSTAR® Cell Culture Flask 50 ml, Greiner BioOne). These cultures grew for three days allowing cultures to reach sufficiently high cell densities (>300,000 cells/ml) for the experiment. Then dispersal systems were inoculated and run for six hours to collect the five measures described above. Two hundred sixty-four dispersal systems (44 lines × 3 densities × 2 replicates) were run in total; one system (replicate 1 of line D17 at 300,000 cells/ml) was defective and hence discarded.

Generation times around two hours are reported in the literature for *T. thermophila* under optimal conditions (35°C, constant shaking for extra O_2 supply, and food ad libitum; Cassidy-Hanley 2012). Under such a rapid population growth, a fortiori, if growth rate is strongly density dependent (i.e., different in start and target tubes), our estimates of dispersal rates could be biased by this population growth. We controlled for this potential bias by (1) using suboptimal culture conditions (27°C, no shaking) to increase generation time, and (2) performing an additional experiment aimed at precisely quantifying, for each genotype, population growth

under the specific conditions of the dispersal experiment and its density-dependent variation: growth rate was measured over six hours after cells had been diluted to a range of densities (from 10,000 to 450,000 cells/ml) in conditions identical to those used in the dispersal experiment except that cells had no possibility to disperse because tubes were not connected. This experiment confirmed that multiple generations were not possible under the experimental settings (median generation time = 5.92 h, ranging from 3.93 to 16.24 h) and allowed us to correct our estimates of dispersal rate for population growth (see Results). Detailed information about how we dealt with the potential bias due to population growth and how we were able to rule it out, can be found in the Supporting Information.

STATISTICAL ANALYSIS

To study the dispersal reaction norms (questions 1 and 2), a two-way crossed ANCOVA was used to test for the effects of genotype (G), initial cell density (E), and their interaction (G × E) on dispersal propensity. 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 genetic lines 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). Consequently, the results cannot be generalized to the species as a whole because the observed variation may not be representative in an ecologically relevant sense. Density was treated as a continuous covariate. The dispersal propensity was log-transformed and visual inspection of residuals indicated no lack of normality and homogeneity of variance. The density was centered on the 200,000 cells/ml density to test for genetic line differences at the center of the tested E range. To assess the strength of association between the response and the effects of G, E, and G × E, effect sizes were calculated using the ω^2 measure (Quinn and Keough 2002) that allows partitioning the total variation into the portion of variation explained by each effect.

To study *condition-dependent dispersal* (question 3), we compared the phenotypic traits of disperser and resident fractions, dispersers being defined as cells present in the target tube and residents being the cells in the start tube at the end of the experiment. In a first step, a three-way crossed ANCOVA was used to test for the effects of genotype (G), initial cell density (E), and tube (dispersers vs. residents) on shape, size, and activity (arcsine transformed). Genetic line, density, and tube were modeled as fixed effects. In a second step, we used Spearman's rank correlation to relate the differences in morphology and activity (arcsine transformed) between the disperser and resident fractions to the dispersal propensity; to do so, we calculated for each dispersal system the difference between tubes in each of the response variables as a proportional change, that is $(\text{observed}_{\text{target}} - \text{observed}_{\text{start}})/\text{observed}_{\text{start}}$.

Because cells in the start and the target tubes differed in density after dispersal took place, we checked for a potential confounding effect, namely that phenotypic differences between dispersers and residents were due to the altered density rather than dispersal status. In the control experiment mentioned above to derive the density-dependent growth rates, the same three phenotypic traits (size, shape, and activity) were measured for cells six hours after they had been diluted to a range of densities (from 10,000 to 450,000 cells/ml). For every genetic line separately, we regressed the phenotypic trait against density (in case of activity an arcsine transformation was used and density log-transformed to linearize the relationship). The values of the trait were first centered to a mean of 0, so that they expressed the deviation from the mean trait due to density. Overall, these regressions showed some significant positive effect of cell density on size, shape, and activity (size = 909.64 + 0.313 × density, $R^2 = 0.10$; shape = 1.535 + 0.0005 × density, $R^2 = 0.23$; arcsin(activity) = 0.704 + 0.111 × log(density), $R^2 = 0.32$; respectively; all $P < 0.001$. These R^2 values were evaluated over all genotypes pooled, hence the considerable variation among genotypes in these traits explains why they can appear relatively low; similar regressions for each genotype showed higher R^2 values [about 0.4 on average] for all traits). Hence, we corrected the values of size, shape, and activity observed in the dispersal experiment before running the two analyses presented above, by adding to every value the expected deviation from the mean due to density, as computed from a regression line computed for that genetic line. The remaining differences between residents and dispersers should therefore be a conservative estimate of the differences related to the dispersal process and not to the fact that cell density itself differed between start and target tubes.

