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Transgenerational plasticity of dispersal-related traits in a ciliate: genotype-dependency and fitness consequences

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Phenotypic plasticity, the ability of one genotype to produce different phenotypes in different environments, plays a central role in species' response to environmental changes. Transgenerational plasticity (TGP) allows the transmission of this environmentally-induced phenotypic variation across generations, and can influence adaptation. To date, the genetic control of TGP, its long-term stability, and its potential costs remain largely unknown, mostly because empirical demonstrations of TGP across many generations in several genetic backgrounds are scarce. Here, we examined how genotype determines the TGP of phenotypic traits related to dispersal, a fundamental process in ecology and evolution. We used an experimental approach in *Tetrahymena thermophila*, a ciliate model-species, to determine if and how phenotypic changes expressed following a dispersal treatment are inherited over multiple generations. Our results show that morphological and movement traits associated with dispersal are plastic, and that these modifications are inherited over at least 35 generations. The fitness costs and benefits associated with these plastic changes are also transmitted to further generations. We highlight that the genotype modulates the expression and reversibility of transgenerational plasticity of dispersal-related traits and its fitness outcomes. Our study thus suggests that genotype-dependent TGP could play an important role in eco-evolutionary dynamics as dispersal determines gene flow and the long-term persistence of natural populations.

Keywords: dispersal, experimental microcosms, movement, protist, *Tetrahymena*, transgenerational plasticity

Introduction

Transgenerational plasticity (TGP) has been proposed as a fundamental mechanism promoting evolution of the living world (Uller 2008, Herman and Sultan 2011). TGP occurs when abiotic (Galloway and Etterson 2007, Marshall 2008,

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Heckwolf et al. 2018) and biotic (Dantzer et al. 2013) environmental conditions alter the phenotype of parents and when those changes then affect offspring phenotypic expression (i.e. parental effects), with the possibility of persisting effects for multiple generations (i.e. grand-parental effects and more). For instance, parents can produce young with phenotypic characteristics that increase their fitness when exposed to similar environmental conditions (i.e. adaptive TGP; Dantzer et al. 2013). Alternatively, phenotypic modifications induced by TGP may be neutral, or decrease offspring performance via transgenerational costs (i.e. maladaptive TGP; Marshall 2008). The ability to transmit and express an advantageous phenotype at the next generation(s), or to mitigate the costs of TGP, could depend on the genetic background (Herman and Sultan 2016), similar to phenotypic plasticity in general. Indeed, evolution of reaction norms (slope and curvature) and the mitigation of plastic costs can depend on specific genetic variants (i.e. G × E interactions; Gerken et al. 2015), epigenetic marks under strict or partial genetic control (Kooke et al. 2015), and the regulation of gene expression (Murren et al. 2015). However, with the exception of the predictions from few theoretical models (see for instance Greenspoon and Spencer 2018), the role of genetic background in TGP evolution remains poorly understood (see however Alvarez et al. 2020), despite its critical importance for the ability of the living to cope with current global change (Guillaume et al. 2016, Donelson et al. 2018).

Dispersal, the movement of individuals potentially leading to gene flow (Ronce 2007), is a highly relevant candidate for investigating TGP mechanisms. Dispersal is a complex, multidimensional process, which can be plastic at all its stages (emigration, transience and immigration; Clobert et al. 2009, Cote et al. 2017) and under partial genetic control (Saastamoinen et al. 2018). Environmental factors composing habitats and their surrounding matrix can influence dispersal metrics (e.g. dispersal rate or dispersal distance; Hanski and Gaggiotti 2004, Baguette et al. 2013) as well as the expression of a broad range of dispersal-related traits (e.g. morphology, behavior and physiology; Bonte et al. 2012, Ronce and Clobert 2012). In addition, dispersal movements per se can plastically change phenotypic traits, for instance due to different environments between patches and matrix, or energy allocation (Bonte et al. 2012, Winandy et al. 2019, Jacob et al. 2020). Dispersal evolution is determined by the balance between the fitness benefit of moving, for instance to escape local detrimental conditions for survival or reproduction, and the related costs (Clobert et al. 2009, Bonte et al. 2012). Dispersal is especially constrained by direct (e.g. energy and time) costs incurred during the displacements in the landscape matrix and indirect costs associated with the expression of phenotypic traits facilitating dispersal (Bonte et al. 2012). These associations between dispersal and other traits are called 'dispersal syndromes' (Ronce and Clobert 2012) and may result in tradeoffs when traits are negatively correlated with fitness components, notably due to gene pleiotropy (Saastamoinen et al. 2018). Interestingly, dispersal syndromes can result from plasticity expressed before, during or after dispersal (Cote et al. 2017).

Previous studies suggested that TGP may facilitate the transmission of traits across generations that improve dispersal in a given environmental context (MacKay and Wellington 1976, Massot et al. 2002, Bestion et al. 2014, Bitume et al. 2014), while offering the possibility to reverse or explore other phenotypic states if the environment changes again (Saastamoinen et al. 2018). Adaptative TGP in dispersal can emerge 1) if the expression of a plastic dispersal rate or dispersal trait state optimizes the dispersal process, 2) if the descendants of those dispersing and/or non-dispersing individuals have advantage to adopt the same strategy as their ancestors and 3) if offspring information gathering costs are greater than the cost of TGP expression. TGP in dispersal could also be neutral or maladaptive especially in cases where a rather stable mechanistic pathway is activated as a consequence of dispersal at any of its stage (e.g. epigenetic mark). In absence of empirical evidence, one might expect that TGP for any component of the complex dispersal process could occur in concert with the transmission of its fitness consequences across generations. In addition, the genetic background of parents could affect the ability to transmit dispersal-related traits and could modulate fitness costs associated to the expression of those traits across generations. However, these hypotheses have not been yet tested due to difficulties in studying TGP across many generations and across different genotypes.

Here, we investigated the genotype-dependency and fitness consequences of TGP by comparing dispersal-related traits in cells engaged in dispersing or non-dispersing behaviors. We used the ciliate *Tetrahymena thermophila*, which represents an excellent biological model to study TGP for dispersal for many reasons. This species reproduces clonally in standard axenic laboratory conditions (Nelsen 1978, Bell and Stein 2017), with the availability of several genotypes showing different degrees of movement plasticity (Schtickzelle et al. 2009, Pennekamp et al. 2014, 2019, Jacob et al. 2016a). A standard procedure using two-patches microcosms connected by a corridor allows to quantify many aspects of dispersal such as the architecture of dispersal syndromes (Fjerdingstad et al. 2007), the causes of dispersal (Pennekamp et al. 2014, Fronhofer et al. 2018), the cooperation–colonization tradeoff (Fjerdingstad et al. 2007, Jacob et al. 2016a), range expansions (Fronhofer and Altermatt 2015, Fronhofer et al. 2017) or (meta)population and community dynamics (Fox et al. 2013, Jacob et al. 2019). In such an experimental system, microenvironmental variation occurring throughout dispersal assays (for instance spatial and temporal variation of cell density during assays or patch versus corridors conformations) as well as dispersal per se can induce plastic changes in phenotypic traits that might distinguish dispersing from non-dispersing cells, even when resource is unchanged. It was recently shown that dispersal syndromes in *T. thermophila* are partly the result of plastic changes occurring when cells disperse toward a new experimental patch (Jacob et al. 2020, Junker et al. 2021). These plastic dispersal syndromes at the within generation level

involve traits related to morphology, movement and demography that strongly differ among genotypes (Nelsen 1978, Fjerdingstad et al. 2007, Pennekamp et al. 2014, Jacob et al. 2016a, 2020). For instance, cells can plastically change their phenotypes from pyriform-shaped cell to elongated rapid-swimming cell with dense ciliation, some cells can develop a long caudal cilium, and oral apparatus can be replaced (Nelsen 1978, Junker et al. 2021). Some of these environmentally-induced characteristics (e.g. via starvation; Nelsen 1978, Nelsen and Debault 1978) can facilitate dispersal (like cell shape in Fjerdingstad et al. 2007, Pennekamp et al. 2014, Jacob et al. 2016a, Junker et al. 2021) and/or help escape stressful environmental conditions. The elongated rapid-swimming phenotype is only partially reversible over cell lifetime (Nelsen 1978) and has decreased growth rate (Schtickzelle et al. 2009, Jacob et al. 2016a), suggesting fitness cost related to dispersal. It is however unknown if and how this intragenerational plasticity (IGP) translates into transgenerational effects.

We performed six successive 4-h dispersal trials in the above-described two-patch systems separated by ~35 cell divisions in common garden to produce two cell lines, dispersing versus non-dispersing cells, in four isogenic strains (negligible genetic variation inside a strain, Fig. 1). Before

