Supplementary Information

S1 Growth characteristics of long-term selection lines: $r_0$ and $N$

In growth assays, intrinsic population growth rate ($r_0$) was measured after 21 (year 1), 78 (year 2) and 160 (year 3) dispersal / growth cycles. Equilibrium density ($\bar{N}$) was taken for each selection line at the end of the 1-week growth period at each cycle during the long-term experiment. Here we averaged $\bar{N}$ for each line over 9-10 cycles in year 1 (cycle 15-25), year 2 (74-84) and year 3 (154-163).

**Fig. S1.** (A) Intrinsic population growth rate ($r_0$) and (B) mean density at the end of the cycle ($\bar{N}$) from core, front and control treatments (respectively in blue, red and grey). Full symbols represent the mean values for each selection line ($n = 15$), different symbols (circle, star, cross) refer to the three different years where measurement were taken. Shaded panels show means and 95 % confidence intervals of the model predictions.

**Script logistic model fitting ($r_0$)**

We use the classical logistic population growth model to estimate population growth rates:

$$\frac{dn}{dt} = r_0 (1 - (N/K)) N$$

where $N$ is the population size, $r_0$ is the intrinsic rate of increase and $K$ the carrying capacity. Note that while we prefer the original formulation by Verhulst which includes the intraspecific competition coefficient (for a detailed discussion, see Mallet 2012) we here use the r-K formulation because $K$ is often numerically easier to fit than alpha.
We fit this model using trajectory matching (Rosenbaum & Fronhofer 2022) to data using Stan and the rstan package (version 2.21.5). We follow established pipelines (Rosenbaum & Fronhofer 2022). In brief, the Bayesian approach allows us to avoid known issues of likelihood ridges (Clark et al. 2010) that are known to be an issue when using least-squares approaches. We use somewhat informative priors based on prior knowledge of the system. Priors are: \( r \sim \text{halfnormal}(0, 0.25) \), \( K \sim \text{halfnormal}(100, 500) \) and \( N0\text{sim} \sim \text{halfnormal}(0,20) \). Note that we also estimate the starting density \( (N0) \) which avoids that the first data point has an extreme effect on the rest of the fit. We tested the model with 3 chains which showed mixing. Subsequently we used a chain length of 10,000 with a warm-up of 1000 for the final fits. We also checked fit using Rhat which was consistently satisfactory.

```r
rm(list=ls())

# load some packages
library(rstan)
library(deSolve)
library(coda)
library(vioplot)
library(loo)

# load data
data<- read.table(file="file_name.txt", header=T)

# declare stan models

# stan rK model
stanmodelcode_rK = '
// function that calulates r-alpha logistic population growth
functions{

```
real[] odemodel(real t, real[] N, real[] p, real[] x_r, int[] x_i){
  // p[1]=r0, p[2]=K
  real dNdt[1];
  dNdt[1] = p[1] * (1 - (N[1]/p[2])) * N[1];
  return dNdt;
}
}

data{
  int n;
  real log_N0;
  real log_N[n];
  real t0;
  real t[n];
}

transformed data {
  // not used here
  real x_r[0];
  int x_i[0];
}

parameters{
  real<lower=0> r;
  real<lower=0> K;
  real<lower=0> N0sim;
  real<lower=0> sdev;
}

transformed parameters{
  // all of this was in the model section previously
}
// I moved it here to be able to get waic because Nsim needs to be accessible in "generated quantities {}"

real p[2];
real Nsim[n,1]; // simulated values, matrix. dim1 = time, dim2 = dim_ODE = 1
real N0sim_dummy[1]; // just a dummy because the ODE solver requires real[] instead of real

// parameters for integrator
p[1] = r;
p[2] = K;
N0sim_dummy[1] = N0sim; // see above

// integrate ODE
Nsim = integrate_ode_rk45(odemodel,N0sim_dummy,t0,t,p,x_r,x_i);
}

model{
  // priors
  // note: it can be VERY helpful to estimate parameters on logscale,
  // especially if they have different orders of magnitude. here it works on regular scale, though.
  r ~ normal(0,0.25);
  K ~ normal(100,500);
  N0sim ~ normal(0,20);
  sdev ~ cauchy(0,1);

  // likelihood, normal (maybe lognormal helpful)
  log_N0 ~ normal(log(N0sim),sdev);
  for (i in 1:n){
    log_N[i] ~ normal(log(Nsim[i,1]),sdev);
  }
}
// calculate log lik to get waic
// from loo R package description

generated quantities {
  real log_lik[n];
  for (nn in 1:n){
    log_lik[nn] = normal_lpdf(log_N[nn] | log(Nsim[nn,1]), sdev);
  }
}