ANCOVAs were carried out in SAS 9.3 (www.sas.com), and all remaining analyses in R 2.15.2 (R Development Core Team 2012).

Results

DISPERSAL REACTION NORMS

We first analyzed how dispersal propensity varied between genetic lines and densities of kin using a two-way crossed ANCOVA (genetic line × density). Genetic lines varied substantially in their dispersal propensity over a six-hour period (genotype effect: $F_{43,175} = 22.51$, $P < 0.001$), ranging from 15 to 95% across all densities (Fig. 1). Overall, they showed a negative density-dependent modulation of their dispersal propensity (density effect: $F_{1,43} = 68.92$, $P < 0.001$), dispersing less when exposed to higher densities (dispersal propensity across all lines decreased from 70% at 100,000 cells/ml to 60% at 300,000 cells/ml). The

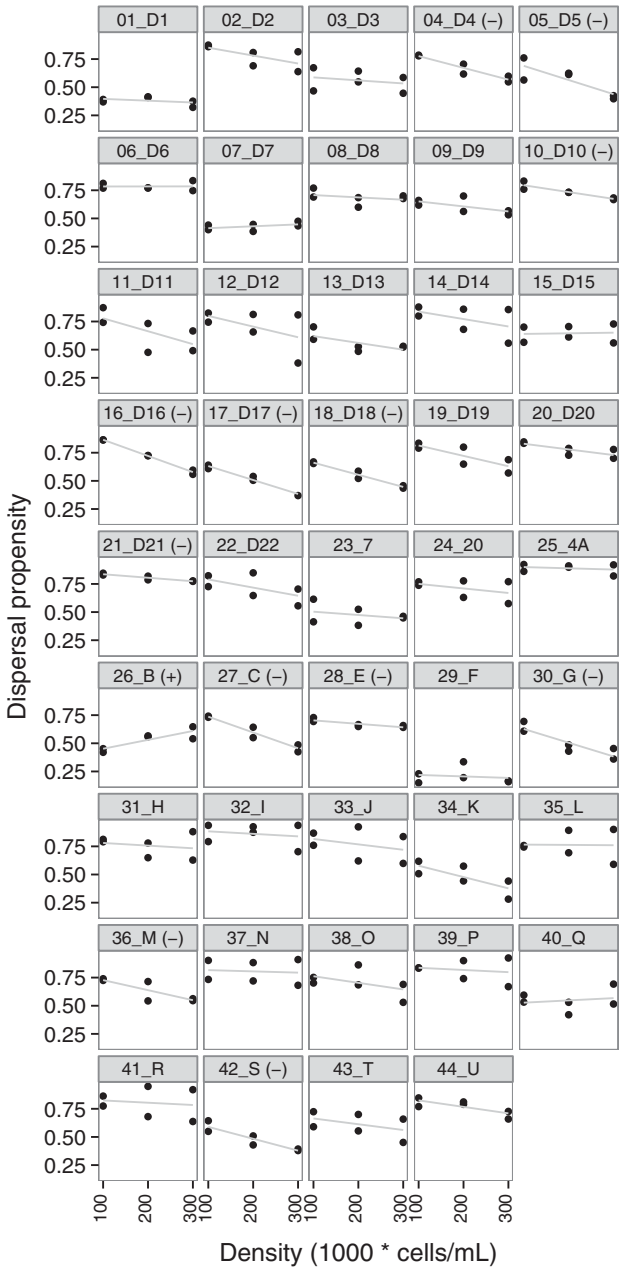

Figure 1. Dispersal propensity (i.e., proportion of cells found in the target tube after six hours) on the Y-axis as a function of density for each genetic line. Each point represents one independent dispersal system. The direction of the density-dependent dispersal reaction is indicated in parentheses after the genotype designation when significantly different from zero at the 0.05 alpha level. Generally, genetic lines showed either negative density-dependent or there was no evidence for density-dependent dispersal; only line B was an exception showing consistently positive density dependence.