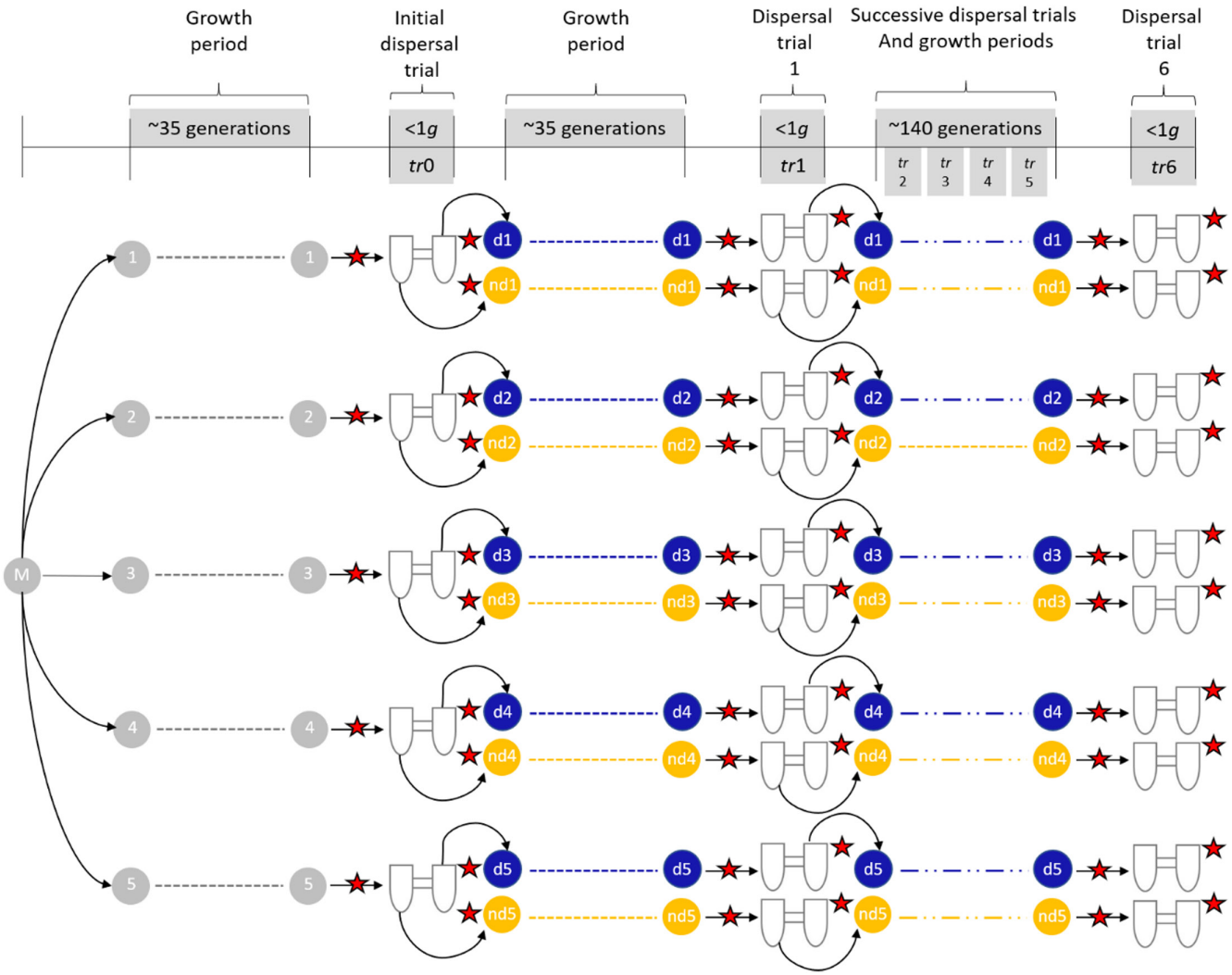

Figure 1. Experimental design to test for transgenerational plasticity of dispersal-related traits. For our four genotypes (D3, D4, D6 and D9), we generated five initial replicated populations (1–5) from a mother culture (M) obtained from the isolation of a single cell (i.e. isogenic population). For simplicity, only one genotype is represented. The five replicated initial populations were cultivated over a seven-days period (~35 generations). Next, we performed the initial dispersal trial (*tr*0) from which we produced one subpopulation (from 1 to 5) with dispersing ancestors (*d*) and one subpopulation with non-dispersing ancestors (*nd*). The trial lasted 4 h, which is less than the time of a cell generation (< 1*g*). After a seven-days period of growth (again ~35 generations), we performed another dispersal trial (*tr*1). We kept and cultivated dispersing cells and non-dispersing cells for *d* and *nd* subpopulations respectively. This procedure was repeated five more times to obtain a total of six dispersal trials. Phenotypic measures (cell size, cell shape, movement linearity and velocity) and fitness measures (growth rate) were performed just before and just after dispersal trials (red stars).

the first dispersal trial, we verified the degree of genotype-dependency for a set of morphological (cell size and shape) and movement (velocity and linearity) traits related to dispersal in *T. thermophila*, as well as in fitness using cell growth as a proxy (Orr 2009). Then, during the first dispersal trial, we investigated IGP of dispersal syndromes (Ronce and Clobert 2012, Stevens et al. 2013, Legrand et al. 2016) by examining if and how dispersing and non-dispersing cells differ in their morphological and movement traits. Next, we investigated the existence of TGP by comparing cells before and after common gardens, and we assessed how the genetic background determines the plastic response of these dispersal-related traits during the six additional dispersal trials.

We tested the hypotheses that 1) the dispersal status of ancestors affects the phenotype of descendants across several generations through TGP, and 2) the strength of immediate and transgenerational plastic response varies with the genetic background. We also examined 3) if the observed TGP was gradual or stable when repeated dispersal trials are experienced by ancestors (Vastenhouw et al. 2006, Remy 2010, Sentis et al. 2018). Finally, we tested 4) whether dispersing cells incur a fitness cost (Bonte et al. 2012) at the first dispersal trial and whether this cost is cumulative through dispersal assays, and modulated by the genotype.

Material and methods

Model species, culture conditions and experimental microcosms

Tetrahymena thermophila is a 30- to 50-µm ciliated unicellular eukaryote naturally living in freshwater ponds in North America, which alternates sexual and asexual phases depending on environmental conditions. The species is a model organism in cell and molecular biology, and its maintenance under laboratory conditions benefits from decades of experience (Collins 2012).

We used four genotypes originally sampled and kindly provided by F. P. Doerder between 2002 and 2008 in North America (genotype D3, D4, D6 and D9; Pennekamp et al. 2014), and bred uniquely under clonal conditions. To control for genetic variation while testing whether TGP explains experimental patterns, mother cultures were established from the isolation of a single cell for each genotype, and these cultures were then split into five replicates (Fig. 1). Experiments were also limited to six weeks with one dispersal trial per week (~200 asexual generations for the entire experiment). This procedure prevents the possibility that pre-existing genetic variation explains the observed phenotypic pattern during experiment and excludes a major role of new genetic variation. The origin of the phenotypic heterogeneity in these mother cultures despite absence of genetic variation is discussed in the Supporting information. Before and during the experiment, cells were all cultivated in the same standard conditions: 23°C in climatic chambers in 0.3× synthetic liquid growth media (0.6% Difco proteose peptone, 0.6% yeast extract) as described in previous studies (Fjerdingstad et al. 2007, Schtickzelle et al. 2009, Jacob et al. 2015). In these conditions, the cell division time is around 4–6 h (~5 generations per day). All manipulations were performed in sterile conditions under a laminar flow hood.

To examine IGP and TGP of dispersal related-traits, we used simple two-patch systems (Supporting information): two habitat patches consisting of 1.5 ml tubes were connected by a corridor made of a 2.5-cm long silicone tube with a 4 mm-internal diameter, the entire system being filled with synthetic media, meaning that the dispersal set-up is clueless (Fjerdingstad et al. 2007, Schtickzelle et al. 2009, Jacob et al. 2016a). The patch in which cells are inoculated is called the departure patch (cells are pipetted in the departure patch and homogenized), while the second, initially free of cells, as well as the corridor, is called the arrival patch. This experimental system allows quantifying dispersal per se. It has been shown that emigration is linked to movement variation and cell activity when traits are measured after dispersal assays (Pennekamp et al. 2019). However, no relation exists between dispersers' movement characteristics measured just after dispersal and innate cells' movement characteristics (i.e. routine movements of cells unexposed to dispersal assays, Junker et al. 2021). As a result, the dispersal events quantified in two-patch systems are likely not the simple by-products of foraging movements (Junker et al. 2021).

Protocol of successive dispersal trials and common garden

We performed an experimental procedure of repeated dispersal trials to investigate how the phenotype of cells is affected by the dispersal status of their ancestors and how the number of experienced trials affects the phenotype of descendants. Before the first dispersal trial, for the four genotypes, we isolated by hand-pipetting one mother cell that reproduced clonally over a seven-day period in one 2 ml well of a 24-well plate. From this initial mother culture, we made five replicates (i.e. initial populations) that were cultivated over another seven-days period (~35 cell divisions; Fig. 1).

These 20 populations (five replicates in four genotypes) then experienced an initial dispersal trial (*tr*0) that allowed producing one subpopulation with dispersing ancestors and one subpopulation with non-dispersing ancestors. To do so, a normalized fraction of ~100 000 cells from initial populations was placed in the departure patch of dispersal systems, while corridors were closed with clamps. Next, corridors were opened and cells may either stay in the departure patch, or disperse to the arrival patch, over a 4-h period (less than one generation). During the experiment, cell movement capacities might theoretically lead to a homogenous distribution, i.e. equal spatial distribution within the two-patches system within a few minutes after inoculation if assuming perfectly straight movement (Laurent et al. 2020). However, such straight movements and the resulting equal spatial distribution have never been observed, and previous studies showed that movements between patches clearly deviates

from random cell diffusion likely as a result of dispersal decisions (Jacob et al. 2018, Laurent et al. 2020). Mean dispersal rates, the proportion of dispersing cells overall cells in the system, are available for each genotype at each time point in the Supporting information. After this period, the corridors were clamped and we inoculated a new separate population in common garden conditions for these 40 subpopulations (five replicates, four genotypes, two dispersal treatments). Common gardens consisted of 2 ml wells of a 24-well plate without dispersal possibility filled with standard medium, in which a normalized fraction of ~1000 cells could grow during seven days (around 35 generations) at 23°C.

After this first dispersal trial and the following common garden, the 40 subpopulations were subjected to a new dispersal trial and common garden (*tr*1). The procedure was then repeated, leading to a total of six trials (*tr*1 to *tr*6), all interspersed with separate common gardens hosting the dispersing cells and non-dispersing cells in the subpopulations with dispersing and non-dispersing ancestors respectively (Fig. 1).

Phenotype and fitness measurements

Four phenotypic traits (morphology: cell size and shape; movement: velocity and linearity, below) were measured in initial populations. Then, from trials *tr*0 to *tr*6, the same traits were measured just 'before' and 'after' each dispersal trial. The 'before' measurement was used to quantify traits after the seven days of the common garden, i.e. around 35 generations. This seven-days period ensures that genotypes all reached the stationary phase of their logistic growth. The normalization of measurements at the same phase is important because the morphology of cells changes along the growth cycle in axenic *Tetrahymena* cultures (Taylor et al. 1976). The 'after' measurement was used to quantify traits at the exact time of dispersal. Cell size (area in μm^2) and shape (cell major/minor axis ratio of a fitted ellipse), as well as velocity ($\mu m\ s^{-1}$) and movement linearity (distance in straight line/effective distance covered), were measured using on automated analysis of digital images and videos (Supporting information, Pennekamp and Schtickzelle 2013, Pennekamp et al. 2015). All phenotypic measurements were performed in standardized conditions: for each sample of cells, we considered five technical replicates (10 µl) pipetted into one chamber of a multi-chambered counting slide (Kima precision cell 301890), and took digital pictures under dark-field microscopy (Pennekamp and Schtickzelle 2013). Data from the five technical replicates were pooled in all analyses. We used ImageJ (ver. 1.47, National Institutes of Health, USA) and BEMOVI (Pennekamp et al. 2015) software to measure morphological and movement variables.