# functions

# rK model as ODE
ode.model = function(t,N,p){
  with(as.list(p),{
    dNdt = p[1] * (1 - (N/p[2])) * N
    return(list(dNdt))
  })
}

# stan options
chains = 1
rstan_options(auto_write = TRUE)
options(mc.cores = chains)
iter  = 10000
warmup = 1000
thin  = 1

# compile models
s_model_rK = stan_model(model_code=stanmodelcode_rK)
S2 Swimming behaviour

**Material and methods.** We collected data on swimming behaviour for each selection line in year 1 (cycles 53, 54, 55, 58, 74) and year 2 (cycles 103 and 116), using an automated video analysis pipeline (Fronhofer & Altermatt 2015; Pennekamp et al. 2015). To this end, 120-µL samples (ca. 20 individuals) from populations at equilibrium were placed on a microscope slide and videos were recorded under a stereomicroscope (Perfex SC38800 camera; settings: frames per second: 15; duration: 5 s; total magnification: 10x). One video per selection line and cycle was recorded, except for cycle 74 (n=4). We analysed the videos using the “bemovi” (Pennekamp et al. 2015) package (see script below), which provided estimates of individual Paramecium swimming speed and the tortuosity of swimming trajectories, an indicator of changes in the swimming direction (standard deviation of the turning angle distribution). In an additional assay (year 3), we assessed the vertical distribution of *Paramecium* during dispersal assays. In these assays, we determined the density of individuals in the ‘resident’ tube from 100 µl samples, just before opening the connection with the (empty) neighbour tube. Sixty minutes after the connections between the two patches were opened, we gently inserted a pipette into the ‘resident’ tube and sampled 200 µL at the height of the connection tube (i.e., the entry point of the dispersal corridor in the 2-patch systems); the number of individuals in these samples were counted under a dissecting microscope.

Statistical analyses were performed in R (ver. 4.2.0) and JMP 14 (SAS Institute Inc. 2018). We analysed swimming speed and tortuosity (averages per line) using linear mixed effects models. We considered evolutionary treatment (core, front, control), year, experimental cycle and line (nested within treatment) as explanatory variables. We additionally investigated correlations across lines and years. We analysed the vertical distribution with generalised linear models (GLM) with binomial error distribution correcting for overdispersion. We considered the proportion of individuals present at the height of the connecting tube sixty minutes after the connection was opened on the total number of individuals present before the opening as response variable, and treatment as explanatory variable.

**Results.** Paramecium from range front lines had a significantly lower swimming speed (-41%) than those from the range core and control treatments (treatment: F2,12 = 33.5; p < 0.001; Fig. S2). Across lines and years combined, swimming speed in the assays was negatively correlated with dispersal rate observed in the selection lines (r = -0.72, n = 30, p < 0.0001), meaning that lines with a higher
dispersal generally had a lower swimming speed. Tortuosity of swimming trajectories were negatively correlated with swimming speed (all replicates: $r = -0.29$, $n=135$, $p = 0.0005$), but did not significantly differ among treatments ($p > 0.545$). The vertical distribution in the water column was significantly different for *Paramecium* from range front lines than core or control (treatment: $\chi^2 = 9.80; p = 0.007$; Fig. S2.1). One hour after opening the connections in the 2-patch systems, the proportion of individuals from the front lines near the connecting corridor was three to four times higher than the other treatments (Fig. S2.1).

**Fig. S2.** Estimates of the (A) swimming speed and (B) tortuosity of swimming trajectories from core (blue), front (red) and control (grey) treatments. Full symbols represent the mean values for each selection line ($n = 15$), with different symbols (point, star) corresponding to the first two years of the study. No measurements were taken in the year 3. Shaded panels show means and 95% confidence intervals of the model predictions.

**Fig. S2.1** Vertical distribution for core (blue), front (red) and control (grey) treatments. This correspond to the proportion of individuals at the height of the connecting tube between the 2 patches sixty minutes after...
the connection was opened on the total number of individuals before the opening. Full symbols represent the values for each selection line, shaded panels show means and standard error of the model predictions.