interaction between genotype (G) and density (E) was significant ($F_{43,175} = 1.53$, $P = 0.028$), indicating that genetic lines differed in the effect of kin density on dispersal. Of the 44 genotype slopes tested, 12 showed significant negative density dependence, one showed significant positive density dependence, whereas there was no evidence for density dependence in the 31 others (Fig. 1). Although only 13 of the 44 slopes were significant, 39 of 44 *T. thermophila* genetic lines had negative and only five positive reaction norm slopes of dispersal with conspecific density. The ANCOVA accounted for 80% of the observed variation in the dispersal propensity; most of the variation in dispersal propensity was due to genetic variation among clonal lines (73%), followed by the environment effect (5%), and the interaction between genotypes and the environment (2%). Because such variance partitioning can be sensitive to the number of levels per factor (Petraitis 1998; Quinn and Keough 2002), we checked for potential bias using a down-sampling procedure equalizing the number of genotypes from 44 to 3. With an equal number of levels for G and E, G accounted on average for 43%, the environment for 13%, and the G × E interaction for 2% of the explained variation (for details on the down-sampling simulation, see the Supporting Information). The larger number of genotypes inflated the high proportion of variance in dispersal explained by G, but nevertheless the absolute ranking of the three components and the larger impact of G remained the same, meaning they were clearly not an artifact of the experimental design.

Applying the same analysis on dispersal rates corrected for population growth, as quantified per genotype and tube, very similar results were obtained, leading to identical conclusions (see Supporting Information).

PHENOTYPE DIFFERENCES BETWEEN DISPERSERS AND RESIDENTS

Genetic lines significantly differed for size, shape, and activity, suggesting genetically based trait differences between genotypes (as previously shown by Fjerdingstad et al. 2007). Shape and activity further differed between the three densities, but in different and opposing ways among genotypes (significant line × density interactions for all traits; Table 1).

Significant differences between disperser and resident cells were found for size, shape, and activity, but in slightly different ways. For the three traits, these differences varied among the 44 genetic lines (significant line × tube interactions) and the three densities (significant density × tube interaction). A general pattern (significant main tube effect) was clear concerning all three traits; for the majority of the 132 genetic line × density combinations, disperser cells were significantly smaller, more elongated, and active than resident cells (Fig. 2). Additionally, we found that more dispersive genotypes showed a stronger cell size reduction ($r = -0.39$, $P < 0.001$; Fig. 2A) and activity gain

Table 1. Statistical analysis (three-way crossed ANCOVA) of size, shape, and activity according to genetic line, density (continuous covariate), and tube (start vs. target) effects. Significant effects are shown in bold.

		Size		Shape		Activity	
Factor	df for *F*-test	*F*	*P*	*F*	*P*	*F*	*P*
Line	43,350	142.36	**<0.001**	108.39	**<0.001**	13.88	**<0.001**
Density	1,43	2.26	0.134	12.95	**<0.001**	4.02	**0.046**
Tube	1,43	378.91	**<0.001**	10.89	**<0.001**	77.44	**<0.001**
Line × density	43,350	1.97	**0.001**	1.43	**0.044**	1.71	**0.005**
Line × tube	43,350	7.09	**<0.001**	4.63	**<0.001**	3.18	**<0.001**
Density × tube	1,43	7.41	**0.007**	15.73	**<0.001**	5.08	**0.025**
Line × density × tube	1,350	0.81	0.797	1.26	0.133	0.64	0.962