We measured cell fitness just before and just after the dispersal trial for both dispersing and non-dispersing cells at three dispersal trials (*tr*0, *tr*1 and *tr*6) using standard population growth analyses. Small numbers of cells (~100 cells) were transferred in four technical replicates into 96-well plates filled with 250 µl of fresh growth media. Cultures were maintained at 23°C and absorbance measurements at 550 nm were performed every 2 h for two weeks using an automated microplate reader (Tecan Infinite Spectrophotometer with a Connect robotized arm). We then computed the growth rate as the maximum slope of logistic population growth through time and the maximal population density as the density reached at the plateau by smoothing the absorbance data using general additive model (gam package; Hastie 2018), and fitting a spline-based growth curve using the grofit package of R (*gcfit* function; Kahm et al. 2010). For simplicity, we present results on growth rate, the most frequently used fitness proxy (Orr 2009), given that results were qualitatively similar using maximal density (data not shown).

Our analyses highlighted that genotype have distinct growth characteristics and dispersing and non-dispersing cells differ in growth pattern, meaning that densities in common gardens depend on biological replicates and time. Therefore, we examined the effect of density dependence on phenotypic traits and dispersal rate after the seven-days growth periods (Supporting information). We did not find any significant effect of cell density on velocity and shape (the two traits influenced by TGP in our study). Furthermore, although density influenced dispersal rate, this effect was similar in cells with dispersing and non-dispersing ancestors (Supporting information), indicating that density did not interact with our experimental treatment. Therefore, we assumed that density has negligible influence on TPG of dispersal in our experimental system.

Statistical analyses

Initial heterogeneity and covariation of velocity, movement linearity, cell shape, cell size and growth rate

Trait covariation (model 1 in the Supporting information)

First, we assessed the initial relationships between the four phenotypic traits (i.e. cell shape, cell size, movement velocity and linearity). We used Pearson's correlation tests on phenotypic measurements recorded prior the first dispersal trial (*tr*0) to assess between-traits covariation pattern. Furthermore, linear mixed models were used to examine correlations between cell growth rate and the four phenotypic traits at *tr*0. Cell growth rate was treated as the dependent variable whereas the phenotypic trait was introduced in the model as an explanatory term. The dependent variable was log-transformed and the explanatory variable was z-scored. The strain and the replicate were introduced as random effects (i.e. random intercepts) in the model. For all analyses implicating linear mixed models, we used restricted maximum likelihood optimization. Normality of the residuals was examined graphically using a quantile–quantile plot. We used a likelihood ratio test to assess the significance of the relationship, meaning that we compared the models with and without the explanatory term. We calculated marginal R^2 to quantify the proportion of variation explained by the explanatory variable only.

Effect of genotype on cell phenotype and fitness (model 2 in the Supporting information)

We evaluated the influence of the genetic background on the four phenotypic traits and cell growth rate before the first dispersal trial at *tr*0. We used linear mixed models in which the

log-transformed phenotypic traits were introduced as dependent variables, cell genotype as the explanatory variable, i.e. a discrete variable with four modalities, and the replicates as random effects.

Intragenerational plasticity of dispersal-related traits and its fitness consequences after the first trial

Dispersal syndrome and dispersal-related fitness cost (model 3 in the Supporting information)

We investigated IGP of dispersal syndromes and its fitness costs by comparing the distributions of phenotypic traits between dispersing and non-dispersing cells. We examined how morphology and movement behavior correlate with cell dispersal status within one generation after *tr*0 – the test lasted 4 h, namely less than one generation in *T. thermophila*. We made general analysis where all genotypes were combined. We used linear mixed models where the log-transformed phenotypic traits were introduced as dependent variables, the cell dispersal status as the explanatory variable (i.e. a discrete variable with two modalities, dispersing versus non-dispersing), and the genotype and replicate as random effects. We did not investigate IGP during the other dispersal trials (*tr*1 to *tr*6) because during our experimental procedure, we only kept and cultivated dispersing cells and non-dispersing cells for *dispersing* and *non-dispersing* subpopulations respectively (Fig. 1). In addition, measurements of IGP from *tr*1 to *tr*6 were supposedly impacted by TGP resulting from the previous dispersal trials, complicating IGP interpretations at any dispersal trial other than *tr*0. Significant phenotypic differences between the two types of cells after a 4-h dispersal treatment can be due to plastic mechanisms expressed during the treatment, to the sorting of phenotypic variation already present in mother cultures (Supporting information), or to selective mortality during the dispersal assay (Junker et al. 2021). The existence of a wider phenotypic distribution in addition to specific trait correlation patterns after the dispersal trial (i.e. dispersing and non-dispersing cells) compared with before (i.e. in mother cultures) would distinguish the plastic scenario from the two others (Junker et al. 2021).

Transgenerational plasticity of dispersal-related traits, dispersal rates and fitness consequences: genotype-dependency and reversibility

Effect of genotype and ancestor dispersal status on descendant dispersal rates, phenotype and fitness (model 4 in the Supporting information)

We examined the effect of ancestor dispersal status (dispersing versus non-dispersing lines coded as a discrete variable) on dispersal rates, phenotypic traits and fitness of descendants reared under common garden conditions during a seven-days period (~ 35 cell divisions). This experimental set-up therefore allowed focusing on TGP with a limited confounding effect of IGP. First, we performed a general analysis using linear mixed models where log-transformed dispersal rates, phenotypic traits and fitness were introduced as dependent variables, and the ancestor dispersal status as discrete explanatory variable. The genotype, the replicate and the number of dispersal trials experienced by ancestor were included in the model as random effects (model 4.1 in the Supporting information). Then, to examine how the genotype determines the persistence of dispersal phenotypes in descendants, we built a model including the interaction *genotype* × *ancestor dispersal status* (G×E, model 4.2 in the Supporting information) and evaluated the support of the interaction term using a likelihood ratio test. Lastly, we conducted a partial analysis where we analyzed the four genotypes separately (model 4.3 in the Supporting information) to get R^2 calculated for each genotype.

Effect of the number of successive dispersal trials on descendant phenotype and fitness (model 5 in the Supporting information)

We retrieved the same linear mixed models used to investigate the effect of ancestor dispersal status on phenotype and fitness (model 3 in the Supporting information), but the number of dispersal trials experienced was removed from the random effects and introduced in the fixed part of the model. For the phenotypic traits, the number of dispersal trials (from 0 to 6) was incorporated as a continuous variable. We tested additive and interactive effects (ancestor dispersal status × number trials) of the variable, and considered both linear and logarithmic relationships; a likelihood ratio test was performed to compare the two relationships. For cell fitness, the number of dispersal trials (0, 1 and 6) was entered in the model as discrete variable, and both additive and interactive effects were examined.

Reversibility of transgenerational plastic changes (model 6 in the Supporting information)

We examined how stable were the transgenerational changes of cell phenotype by comparing the phenotype of cells measured after each dispersal trial and the phenotype of their descendants after ~ 35 asexual generations (seven days) in common garden (model 6.1 in the Supporting information) in dispersing lines. We used linear mixed models where log-transformed phenotypic traits and fitness were introduced as dependent variables, and the type of cell (i.e. ancestor after dispersal trial versus descendants) as explanatory variable. We included the genotype, the replicate and the number of dispersal trials experienced by ancestor as random effects. Next, to investigate how the genotype determines reversibility of transgenerational plastic changes, we built a model including the interaction *genotype* × *type of cell* (ancestor versus descendant) (model 6.2 in the Supporting information) and evaluated the support of the interaction term using a likelihood ratio test. Lastly, we conducted a partial analysis where we analyzed the four genotypes separately (model 6.3 in the Supporting information) to get R^2 calculated for each genotype.

Results

Initial heterogeneity and covariation of velocity, movement linearity, cell shape, cell size and growth rate

Before the initial dispersal trial (*tr*0, Fig. 1), we examined the covariation among the four tested dispersal-related traits

within mother cultures (see the Supporting information for a discussion on the origin of this phenotypic variability). We found that velocity was positively correlated to movement linearity and cell shape in the four genotypes, although the strength of the correlation varied among genotypes (Fig. 2); the fastest cells had the most linear movements and show higher elongation. Furthermore, velocity was positively correlated with cell size in genotypes D3, D4 and D6 and negatively correlated with cell size in D9 (Fig. 2). Movement linearity was also positively correlated to cell size in the four genotypes, although the strength of the correlation differed among genotypes (Fig. 2). We also measured cell growth rate estimated from 15 days (~75 generations), a common fitness proxy in *T. thermophila* (model 1). Growth rate was negatively correlated to cell shape ($R^2 = 0.45$, $\chi^2 = 4.15$, $p = 0.04$), but no significant relationship was found with cell size ($R^2 = 0.14$, $\chi^2 = 1.21$, $p = 0.27$), linearity ($R^2 = 0.08$, $\chi^2 = 0.69$, $p = 0.40$) and velocity ($R^2 = 0.12$, $\chi^2 = 0.98$, $p = 0.32$).

We then examined the effect of genotype identity on the four phenotypic traits (model 2). Genotype explained 46% of cell size variation ($\chi^2 = 90.31$, $p < 0.0001$), 26% of cell shape variation ($\chi^2 = 64.67$, $p < 0.0001$), 7% of movement linearity variation ($\chi^2 = 49.39$, $p < 0.0001$) and 5% of velocity variation ($\chi^2 = 11.47$, $p = 0.009$). It also explained 87% of variation in growth rate ($\chi^2 = 44.65$, $p < 0.0001$). These results indicate strong phenotypic differences between the genetic backgrounds used in our experiments.