**Discussion.** Similar experiments with the ciliate *Tetrahymena thermophila* (Fronhofer & Altermatt 2015; Pennekamp et al. 2019) or with other organisms (Philips et al. 2006; Lombaert et al. 2014; Szűcs et al. 2017; Weiss-Lehman et al. 2017) showed that range front populations can evolve enhanced movement ability. In our case, we find an opposite trend, with *Paramecium* from range front treatments swimming more slowly than those from the core. This pattern seems to be characteristic for this organism, as it is in line with other studies, where high-dispersal lines also have a lower swimming speed (Zilio et al. 2021; Zilio et al. 2023). This counterintuitive observation suggests that the speed of movement is not the only aspect of dispersal-relevant swimming behaviour. Indeed, the lower swimming speed of the range front lines was associated with higher tortuosity, which is the tendency of the individuals to change directions while swimming. Moreover, *Paramecium* show a characteristic not-random vertical distribution in culture conditions (Fels et al., 2008), and here *Paramecium* from the range front treatment were considerably more likely to be found next to the dispersal corridor (rather than at the bottom of the tube). This might be the consequence of a more exploratory swimming behaviour and would also readily explain their higher dispersal rates, i.e., the likelihood to swim through the corridor into the neighbour tube. Different components of movement (Ricci, 1989) or proxies should be therefore considered when investigating the mechanism or the proximate cause of dispersal.

**Script video analysis**

```r
# R script for analysing video files with BEMOVI (www.bemovi.info)
rm(list=ls())
# load package
library(devtools)
install_github("efronhofer/bemovi", ref="experimental")
library(bemovi)
```

---

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# VIDEO PARAMETERS

# video frame rate (in frames per second)
fps <- 15

# length of video (in frames)
total_frames <- 150

# magnification
# this parameter sets "measured_volume" and "pixel_to_scale" for Perfex Pro 10 stereomicroscope with #Perfex SC38800 (IDS UI-3880LE-M-GL) camera and sample height = 0.5mm
# if other devices are used, set the two parameters manually
# possible values: 0.8, 1, 2, 3
magnification <- 1

# specify video file format (one of "avi","cxd","mov","tiff")
# bemovi only works with avi and cxd. other formats are reformated to avi below
video.format <- "avi"

# setup
difference.lag <- 10
thresholds <- c(90,255) # don't change the second value

# FILTERING PARAMETERS
# min and max size: area in pixels
particle_min_size <- 5
particle_max_size <- 1000

# number of adjacent frames to be considered for linking particles
trajectory_link_range <- 3

# maximum distance a particle can move between two frames
trajectory_displacement <- 30

# these values are in the units defined by the parameters above: fps (seconds),
#measured_volume (microliters) and pixel_to_scale (micrometers)

filter_min_net_disp <- 500
filter_min_duration <- 1
filter_detection_freq <- 0.1
filter_median_step_length <- 5

# MORE PARAMETERS (USUALLY NOT CHANGED)

# set paths to ImageJ and particle linker standalone
IJ.path <- "/home/user_name/bin/ImageJ"
to.particlelinker <- "/home/user_name/bin/ParticleLinker"

directories and file names
to.data <- paste(getwd(),"/",sep="")
video.description.folder <- "0_video_description/
video.description.file <- "video_description.txt"
raw.video.folder <- "1_raw/
particle.data.folder <- "2_particle_data/
trajectory.data.folder <- "3_trajectory_data/
temp.overlay.folder <- "4a_temp_overlays/
overlay.folder <- "4_overlays/
merged.data.folder <- "5_merged_data/
ijmacs.folder <- "ijmacs/"

# RAM allocation
memory.alloc <- c(60000)

# RAM per particle linker instance (in MB)
memory.alloc.perLinker <- c(3000)

# VIDEO ANALYSIS
# identify particles
locate_and_measure_particles(to.data, raw.video.folder, particle.data.folder,
  difference.lag, thresholds, min_size = particle_min_size, max_size =
  particle_max_size, IJ.path, memory.alloc)

# link the particles
link_particles(to.data, particle.data.folder, trajectory.data.folder, linkrange =
  trajectory_link_range, disp = trajectory_displacement, start_vid = 1, memory =
  memory.alloc, memory_per_linkerProcess = memory.alloc.perLinker)

# merge info from description file and data
merge_data(to.data, particle.data.folder, trajectory.data.folder,
  video.description.folder, video.description.file, merged.data.folder)

# load the merged data
load(paste0(to.data, merged.data.folder, "Master.RData"))

# filter data: minimum net displacement, their duration, the detection
# frequency and the median step length
trajectory.data.filtered <- filter_data(trajectory.data, filter_min_net_disp,
  filter_min_duration, filter_detection_freq, filter_median_step_length)

# summarize trajectory data to individual-based data
morph_mvt <- summarize_trajectories(trajectory.data.filtered, calculate.median=F,
  write = T, to.data, merged.data.folder)

# get Sample level info
summarize_populations(trajectory.data.filtered, morph_mvt, write=T, to.data,
  merged.data.folder, video.description.folder, video.description.file, total_frames)