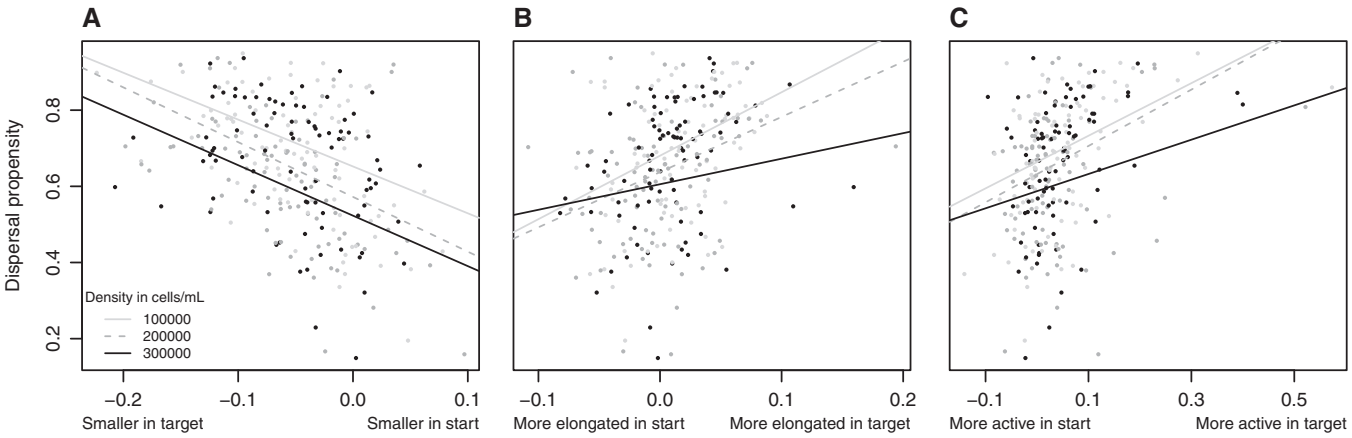

Figure 2. Relationship between the dispersal propensity (i.e., proportion of cells found in the target tube after six hours) and relative differences in cell phenotypes between start and target tubes. Panel A depicts the relationship between dispersal and size, panel B between dispersal and shape, and panel C between dispersal and activity. Each point represents one independent dispersal system (44 genetic lines × 3 densities × 2 replicates), where gray scales indicate the density treatment. The lines are visual aids to indicate the general trends, while the underlying analysis was based on Spearman rank correlations and therefore is not necessarily linear. The more dispersive a genotype was, the smaller, more elongated, and more active the cells were in the target tube.

($r = 0.37$, $P < 0.001$; Fig. 2C) compared to less-dispersive lines. For shape the pattern was less clear with a positive correlation between the difference in shape between tubes with dispersal propensity ($r = 0.38$, $P < 0.001$); however, in dispersive lines, disperser cells were more elongated, whereas in less-dispersive lines, resident cells were more elongated (Fig. 2B).

Comparing the initial cell morphology between the two replicates before dispersal took place may provide insight whether changes in cell size and shape after dispersal are a cause or consequence of dispersal. If differences in population level cell size between replicates are a cause of dispersal, we should see more dispersal in the replicate with cells being smaller prior to dispersal. A chi-square test testing whether the ratio of genetic lines with smaller cells in one of the two replicates dispersed more during that replicate met a 1:1 ratio, that is, indicating no association of size with dispersal, was rejected at all densities (for 100,000, 200,000, and 300,000 cells/ml, respectively: $X_1 = 9$, $P = 0.003$; $X_1 = 6.182$, $P = 0.009$; $X_1 = 9$, $P = 0.003$). Shape differences between replicates before dispersal did not indicate an association with dispersal, while activity could not be assessed because no before experiment measures were available.

The same analysis on traits not corrected for density yielded similar results (main density effect became significant for size, while interactions between genetic line × density turned non-significant for shape and activity).

Discussion

Dispersal is a complex trait that is most likely influenced by a combination of genetic and environmental effects. Using a controlled reaction norm approach (Ronce 2007; Clobert et al. 2009), we were able to tease apart the relative contributions of the genotype, the environment, and the G × E on observed dispersal variation. Most of the variation in dispersal propensity was explained