Intragenerational plasticity of dispersal-related traits and its fitness consequences after the first trial

Next, we investigated IGP of dispersal syndrome by performing an immediate quantification of the association between the dispersal status of cells and phenotypic traits just after the first dispersal trial (*tr*0; model 3). Dispersing cells were more elongated ($R^2 = 0.02$, $\chi^2 = 61.94$, $p < 0.0001$) and swam faster ($R^2 = 0.03$, $\chi^2 = 77.69$, $p < 0.0001$) than non-dispersing cells (Fig. 3), with ranges of phenotypic values generally wider than the ones of mother cultures. By contrast, dispersing and non-dispersing cells did not significantly differ in terms of size ($R^2 = 0.001$, $\chi^2 = 2.18$, $p = 0.13$) and movement linearity ($R^2 = 0.001$, $\chi^2 = 2.14$, $p = 0.16$), although in mother cultures, the longest cells are the biggest and those swimming the more linearly. This result indicates that some trait correlations observed in mother cultures were altered after the dispersal trial. In addition, fitness differed between the dispersing and non-dispersing cells: dispersing cells had lower growth rate than non-dispersing ones ($R^2 = 0.04$, $\chi^2 = 15.48$, $p < 0.0001$). Moreover, trait mean and variance differed between cells from mother cultures and cells from dispersal trials (dispersing + non-dispersing cells) in most trait × genotype combinations (Supporting information). Interestingly, for all genotypes, variance of cell velocity, cell size and movement linearity was higher in cells from dispersal trials than in cell from mother cultures. These shifts in trait distribution, occurring within a very short time period (less than 4 h), are necessarily caused by intragenerational plasticity.

Altogether, our results highlight the existence of a plastic dispersal syndrome. We indeed observe non-overlapping shifts in trait distributions between mother cultures and cultures exposed to dispersal at the within generation-level, and different trait-trait correlation patterns separating dispersing and non-dispersing cells from those observed in mother cultures. As cell size and movement linearity did not differ between dispersing and non-dispersing cells, we focused further analyses on this plastic dispersal syndrome on cell velocity and shape.

Transgenerational plasticity of dispersal-related traits and dispersal rate: genotype-dependency and reversibility

We tested for the persistence of plastic trait divergence between dispersing and non-dispersing cells following each dispersal trial after ~35 asexual generations in common garden conditions in the whole dataset (i.e. existence of TGP, model 4.1; Fig. 1). The dispersal status of cell ancestors, i.e. cells from the dispersing versus non-dispersing selected lines, affected descendants' velocity ($R^2 = 0.05$, $\chi^2 = 8.58$, $p = 0.003$) and shape ($R^2 = 0.01$, $\chi^2 = 8.58$, $p = 0.03$). Cells with a dispersing ancestor recurrently had a higher velocity and a more elongated shape than those with a non-dispersing ancestor even after ~35 asexual generations of common garden (Fig. 4).

We then examined how the strength of the effect of ancestor dispersal status on phenotypic traits differed among genotypes (i.e. genotype-dependency of TGP, model 4.2 and 4.3). The interaction genotype × ancestor dispersal status (model 4.2) was supported by the data for velocity ($R^2 = 0.28$, $\chi^2 = 7.95$, $p = 0.04$), and genotype-specific models (model 4.3) confirmed this result. This indicates that the proportion of velocity variation explained by the ancestor dispersal status varied among genotypes (from 0.3% to 13% of velocity variation in D6 and D9 respectively, Supporting information). By contrast, the interaction genotype × ancestor dispersal status (model 4.2) was not significant for cell shape ($R^2 = 0.68$, $\chi^2 = 4.23$, $p = 0.17$), although the proportion of shape variation explained by the ancestor dispersal status varied among genotypes (from 0.01% to 11% of velocity variation in D6 and D9 respectively, Supporting information).

Then, we evaluated the influence of ancestor dispersal status on population-level dispersal rate (i.e. TGP on dispersal rates, model 4.1). The model where genotype was coded as a random effect indicated that ancestor dispersal status had a negligible effect on dispersal rate as whole ($R^2 = 0.002$, $\chi^2 = 0.59$, $p = 0.44$). However, further analyses showed that the direction and amplitude of the ancestor dispersal status effect on dispersal rate differed among genotypes; the interaction genotype × ancestor dispersal status (model 4.2) was strongly supported by the data ($R^2 = 0.14$, $\chi^2 = 15.36$, $p = 0.001$). In genotype D9 where ancestor dispersal status had the strongest effect on cell elongation and velocity (Supporting information), dispersal rate was higher in cell populations with dispersing ancestors than in populations with non-dispersing ancestors ($R^2 = 0.07$, $\chi^2 = 4.60$, $p = 0.03$). By contrast, in

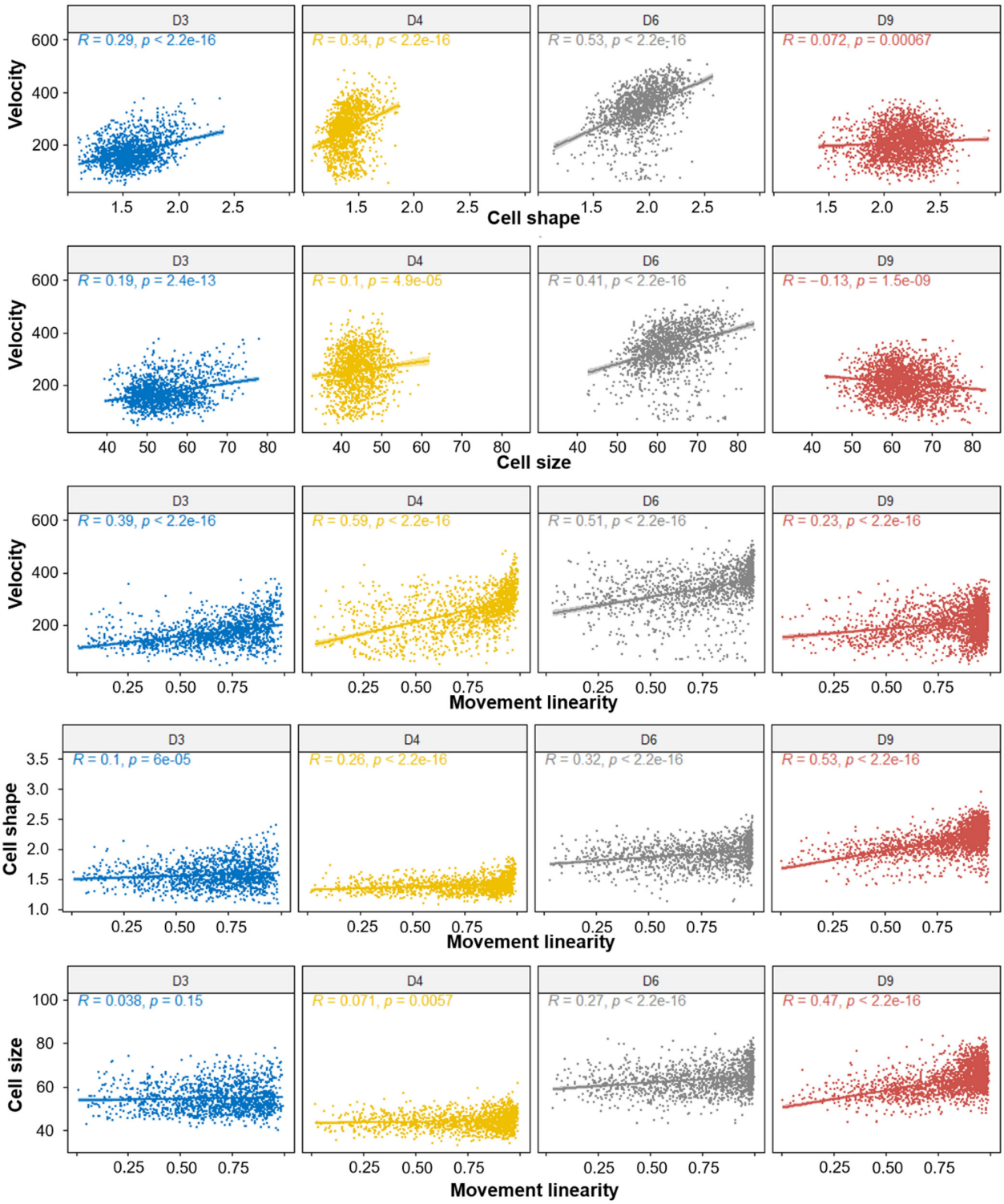

Figure 2. Covariation between cell traits [i.e. velocity (µm s^{-1}), movement linearity (distance in straight line/effective distance covered) and cell size (area in $µm^2$) and shape (cell major/minor axis ratio of a fitted ellipse)] in the four genotypes (D3, D4, D6 and D9) considered in our study before the first dispersal trial. The five replicates for each genotype were pooled. Pearson correlation coefficient and associated p-value are provided.