# create overlays for validation
create_overlays(trajectory.data.filtered, to.data, merged.data.folder,
raw.video.folder, temp.overlay.folder, overlay.folder, 2048, 2048,
difference.lag, type = "label", predict_spec = F, IJ.path,
contrast.enhancement = 1, memory = memory.alloc)

S3 Trait correlations

Fig. S3. Overall correlation between (A) dispersal - $r_0$, (B) dispersal - $\overline{N}$ and (C) $r_0$ - $\overline{N}$ obtained with Bayesian inference. Symbols are the average values for a given selection line and year, with blue, red and grey corresponding to core, front and control treatment, respectively. Different symbols refer to the three different years: circle (year 1), star (year 2), cross (year 3). The black point represents the ancestral values (overall mean) of the founder population. The ellipses are bound to non-linear space and correspond to the 10, 25, 50, 75 an 95 % CI of the correlation of pairs of traits. The shaded areas in the insert panels represent the posterior distribution of the overall correlation coefficients (across selection treatments and years). The dot-dashed lines show the posterior distribution of the year 1 correlation coefficients, dashed lines of the year 2, and dotted lines of the year 3. The black line in the inserts highlights the 0 value, and thus the absence of correlation.

Script correlation analysis

# Stan code

```
data {
```
int<lower=0> n;
vector[2] x_obs[n];

parameters {
  vector[2] mu;
  vector<lower=0>[2] lambda;
  real<lower=-1,upper=1> r;
}

transformed parameters {
  vector<lower=0>[2] sigma;
  cov_matrix[2] T;

  // Reparameterization
  sigma[1] = sqrt(lambda[1]);
  sigma[2] = sqrt(lambda[2]);
  T[1,1] = square(sigma[1]);
  T[1,2] = r * sigma[1] * sigma[2];
  T[2,1] = r * sigma[1] * sigma[2];
  T[2,2] = square(sigma[2]);
}

model {
  // Priors
  mu ~ normal(0, 10);
  lambda ~ normal(0,1);

  // Data
  x_obs ~ multi_normal(mu, T);
}

# selecting the variable of interest, e.g. trait_1 and trait_2
data <- cbind(dataset$trait_1, dataset$trait_2)

# sampling
samples <- stan(model_code=model_simple,
               data=list(x_obs=data, n = dim(data)[1]),
init=list(list(r=0, mu=c(0, 0), lambda=c(1, 1))),  # If not specified, gives random inits
pars= c("r", "mu", "sigma"),
iter=10000,
warmup = 2000,
chains=1
)

**S4 Founder population trait values and correlations**

Prior to the start of the long-term experiment, we characterised the 20 founder strains for dispersal and population growth characteristics \( r_0, N \), with 3-4 replicates per strain, as described in the main text. Using a Bayesian approach (Rosenbaum et al. 2019), we determined median values and 95% CI for each strain (Table S1, see also section S1). Figure S4 illustrates the (bivariate) trait space occupied by the mix of the strains in the founder population. For example, Fig. S4A shows considerable genotypic variation in both dispersal and \( r_0 \). Certain strains have very high \( r_0 \) and very low dispersal, and several strains have relatively high dispersal and intermediate levels of \( r_0 \). As shown in the main text, these two types of strains are targeted by short-term selection in the range core and front treatments, respectively. There are no strains with very high levels of both dispersal and growth, and such variants also do not seem to evolve in the long term (see Fig. 4), suggesting that this part of the trait is unavailable to the genetic backgrounds used in this experiment.

**Fig. S4.** Trait relationships in the base population (mix of the 20 founder strains) for (A) dispersal - \( r_0 \), (B) dispersal - \( N \) and (C) \( r_0 \) - \( N \). Each point represents the median trait values of a strain with the 95% CI (see also Table S1).
S5 Trait relationships relative to the control treatment

In the main text, Figure 4A-C illustrates short- and long-term trends in pairwise trait associations, in relation to the model predictions. The long-term trends are inferred from the comparison of measurements taken at different time points (year 1, 2, 3, see main text), and we can therefore not a priori exclude the possibility that the (evolutionary) change in a trait is confounded with a measurement year effect. Ideally, to avoid this problem, samples would be frozen each year and all samples measured at the same time in a single assay at the end of the long-term experiment. However, freezing of samples was not possible for our lines. Instead, we accounted for potential year effects by expressing the performance of range core and range front lines relative to the control treatment. This was done by subtracting the means of the control lines from the values of individual core or front lines from the same year. Patterns for these standardized trait associations (Fig. S5) are very similar to those shown in Fig. 4A-C. Thus, our main conclusions regarding the divergence of selection lines were unlikely to be affected by measurement year effects.