by the genotype (43% of phenotypic variation), followed by the environment (13%), whereas the least variation was found for the G × E interaction (2%). This indicates significant genetic differences in the sensitivity of the genotypes to conspecific density, highlighting the importance of dispersal strategies for the fitness and survival of these genetic lines. Previous studies have shown that intraspecific variation in dispersal rates may be an adaptation to variation in among-population environmental conditions such as habitat stability (Ronce 2007). This has been seen previously in planthoppers, with a higher fraction of winged individuals (i.e., potential dispersers) found in more unstable habitats (Denno et al. 1996). Thus different dispersal propensities observed may reflect local adaptation of optimal dispersal propensity in the original environments of our genetic lines. Despite extensive search (Thuillier and Schtickzelle, unpubl. data), we lack detailed information about the previous environments of our *T. thermophila* genetic lines, but the stability of environmental conditions is likely to differ between differently sized ponds or between lentic and lotic habitats. Alternatively, environmental variation could maintain several coexisting strategies analogous to species coexistence (Chesson 2000) or within species alternative social strategies (Oliveira et al. 2008). Collections of *T. thermophila* in the wild often yield multiple genotypes across a small spatial scale (a few cubic meters; P. Doerder, pers. comm. and Doerder et al. 1995), although specific information is lacking for our genotypes. Understanding the cause of variation in dispersal strategies among genotypes will require a targeted study linking the ecology of collection sites with laboratory-based measures of dispersal.

POTENTIAL FOR ADAPTATION OF DISPERSAL PROPENSITY PLASTICITY

Although there were differences in reaction norms between the genotypes, the low variance for the G × E interaction, relative to the G and E components, suggests that adaptation of plastic responses in *T. thermophila* to novel conditions would be slower due to lower standing genetic variation in reaction norms. The higher variance in E (indicating current plastic responses) compared to the G × E interaction indicates that perhaps this may be due to significant prior selection for plastic responses. A similar scenario of reduced genetic variation in plasticity due to prior selection was reported by Charmantier et al. (2008) who compared plasticity of breeding dates in relation to temperature (and hence food availability) between populations of Great tits (*Parus major*) in the United Kingdom and the Netherlands. The authors demonstrate adaptive but reduced genetic variation in plasticity (nonsignificant G × E interaction) in breeding dates for the U.K. population, whereas the population in the Netherlands showed a significant G × E interaction but a less steep slope than the U.K. population. It is concluded that selection on plasticity in the United Kingdom has decreased variation between individuals, whereas on-going selection on reaction norms in the Netherlands has not yet fully decreased the variation (Charmantier et al. 2008).

When estimated across genotypes, dispersal decreased with increased density of kin (i.e., negatively density-dependent) and thus showed variation due to environmental conditions, that is, context-dependent dispersal. Exhibiting plasticity in relation to density concurs with most established theory, which predicts that dispersal should increase with density due to the costs of direct resource competition with conspecifics and also kin (Hamilton and May 1977; Travis et al. 1999). Empirical evidence for density effects on dispersal is somewhat mixed with the majority showing positive density-dependent dispersal (Matthysen 2005) and a few showing negative density-dependent dispersal (Denno and Peterson 1995; Matthysen 2005; Baguette et al. 2011; Fellous et al. 2012). Therefore, evidence from the genetic lines we studied here shows an opposite pattern to that predicted by many theoretical models and the prevailing empirical patterns. *Tetrahymena thermophila* ciliates are known to show some cooperative behavior under harsh environmental conditions by exchanging growth factors that allow cell populations to be maintained at both low and high densities (Schousboe and Rasmussen 1994; Chaine et al. 2010, respectively). Moreover, the density in our single genotype populations represents the density of kin, which may influence both dispersal and cooperation. Indeed, Chaine et al. (2010) found that cooperation is kin-based and that genetic lines are able to orient toward/against kin according to their cooperation strategy. A previous study found a negative association between the degree of cell cooperation and short-distance dispersal, as dispersal is likely to disrupt group structure (Schtickzelle et al. 2009). However, specialized morphs capable of long-distance dispersal are more frequent within cooperative genotypes of *T. thermophila* (Schtickzelle et al. 2009), with similar findings also known for spiders (Corcobado et al. 2012). Therefore, a likely explanation for an overall negative density-dependent pattern of dispersal in our experiments might stem from the density-related effects of cooperation previously detected in *T. thermophila*. However, given the extensive variation in cooperation among genotypes in our system (Schtickzelle et al. 2009), we might expect to find considerable variation in the response of different genotypes to density.