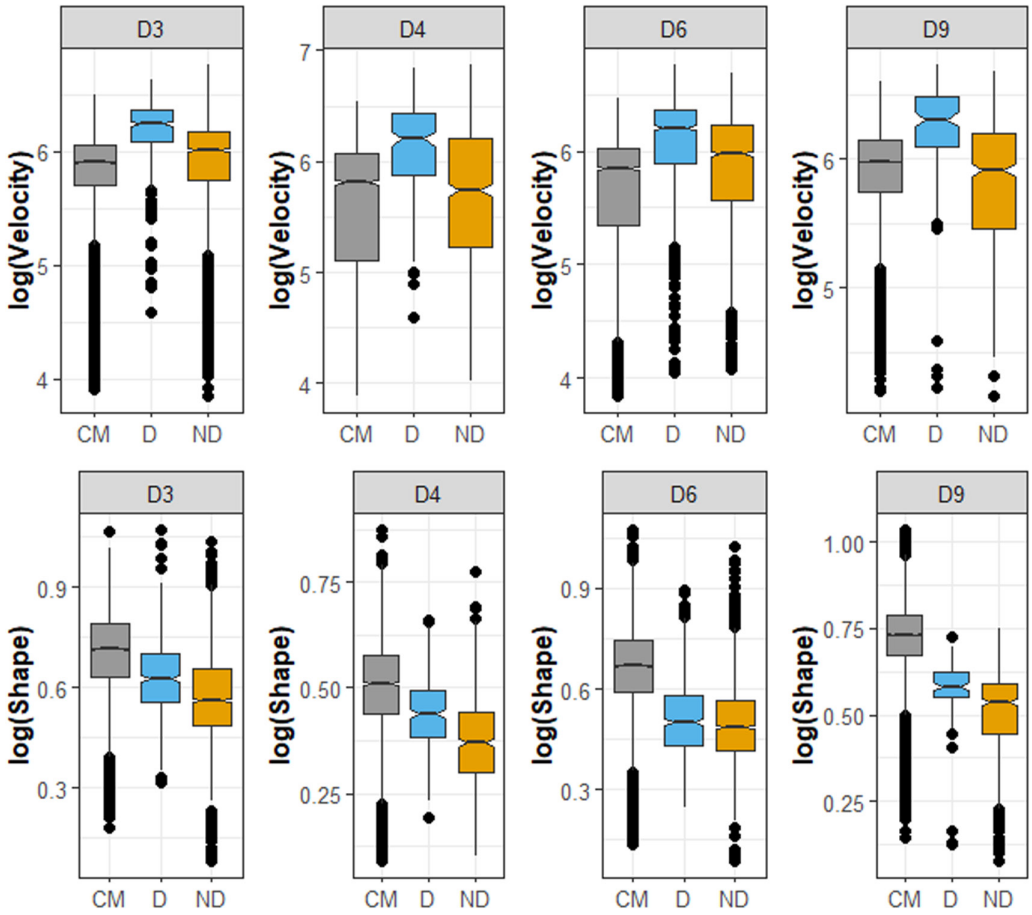

Figure 3. Intra-generational plasticity for dispersal-related traits (i.e. the two traits differing between dispersing and non-dispersing cells at dispersal trial *tr*0): cell velocity (µm s^{-1}) and shape (cell major/minor axis ratio of a fitted ellipse) in the four genotypes (D3, D4, D6 and D9). CM = cell velocity and shape measured prior the dispersal trial in mother cultures (grey), D = cell velocity and shape measured just after the 4 h of the dispersal trial in dispersing cells (i.e. less than one asexual generation; blue), ND = cell velocity and shape measured just after the 4 h of the dispersal trial in non-dispersing cells (yellow). The distribution of trait is based on individual data (and not population data like in further analyses; Supporting information). We show relationships where the effect of the ancestor dispersal status on phenotypic traits was significant with a p-value threshold of p = 0.05. The distribution of population growth cannot be shown because of the limited number of population replicates.

genotype D6 where ancestor dispersal status had the weakest effect on cell elongation and velocity (Supporting information), cell populations with a dispersing ancestor had lower dispersal rate than populations with a non-dispersing ancestor ($R^2 = 0.10$, $\chi^2 = 6.80$, $p = 0.009$). The influence of ancestor status on dispersal rate was marginal in genotypes D3 and D4 (Supporting information).

Increasing the number of dispersal trials experienced by each experimental line did not cause a gradual change of trait values with time (model 5). The shape and velocity differences between the descendants of dispersing and non-dispersing cells appeared at *tr*0 and did not increase nor decrease over the following trials (from *tr*1 to *tr*6, Fig. 5). Accordingly, the association between these phenotypic traits and the number of dispersal trials was better described by a logarithmic relationship than a linear relationship (velocity, $\chi^2 = 35.40$, $p < 0.0001$; shape, $\chi^2 = 18.35$, $p = 0.0001$). In addition, the interaction between 'ancestor dispersal status' and 'number of trials' was not supported by the data for the two phenotypic traits (Supporting information).

Finally, we tested how stable the described transgenerational changes of cell phenotype were by comparing the phenotype of cells measured after each dispersal trial and the phenotype of their descendants after ~ 35 asexual generations in common garden (model 6.1). Cells with a dispersing ancestor had a higher velocity immediately after the trial than after ~35 generations ($R^2 = 0.23$, $\chi^2 = 82.09$, $p < 0.0001$), indicating that this trait was partially reversible under standard environmental conditions (Fig. 6). Yet, the reversibility was not sufficiently strong to eliminate the effect of ancestor dispersal status on descendant phenotype. By contrast, the shape of descendants was more elongated than that of their ancestors ($R^2 = 0.09$, $\chi^2 = 52.19$, $p < 0.0001$), suggesting a slight exacerbation of this trait after ~35 generations (Fig. 6). The phenotype reversibility differed among genotypes (model 6.2 and 6.3): the interaction genotype × type of cells (ancestor

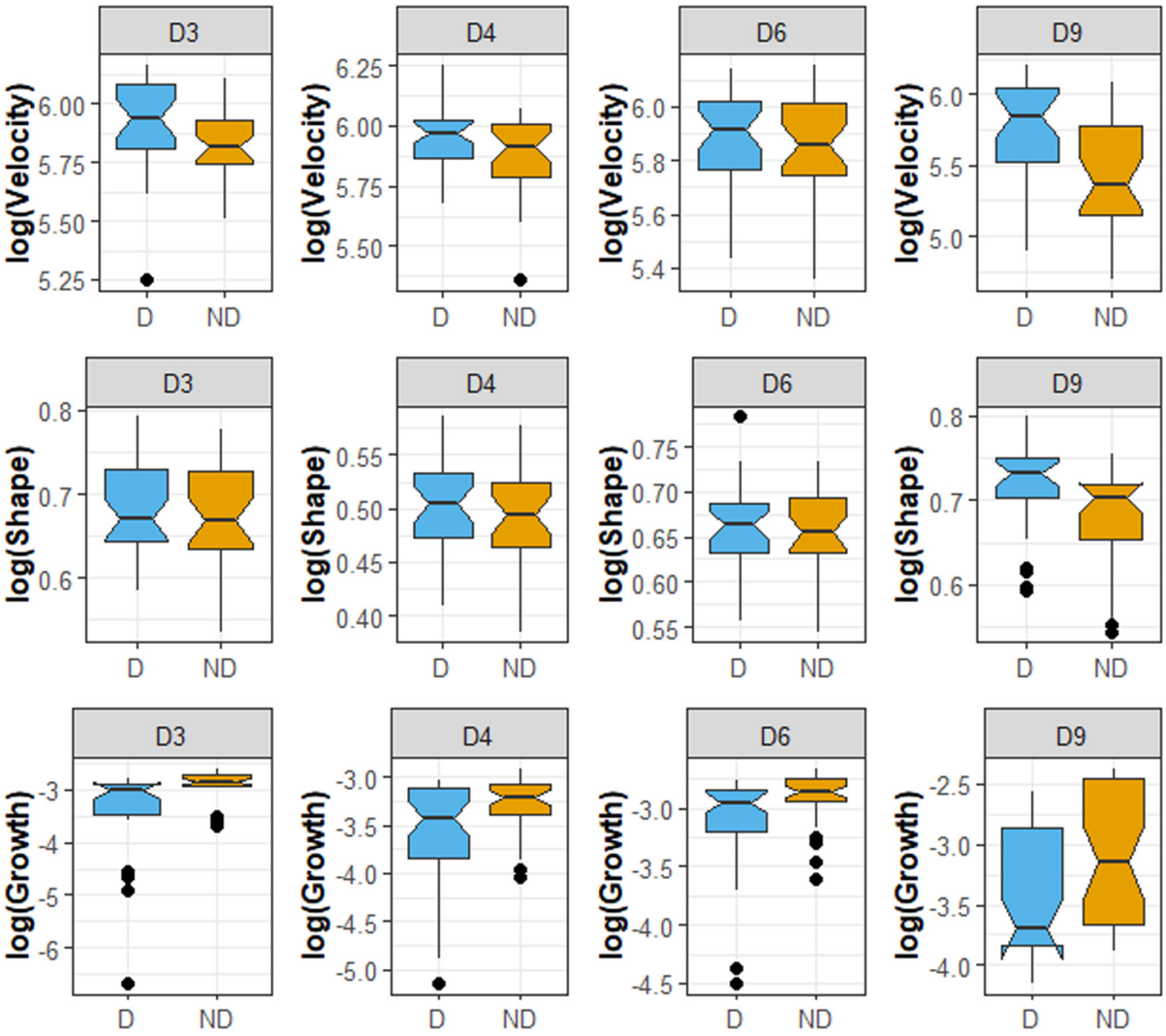

Figure 4. Transgenerational plasticity for dispersal-related traits (i.e. the two traits differing between dispersing and non-dispersing cells at dispersal trial *tr*0): effect of ancestor dispersal status in the whole dataset (dispersing ancestor in blue and non-dispersing ancestor in yellow) on cell shape (distance in straight line/effective distance covered), velocity (µm s^{-1}) and growth rate of descendants kept during ~35 asexual generations in a common garden after each dispersal trials in the four studied genotypes (D3, D4, D6 and D9). We show relationships where the effect of the ancestor dispersal status on phenotypic traits was significant with a p-value threshold of $p = 0.05$.

versus descendant after ~35 generations) was strongly supported by the data (model 6.2; $R^2 = 0.69$, $\chi^2 = 59.84$, $p < 0.0001$). In addition, genotype-specific model (model 6.3) confirmed this result, showing that the type of cells explained from 10% to 54% of velocity decrease in D9 and D6 respectively, and from < 0.01 and 65% of shape variation in D3 and D9 respectively (Supporting information).

Transgenerational fitness consequences of dispersal: genotype-dependency and reversibility

We examined growth rates of dispersing and non-dispersing lines at three dispersal trials (*tr*0, *tr*1 and *tr*6; model 4.1 and Fig. 1). Combining these three times and the four genotypes revealed that cells with a dispersing ancestor had a lower growth than those with a non-dispersing ancestor ($R^2 = 0.09$, $\chi^2 = 33.84$, $p < 0.0001$, Fig. 4), which indicates a transgenerational effect of dispersal trials on descendants' fitness. Looking at temporal trends revealed that growth of cells with dispersing ancestors decreased between *tr*0 and *tr*1 and between *tr*1 and *tr*6 while it increased in cells with non-dispersing ancestors (Fig. 5, $\chi^2 = 24.61$, $p < 0.0001$; model 5).

Our analyses also suggest that the transgenerational fitness cost tended to differ in intensity among genotypes (model 4.2 and 4.3). Although the interaction genotype × ancestor dispersal status (model 4.2) was not supported by the data ($R^2 = 0.28$, $\chi^2 = 4.25$, $p = 0.23$), genotype-specific models (model 4.3) indicated that the proportion of growth rate variation explained by the ancestor dispersal status varied among genotypes: it was twice as important for D3 and D6 (13% and 10% respectively) as for D9 and D4 (6% and 5% respectively) (Supporting information).

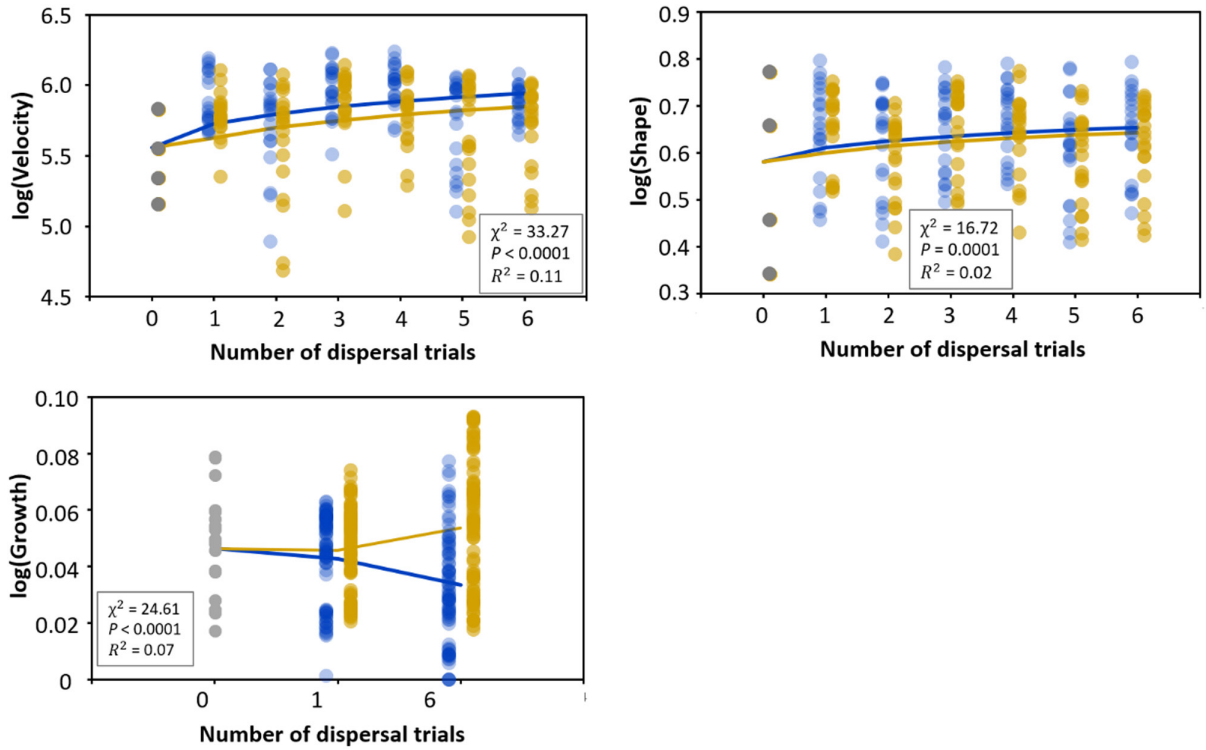

Figure 5. Effect of the number of dispersal trials experienced by ancestors on cell phenotype [i.e. velocity (μm s^{-1}) and cell shape (cell major/minor axis ratio of a fitted ellipse)] and fitness. Cells with dispersing ancestors are shown in blue, cells with non-dispersing ancestors in yellow, and cells from mother cultures in grey. The terms 'ancestor dispersal status' and 'number of trials' were entered in an additive way in the model (the interaction was not supported by the data). We give marginal R^2 of the sum of fixed effects in the mixed model and outputs of the likelihood ratio test (χ^2 and p-value) used to examine the effect of number of dispersal trials on phenotypic traits.

Finally, we found that the fitness consequences of dispersal were weakly reversible (model 6.1) as growth rate was similar just after dispersal trials and ~35 generations later for both dispersing ($R^2 = 0.004$, $\chi^2 = 2.03$, $p = 0.15$) and non-dispersing cells ($R^2 = 0.001$, $\chi^2 = 0.45$, $p = 0.50$) (Fig. 6). Furthermore, the fitness reversibility differed among genotypes (model 6.2 and 6.3): the interaction genotype × type of cells (ancestor versus descendant after ~35 generations in common garden) was strongly supported by the data (model 6.2; $R^2 = 0.21$, $\chi^2 = 20.91$, $p = 0.0001$). Genotype-specific models (model 6.3) also supported this result, indicating that the type of cells explained from < 0.01 to 5% of fitness variation in D4 and D9, respectively (Supporting information).

Discussion

While transgenerational plasticity (TGP) plays a fundamental role in the transmission of environmentally-induced phenotypic variation across generations and can influence adaptation, its long-term stability, potential costs and genetic control remain largely unknown. Thus, our study is one of the very few that aimed at documenting how genotype determines the TGP induced by an experimental dispersal treatment. Using microcosms, we showed that plastic mechanisms can change the distribution of dispersal-related traits for more than 30 asexual divisions (seven days) in the protist *T. thermophila*. While plastic changes in morphology and movement traits distinguishing dispersing and non-dispersing cells were relatively stable across dispersal trials, the fitness consequences of repeated trials were cumulative (Fig. 7). The expression of dispersal-related traits, its reversibility and the associated fitness consequences were all modulated by the genotype (Fig. 7), underlying a potential critical role of the genetic background in the TGP induced by dispersal (Alvarez et al. 2020). As such, we show that genetically-determined inheritance of non-genetic mechanisms could influence key eco-evolutionary processes over tens of generations. Plasticity might thus durably impact both ecological and evolutionary dynamics (Hendry 2016).

Intragenerational plasticity is consistent across dispersal studies in *Tetrahymena*

During the initial dispersal trial, our results confirmed the existence of an IGP of dispersal-related traits and plastic dispersal syndrome in *T. thermophila*. Within the four genotypes, dispersing cells had a more elongated shape and a higher velocity than non-dispersing cells, confirming previous results (Fjerdingstad et al. 2007, Pennekamp et al. 2014, Jacob et al. 2016a). Although our analyses cannot allow us

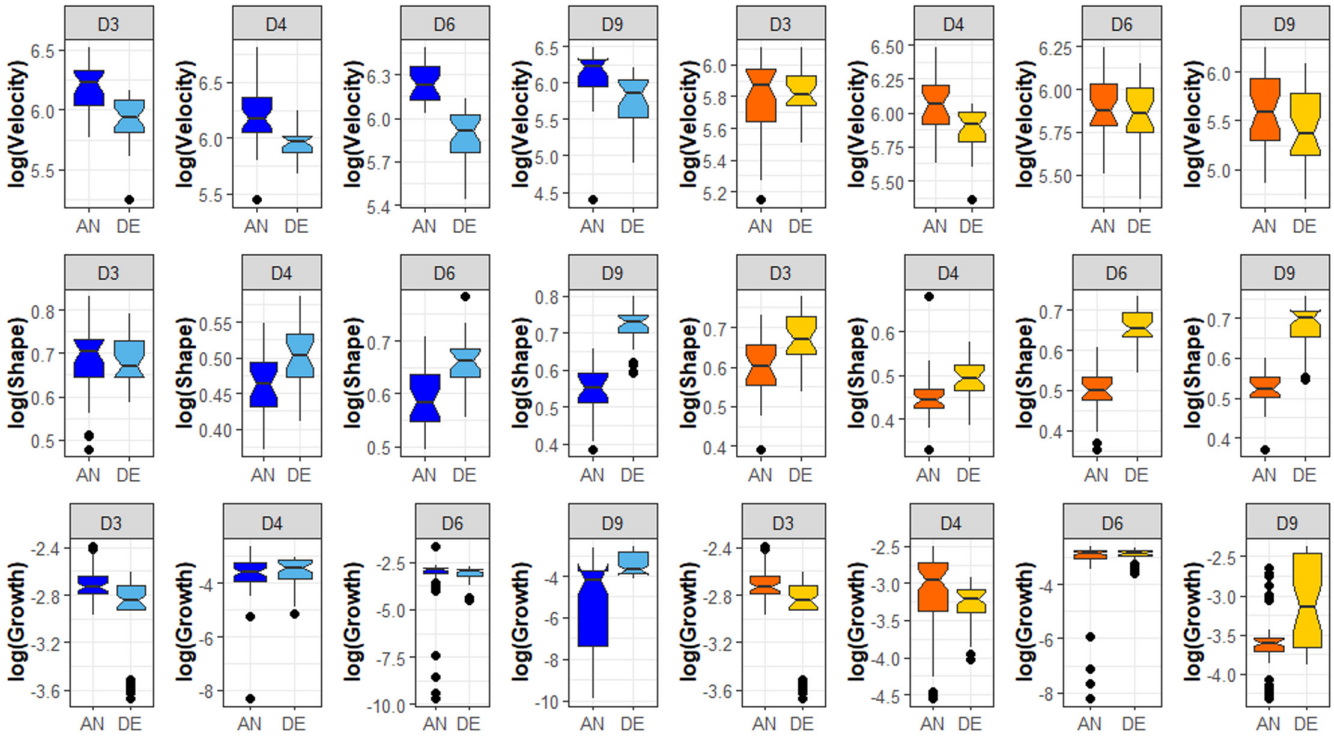

Figure 6. Reversibility of transgenerational plasticity of dispersal-related traits (i.e. the two traits differing between dispersing and non-dispersing cells at *tr*0) and its fitness cost in cells with dispersing (blue) and non-dispersing (orange) ancestors in the four studied genotypes (D3, D4, D6 and D9). We examined two dispersal-related traits, cell shape (cell major/minor axis ratio of a fitted ellipse) and velocity (μm s^{-1}), and one fitness proxy, cell growth, in ancestors (AN) and their descendants (DE). Trait measurement for ancestors was performed just after a dispersal trial, and measurement for descendants was executed just before the next trial, i.e. after ~35 generations.

to determine precisely when these plastic differences are expressed (Jacob et al. 2020) and on which cells (dispersing and/or non-dispersing), trait distributions observed in our experiments nevertheless suggest that these phenotypic changes could mainly occur in dispersing cells (Fig. 3, 4). This interpretation is congruent with previous descriptions of a facultative, inducible phenotypic shift, where cells change from a pyriform-shaped phenotype to an elongated phenotype (Nelsen 1978, Junker et al. 2021).