Examining the significance of slopes for each genotype separately, a decrease of dispersal with conspecific density was significant for 12 genetic lines, nonsignificant for 31 lines, and one genetic line showed significantly increased dispersal with density. Variation in dispersal plasticity may have adaptive value depending on the environment from which our *Tetrahymena* genotypes originate. Plastic behaviors are beneficial in variable environments, especially when environmental variability occurs at time scales shorter than the generation time (Liefting and Ellers 2008). Genotypes living in habitats with more ephemeral resources

should experience stronger variability in population density favoring highly plastic responses in dispersal, whereas genotypes from habitats with a more stable food supply where densities fluctuate less would benefit from less plasticity. The amount of genetic variation in reaction norms suggests that the response to a change in selection on the reaction norms may be slow. New collections are needed to infer whether within site variability in reaction norms is similar to the patterns we find here and could confirm this possibility through experimental evolution, providing a rare investigation of how selection acts on standing variation of reactions norms (Scheiner 2002; David et al. 2004). Such a task would be facilitated because the reaction norms we detected were primarily linear and thus more easily analyzed under an experimental framework (David et al. 2004; Rocha and Klaczko 2012).

DISPERSAL PHENOTYPES AND TRADE-OFFS

Alternative dispersal strategies may arise through adaptations to mitigate the costs of dispersal (Bonte et al. 2011) and improve survival during dispersal or settlement in a new habitat. Such positive combinations of dispersal-related traits are known as dispersal syndromes (Ronce and Clobert 2012). However, negative relationships between traits, such as a trade-off between fecundity and dispersal ability (Roff 1992), are also expected and reported from empirical studies (Johnson 1969; Rankin et al. 1986). The different dispersal propensity observed between our lines may be the result of genetic variation in strategies that result from trade-offs between life-history traits such as competitive ability against dispersal capacity. In the nematode *Caenorhabditis elegans* such a trade-off was proposed to explain the polymorphism of solitary and gregarious genotypes, where the ability to exploit resources was negatively associated with the dispersal propensity (Gloria-Soria and Azevedo 2008). *Tetrahymena thermophila* genetic lines also show a trade-off between the dispersal propensity and maximum cell density they can reach in standard nutrient conditions (Pennekamp 2014) suggesting that genetic lines that are potentially more competitive due to better conversion of resources into biomass are less mobile corresponding to a competition–colonization trade-off (Limberger and Wickham 2011). Likewise, a trade-off with dispersal could be linked to other traits such as social cooperation (Chaine et al. 2010). Such trade-offs could exist either within or between populations as described above.

Recent empirical work has shown that dispersers often have distinct phenotypes from nondispersers (Ims and Hjermann 2001). For example, in naked mole rats dispersers are larger and have a distinct morphology from residents (O'Riain et al. 1996). Likewise, in butterflies highly dispersive individuals have higher flight metabolic rates (Hanski et al. 2004; Niitepõld et al. 2009) and are more active, fly better, and have a distinct morphology compared to residents (Ducatez et al. 2012). Specific phenotypes optimized for dispersal could reduce costs of this strategy and thus make it competitive with nondispersers (Clobert et al. 2009). We found that within genotypes dispersers tend to differ in morphology and activity in addition to life-history traits (Fjerdingstad et al. 2007; Schtickzelle et al. 2009; Pennekamp 2014). However, our experiments also revealed that these differences in morphology and activity between resident and disperser cells vary between genotypes and are also dependent on local density.

Differences in morphology and behavior between residents and dispersers could either reflect a dispersal syndrome or be a consequence of dispersal itself. Our experimental design measures phenotypes on the population level, therefore we do not know the exact phenotype of each individual cell before and after dispersal took place, and thus cannot unambiguously tell if differences are a cause or consequence of dispersal. However, circumstantial evidence supports the view that differences are most likely a cause of dispersal: differences between replicates in cell size before dispersal show that the propensity to disperse was higher in the replicate with smaller cells suggesting that small cell size is a cause rather than a consequence of dispersal.

From a biomechanical point of view, the observed phenotype in the target tube should facilitate dispersal through reduced resistance by smaller and more elongated cells. Indeed, the small cell size of *T. thermophila* leads to low Reynolds numbers and therefore viscous-dominated regimes (Beveridge et al. 2010). Both shape and size are known to influence the drag of solid bodies in fluids as formalized by Stokes' law, in addition to the viscosity of the medium and the speed at which the body moves (Beveridge et al. 2010). Using an equation provided by Beveridge et al. (2010), we calculated the expected drag for a body of prolate ellipsoid shape, which is appropriate for *T. thermophila*. Evaluating this equation for size and shape changes observed between resident and dispersers in our experiment, we found that drag can decrease at the same scale as the shape (i.e., 25% less drag for dispersers than for residents) and also linearly with smaller cell sizes. However, for meaningful comparisons these values need to be expressed in comparison to the total energy expenditure of the individual cell.

Although early research on the energetic costs of movement in protists concluded that the amounts spent on locomotion were negligible (less than 1%) compared to the total metabolic energy expenditure (Fenchel and Finlay 1983), recent research showed that costs can be significant (1–10%) to substantial (10–100%) for small and fast moving ciliates or flagellates (Crawford 1992). Swimming speeds between 100 and 1000 μm/sec and sizes relevant to our *Tetrahymena* clones fall within the range where negligible to significant amounts of total energy expenditure are expected for protists (Crawford 1992). Based on this information, we suggest that the variation observed between residents and dispersers in terms of size and shape can result in different locomotion and consequently dispersal, albeit the energy costs due to different

morphology are probably limited. To fully understand the link between individual morphology, movement, and the population-level dispersal, we would need to quantify the movement behavior and morphology of individual cells by video analysis.

Alternative explanations could be that cells are smaller due to energetic costs associated to the dispersal process or that cells settle in the target due to lower density of conspecifics and hence the size differences result from differential cell division between tubes. However, conditions are nutrient rich throughout our experimental system so that cells are unlikely to be nutrient limited and show motility or energetic costs of dispersal as outlined above.

If our interpretation of morphology and activity differences is correct, the observed differences between tubes reflect *condition-dependent dispersal*, where certain morphological and behavioral properties influence the dispersal propensity of individuals (Clobert et al. 2009). Variation among genetic lines would then suggest that we have extensive genetic variation in the *condition-dependence* of dispersal such that some genotypes are highly condition dependent whereas others are not.

Our experiment evaluates *context* (density of kin) and *condition dependence* (size, shape, activity) simultaneously, which allowed us to examine the relationship between internal and external influences on dispersal. Although the dispersal propensity changed across densities in all genotypes, overall there was no consistent global trend of density on size and activity across all genotypes. There were however differences between residents and dispersers, which suggest *condition-dependent dispersal* across all density contexts. The presence of significant interactions between the tube (i.e., the contrast between residents and dispersers) and both the genotype and the density indicates that the magnitude and direction of differences between residents and dispersers varied among genotypes and across density contexts. In other words, some genetic lines show *condition-dependent dispersal* that is not sensitive to density, whereas other genotypes show an increase or decrease in *condition-dependent dispersal* as density increases.

Such inconsistencies are abundant in the literature and may reflect the interaction between *context*, *condition*, and genetic variation. For example, while under certain contexts individuals with higher body condition dispersed, under different contexts the opposite pattern was found (Matthysen 2012). Although puzzling at first, such inconsistencies in dispersal phenotypes may be explained by different motivations to disperse, linking the concepts of *context*- and *condition-dependent dispersal*. Indeed, individuals that disperse to avoid competition may differ from those that disperse due to other reasons such as kin structure (Clobert et al. 2009). If we also add in the influence of variation among genotypes that we detect in *Tetrahymena*, the complexity of dispersal decisions increases dramatically with just three variables (genotype, environmental context, individual condition) even when populations are composed of genetic clones.

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DATA ARCHIVING

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Overview of the strains used in the experiment.

Table S2. ANCOVA analysis using the dispersal propensity corrected for growth rate differences among genotypes and densities.

Figure S1. Box plot of variance partitioning when three genotypes were randomly sampled from the full set and the explained variation for each effect computed on such reduced data ($n = 1000$ simulation runs).

Figure S2. Histogram of the generation times observed under conditions representative for the dispersal experiment ($N = 44$ genotypes).

Figure S3. Comparison of dispersal reaction norms that were corrected (red) versus those uncorrected for growth (blue).