It has been shown that dispersers' movement characteristics does not correlate with the ones of cells unexposed to dispersal, which suggests that *T. thermophila* dispersal movements in experimental two-patch systems are not a simple by-product of routine movements (Junker et al. 2021). This IGP of dispersal-related traits is thus likely determined by microenvironmental variation occurring throughout dispersal assays, for instance changes in spatial repartition of cells or density in both departure and arrival patches, or variation in temperature, luminosity, oxygen content or area size between the departure patch and the corridor. For instance, Jacob et al. (2020) recently showed that the harshness of the matrix separating habitat patches can modify dispersal-related traits through phenotypic plasticity. Besides, dispersal itself can generate plastic changes: individuals might change their phenotype when dispersing in order to increase their mobility or colonization efficiency, or resulting from costs paid during dispersal (Bonte et al. 2012, Winandy et al. 2019, Jacob et al. 2020). Moreover, the phenotypic and fitness differences between dispersing and non-dispersing cells resulting from IGP, observed at the end of the first dispersal trial, strongly differed among genotypes. This result confirms the findings of previous studies showing that cell genotype regulates dispersal syndromes and costs (Fjerdingstad et al. 2007, Schtickzelle et al. 2009, Pennekamp et al. 2014, Jacob et al. 2016a).

Transgenerational plasticity of dispersal-related traits and dispersal rates

Our study demonstrated that plastic phenotypic variation linked to dispersal is stably inherited when cells are exposed to successive dispersal trials separated by ~35 asexual generations (Fig. 6). It suggests that the initial shift from a pyriform-shaped phenotype to an elongated rapid-swimming phenotype in dispersing cells is likely conserved through non-genetic mechanisms over multiple generations. Our experimental protocol allows us to reasonably assume that the detected phenotypic variation in the descendants results from TGP rather than in genic selection. Indeed, we have eliminated most genetic variation within each replicate at the beginning of the experiment using a single mother cell, which rules out the possibility of selection from standing genetic variation. In addition, the distribution of measured phenotypic traits across non-dispersing and dispersing cells is generally wider and/or skewed after our 4-h environmental treatment (crossing or not the

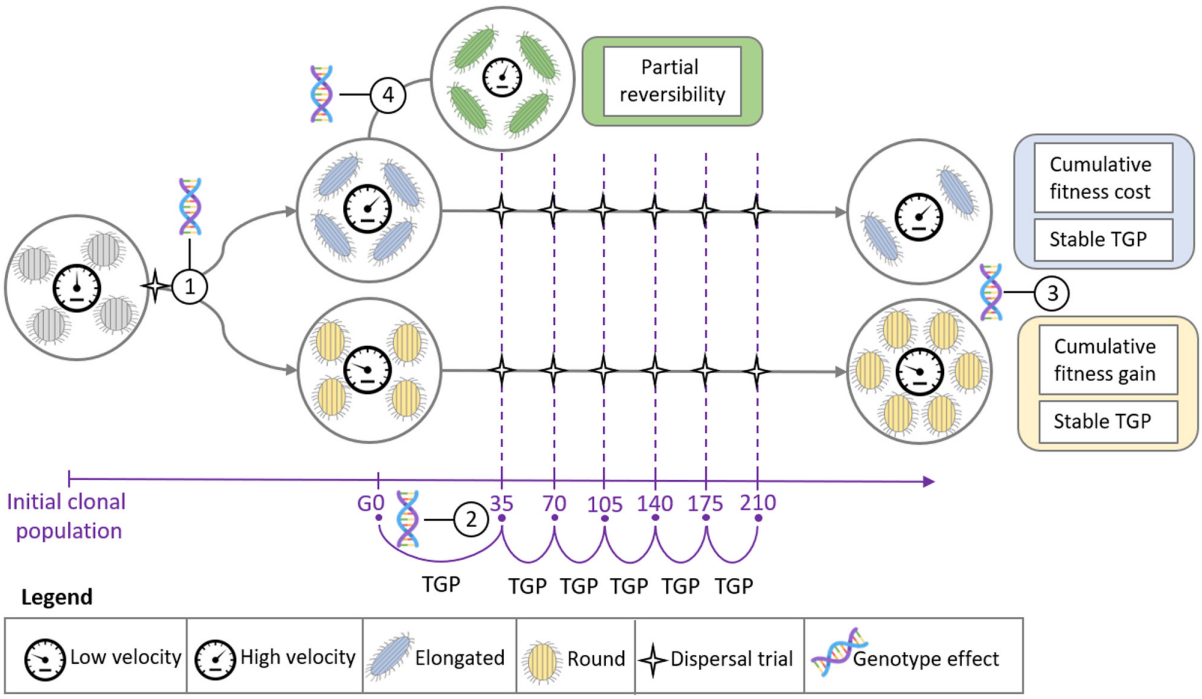

Figure 7. Transgenerational plasticity for dispersal-related traits and its cost in *Tetrahymena thermophila*. At generation 0 (G0), the initial dispersal trial is performed (dispersal trials are represented by the black stars). After the first trial, cells are more elongated and swim faster than in mother cultures, but dispersing cells (in blue) have a more elongated shape and a higher velocity than non-dispersing cells (yellow) due to plastic changes within genotypes. The strength of phenotypic differences between dispersing and non-dispersing cells differ between genotypes (1). The dispersal status of the ancestor affects the phenotype of descendants: cells with a dispersing ancestor conserve a dispersing-like phenotype (elongated and fast) via transgenerational plasticity during whole the experiment. Yet, the strength of this effect depends on cell genotype (2). These phenotypic changes are only partially reversible (in green) after ~35 generations in common garden (velocity slightly decreases while elongation slightly increases, fitness is stable). The number of dispersal trials experienced by the ancestors of a cell does not affect its phenotype: the effect of transgenerational plasticity is not gradual. Indeed, the phenotypic switches appear at the first trial and are then maintained throughout the experiment. By contrast, cells with dispersing ancestors experience a gradual decrease in fitness along with the number of dispersal trials experienced by their ancestors. Likewise, the fitness of cells with non-dispersing ancestors increases with the number of dispersal trials experienced. Genotype modulates this fitness effects of transgenerational plasticity for dispersal (3). Furthermore, phenotypic modifications in both dispersing and non-dispersing cells were partially reversible and reversibility was strongly genotype-dependent (4).

corridor) compared with that of mother cultures (Fig. 3). We also observed that trait-trait associations changed just after the dispersal trial: in mother cultures, the fastest cells are the more elongated, the biggest and those with the most linear movements. By contrast, dispersing cells, which are longer and swim faster than residents, are not the biggest cells nor those with the most directional movements. In case of a sole sorting effect of the treatment among pre-existing phenotypic variation, we would have observed either similar or narrower distributions with identical trait correlations when pooling dispersing and non-dispersing cells compared with mother cultures. Moreover, it seems very unlikely that de novo mutations have been simultaneously recruited and have produced parallel phenotypic shifts in the four genotypes during the seven-days growth period preceding the first dispersal trial. Therefore, the phenotypic changes observed after the first dispersal trial, and maintained at least during ~35 generations, are likely due to transgenerational plastic mechanisms.

At first glance, trait variance explained by ancestor dispersal status might appear low (from 1 to 13% depending on the trait and genotype). However, dispersal is a multifaceted process for which tens (or more) phenotypic traits are involved (Clobert et al. 2009), those traits being themselves involved in other biological functions. Therefore, it might not be surprising that, focusing on four candidate traits, two were not affected by our simple experimental conditions, and the two others show moderate responses. Besides, cell shape and velocity are involved in numerous other fundamental cell functions (e.g. feeding, mating, osmoregulation), which certainly impose constraints on their variance.

Dispersal treatment produced more consistent effects on phenotypic traits than on dispersal rates across genotypes (Supporting information). In genotype D9 where ancestor dispersal status had the strongest effect on cell elongation and velocity, dispersal rate was higher in cell populations with dispersing ancestors than in populations with non-dispersing ancestors. By contrast, in genotype D6 where ancestor dispersal status had the weakest effect on cell elongation and velocity, we found the oppositive pattern. In addition, the influence of ancestor status on dispersal rate was negligible in genotypes D3 and D4. To date, the underlying mechanisms (e.g. genotype-specific evolutionary potential of dispersal

rates and/or energy allocation tradeoffs across generations after exposure to dispersal) causing these genotype-dependent patterns are still unidentified. However, predictions on the effects of artificially selecting dispersing and non-dispersing lines in an isogenic pool on dispersal rates are not straightforward. The benefits to produce dispersing descendants for parents having dispersed and settled in a suitable environment are probably low, and we would rather expect selection to favor a decrease in descendants' dispersal rate as long as local conditions remain stable. In any case, the outcome of selection will depend on the non-genetic and genetic heritability of dispersal rate. Unfortunately, heritability estimates of dispersal rates and other dispersal metrics are rare, the vast majority of studies focusing on heritability of dispersal-related traits (Saastamoinen et al. 2018). Joint evolution of increased dispersal propensity and ability was observed following artificial selection of dispersing fruit flies in a case where resource was absent from the source patch and dispersal costs were low (Tung et al. 2018). In spider mites, dispersal distance was unaffected by artificial selection unless population density was low, with maternal effects strongly influencing the response to selection (Bitume et al. 2011). On top of this context-dependency, the response to selection of dispersal metrics can also depend upon the species and sex (Ogden 1970, Zwoinska et al. 2020).

Examples of TGP observed for more than a few generations are not frequent and mostly found in other (partially) asexual species (Vastenhouw et al. 2006). The balance between the frequency of environmental changes and generation time generally differs between short-living and long-lived organisms. Indeed, many changes in environmental conditions would be perceived at the within-generation level in long-living organisms, and at the between-generation level in species with short lifespan such as microbes. Selection on TGP might therefore differ according to organism's lifespan, resulting in selection for longer transmission of phenotypes in short-living species. Clearly, one should also keep in mind that it is easier to experimentally observe TGP in short-living clonal species than in long-living sexually reproducing species. The intensity and frequency of environmental shifts perceived by *T. thermophila* in nature is largely unknown (the species live in freshwater ponds), and should be investigated to feed this debate. Here, we demonstrate that TGP over tens of asexual generations can influence trait related to dispersal, an eco-evolutionary force that could act to enhance gene flow. Future research should determine if TGP for dispersal-related traits occurs also across sexual generations in this ciliate.

Transgenerational plasticity of dispersal-related fitness costs

We observed that cell growth (a proxy for cell fitness) progressively decreases with increased number of dispersal trials experienced by ancestors, indicating a transgenerational cumulative fitness cost (i.e. decrease in cell growth) associated with dispersal; we found the opposite pattern in non-dispersing cells (Fig. 6). Prior to the first dispersal trial, we also showed that growth of elongated cells was slower than that of rounder cells (model 1). As dispersing cells are more elongated than non-dispersing ones, their growth could be also slower, provided that the same growth-elongation rules hold for innate and plastic phenotypes. However, our results showed that cell elongation does not increase with increased number of dispersal trials (morphological plasticity is expressed at the first trial and then trait distributions do not change). Overall, these results indicated that the number of dispersal trials likely has a larger influence on fitness variation than cell shape per se. Furthermore, fitness differed on average by 9% between dispersing and non-dispersing cells, suggesting that transgenerational plasticity of dispersal-related traits can strongly impact evolutionary dynamics.

To the best of our knowledge, cumulative fitness costs of plasticity across ~200 generations have never been described in the context of dispersal. While fitness dynamics should be built on more time points and for more generations in the future, our result is of utmost importance because differential costs and benefits associated with dispersal syndromes can drive their coexistence (Bonte et al. 2012). *Tetrahymena thermophila* thus offers an interesting system to test a series of predictions and calibrate models on the role of plasticity, dispersal and their costs and benefits on eco-evolutionary dynamics (Scheiner and Holt 2012, Scheiner et al. 2017). Future work in ciliates and other taxa should also determine the tipping points at which TGP costs of dispersal-related traits would alter colonisation and/or (meta)population dynamics (Doebeli and Ruxton 1997).

This fitness difference between dispersing and non-dispersing cells could affect the TGP pattern observed in our study. Indeed, densities of non-dispersing and dispersing cells, while normalized at the beginning of the common garden, will differ at its end. Completely identical common gardens are generally difficult, if not impossible, to achieve. The introduction of two different plastic phenotypes will irremediably modify their initially controlled environments. Controlling each factor potentially influencing TGP patterns at each generation is therefore a challenge for any study (Wadgymar et al. 2018). In our experiment, strains have distinct growth characteristics and the growth difference between cells with dispersing and non-dispersing ancestors gradually increases with the number of dispersal trials, which could impact on TGP results. However, we showed that density differences between genotypes have negligible effects on dispersal-related trait values (Supporting information). Further, plastic differences in cell shape and velocity appeared just after the first trial and remained highly stable over all trials despite time-dependent growth differences induced by the accumulation of dispersal treatment. We therefore consider unlikely that differences in cell density over time explain a significant part of the described TGP pattern.

Reversibility of transgenerational plasticity of dispersal-related traits and its fitness consequences

In our experiments, plastic changes were only partially reversible between the dispersal trials. Velocity measured just after each trial was weakly lower after ~35 generations in common

garden, but still higher in dispersing cells with a dispersing ancestor than in non-dispersing cells with a non-dispersing ancestor. Dispersing cells with a dispersing ancestor were even more elongated after the common garden, which might be due to the dispersal treatment itself, or to undetermined effects resulting the interaction between ancestor dispersal status and our common garden conditions. The degree of these phenotypic modifications during the common garden was genotype-dependent, with the highest variability between the genotype reversibility for velocity. Finally, the fitness difference between dispersing and non-dispersing cells was not affected by the common garden. Such limited reversibility of phenotypes suggests either that the mechanisms responsible for this dispersal plasticity present a time-lag to fully reverse the phenotypes, or that the environmental cues triggering the phenotypic reversibility are not entirely reliable (the two hypotheses being non-exclusive).

Potential molecular mechanisms underlying transgenerational plasticity of dispersal

In absence of substantial genetic variation within the four clonal cell lines, the described inheritance of dispersal-related traits should mainly rely on non-genetic factors causing transgenerational modifications of gene expression, as already demonstrated (Devanapally et al. 2015). However, the underlying molecular mechanisms responsible for modulating TGP over tens of generations remains largely unexplored, thus representing interesting trail for future investigations. We speculate that already described molecular mechanisms, some specific to ciliates, could explain such pattern. In *T. thermophila*, epigenetic mechanisms as DNA methylation (Chung and Yao 2012), microRNA (Mochizuki 2012) or histone modifications (Morris et al. 2007) might allow the transmission of changes in cell shape and velocity across clonal generations. As the ciliate somatic genome is highly polyploidized (~45 copies in *T. thermophila*, Doerder et al. 1992), epigenetic modifications induced before or during the dispersal process could cause differences in the expression of specific copies of homeologous genes coding for dispersal-related traits (Liu and Adams 2007). In our experimental design, the absence of sexual reproduction, and therefore the lack of meiotic reprogramming of epimarks, should facilitate the transgenerational inheritance of epigenetic variants regulating the expression of homeologous genes (Heard and Martienssen 2014), and could thus foster the TGP for dispersal-related traits. In *T. thermophila*, copy number variation (CNV) can generate adaptive plastic responses under stressful conditions with a time lag of at least a few generations (de Francisco et al. 2018). While it should be excluded that CNV explains the initial phenotypic changes in our experiment (cells are different from mother cultures in both dispersing and non-dispersing lines at the first trial), it might be possible that epigenetic modifications followed by CNV act in concert to maintain the observed TGP. Future effort should be devoted to test whether the time lag associated with copy number regulation might account for the partial reversibility of phenotypes observed, as well as progressive elimination of mRNA, microRNA or other intracellular molecules potentially responsible for TGP through cell divisions.

Genotype-dependency of transgenerational plasticity of dispersal-related traits and its fitness cost

Our study showed that genetic background explained the differential persistence and reversibility of dispersal-related traits during ~35 asexual generations (Fig. 6). As well, cell genotype significantly modulated the transgenerational fitness cost of dispersal. Indeed, genotype (D9) having the highest intra- and transgenerational plasticity for dispersal-related traits showed the highest reversibility for the fitness effects. This suggests that genotypes able to plastically express specialized dispersing phenotypes might evolve mechanisms to reduce the associated transgenerational costs. Our results therefore revealed that G × E interactions drive the TGP for dispersal-related traits and its cost in *T. thermophila*. Phenotypic tradeoffs are usually observed in the context of dispersal (Bonte et al. 2012), but our results additionally point out to an original dependency on the genetic background. A genetic control of TGP has rarely been documented (see however Devanapally et al. 2015, Vu et al. 2015, Alvarez et al. 2020), and could be caused by the genetic determinism of epimarks' transgenerational inheritance. Indeed, methylation variation are usually strongly associated with genetic variants in both *cis* and *trans* (Dubin et al. 2015, Zaghlool et al. 2016), facilitating or constraining the transmission of epimarks over generations (Richards 2006). It is noteworthy that not only genetic factors, but also fixed non-genetic factors such as highly stable epigenetic marks, inherited cytoplasmic characters (Beisson and Sonneborn 1965) or constrained chromosome copy number within the somatic genome (Spring et al. 2013) could explain part of the genotype effect we described here. In the future, comparisons between genomes, epigenomes and transcriptomes of the tested genotypes should provide mechanistic answers. It should also be helpful to understand if the parallelism found between the biological replicates of each genotype and for some traits between genotypes (models all include replicates and genotypes as variables) relies on similar molecular mechanisms.

Conclusion

Our study provides a first evidence of the role of genetic background in the TGP of dispersal-related traits and its associated cost. Further investigations are now necessary to determine how such TGP influences ecological dynamics, and if the benefits to disperse (e.g. kin avoidance, escape of poor local conditions) are sufficient to overweight the costs associated with TGP of dispersal-related traits as revealed in our study. Our work emphasizes the tremendous importance of genotypic variation in the ability of organisms to transmit environmentally-induced phenotypic variation across generations, shedding light on the importance of intraspecific genetic variation in ecological and evolutionary dynamics (Raffard et al. 2019).

Our results provide support to the hypothesis that genotype-dependent TGP could play an important role in the evolution of dispersal-related traits, which could in turn affect this major eco-evolutionary force determining the migration-drift and migration-selection equilibria in natural populations (Slatkin 1987, Lenormand 2002). Genetically-controlled TGP could then impact on biological invasions by allowing a rapid phenotypic specialization maximizing colonization success and speed (Perkins et al. 2013, Ochocki and Miller 2017), despite low genetic polymorphism caused by serial founder effects (Excoffier et al. 2009). More broadly, genotype-dependent TGP could facilitate a rapid adjustment to sudden environmental changes. In this regard, it might be of high concern to determine if the degree of parallelism measured here can also be observed at the inter-specific level. This would help quantifying the importance of plastic mechanisms in biodiversity response to environmental changes.

Speculation and alternative viewpoint

Until now, we have explained the TGP pattern described in our study by the vertical transmission of non-genetic factors allowing the maintenance of a substantial part of the phenotypic variance. However, an alternative non-exclusive mechanism could also be at play: cultural inheritance. It refers to the part of phenotypic variation that is inherited socially, i.e. learnt from others (Danchin et al. 2011). In *T. thermophila*, where generations are overlapping, the observed dispersal-driven plastic changes could be transmitted across generations through cell-cell interactions. In our system, this would mean that after asexual division, some offspring cells would copy the phenotype of parental cell's generation that have not yet divided. To our best knowledge, such an effect acting on dispersal-related trait distributions have never been reported in microorganisms, and even in other organisms. However, some genotypes of *T. thermophila* are indeed cooperative, meaning that they can exchange information in a fitness advantageous manner, especially when they disperse (Jacob et al. 2016b). We think that such socially-dependent mechanism resulting in the 'copying' of the parental phenotype over 35 generations is less probable than the direct transmission of intracellular molecules from parents to daughter cells when considering a unicellular species (see above the paragraph Potential molecular mechanisms underlying transgenerational plasticity of dispersal), but this needs to be tested. Unfortunately, our design does not allow to test for the existence of cultural transmission: 1000 cells were inoculated in the common gardens for which we could not track the occurrence of social interactions. Deciphering the role of one or the other mechanism of non-genetic inheritance in dispersal TGP is an exciting research avenue.

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Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.x0k6djhm1> (Cayuela et al. 2021).

Supporting information

The supporting information associated with this article is available from the online version.

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