![Fig. S5. Trait relationships between (A) dispersal - $r_0$, (B) dispersal - $N$ and (C) $r_0$ - $N$, expressed relative to the control treatment (core/front minus control values), for each of three years. Symbols are the average values for each selection line. Negative values correspond to decreased trait values compared to the control treatment of the same year, positive indicated increased values and 0 corresponds to no changes. Different symbols refer to the three different years: circle (year 1), star (year 2), cross (year 3).](image)

S6 Complementary experiments

After the long-term experiment was completed, additional tests were performed with the evolved selection lines. First, we performed a ‘treatment-reversal’ experiment. To this end, we divided the evolved lines in two new replicates. The first replicate was continued in the original treatment,
whereas the second replicate was subjected to the other (opposite) treatment. This experiment was run for 9 cycles and dispersal measured, following the protocols described in the main text.

Range front lines continued to show higher dispersal than range core lines, when switched to ‘range core’ selection conditions (Fig. S6.1). Conversely, range core lines continued show very low dispersal, when switched to ‘range front’ selection conditions (Fig. S6.2).

Fig. S6.1. Treatment-reversal experiment. (A) Front and core evolved lines exposed for 9 cycles to core treatment. (B) Front and core evolved lines exposed for 9 cycles to front treatment.

Secondly, we wanted to test whether the observed treatment effects in the long-term experiment, as measured in single-line assays, were strong enough to be picked up by selection. To this end, we mixed range core and front lines at different ‘initial’ proportions, and then exposed these mixes (together with pure 100% core and front controls) to a range core or range front treatment for 3 cycles.

Under range core selection (Fig. S6.1A), we find a decrease in dispersal in the mixes, reaching levels as low as those observed for pure range core lines, whereas pure front lines continue to show high dispersal. Conversely, under range front selection (Fig. S6.1B), we find an increase in dispersal in the mixes, reaching levels comparable to values observed for the pure range front lines. Pure range core lines also show an increase in dispersal, but still disperse less than the mixes or the pure front lines. These results indicate a match between selection history and selection treatment, meaning that front lines have a selective advantage under front selection and core lines under core selection. Indeed, in similar experiments (F. Manzi & O. Kaltz, unpublished), we find that such observed phenotypic changes go hand in hand with the fixation of range core or front lines.
**Fig. S6.2.** Mixed-lines experiment, with (A) range core selection or (B) range front selection treatment. Solid connecting lines are pure (100%) evolved core and front selection lines, respectively. Dashed red lines are mixes of core and front lines, with the initial proportion of front lines ranging from 1.6% to 52%. Each dashed line represents a single experimental 'mixed' replicate, the solid 'pure' lines represent averages (± SE) over 3 experimental replicates.

**S7 Strain winning probability**

The model predictions show strong variation in the winning probability among the 20 strains, i.e. the probability of strains fixation in the population for each of three treatments at the end of the experiment. Although the model is deterministic, we parametrize the model with draws from posteriors (section S4 above). Thus, the model takes into account the data uncertainty and gives a distribution of likely outcomes. Details of the model are given in the main text.

**Fig. S7.** Histograms of strain winning probability for Front, Core and Control treatment with a quasi-extinction threshold of 0.7. Strain winning probability corresponds to the fixation probability among the 20 strains in
10000 model runs. The true potential winner candidates with the right COI genotype are highlighted in red for the Front, blue for the Core and grey for the Control treatment.

**S8 Quasi-extinction threshold**

The quasi-extinction threshold implies that strains go extinct if they exhibit densities below this value. The model scenario that visually corresponds best to the observed data, particularly the identity if the “winning” strains, indicates a relatively large extinction threshold (here 0.7), leading to a similar selection on dispersal and growth rate. Under these conditions, selection favours strains with high dispersal but also a relative high growth rate. When running additional model scenarios with decreased quasi-extinction threshold, selection for growth rate overrides selection for dispersal. Despite the bottlenecks occurring during the dispersal phase, strains with low dispersal can still reach the new patch and regrow to high density. Under these alternative conditions few extinctions occur and all strains can reach the new patch, but it is the strain with the highest growth rate (AMF_11_1A) that becomes fixed in all treatments.

**Fig. S8.1.** Histograms of strain winning probability for Front, Core and Control treatment with a quasi-extinction threshold 0.001.
**Fig. S8.2.** Histograms of strain winning probability for Front, Core and Control treatment with a quasi-extinction threshold 0.1.

**Fig. S8.3.** Histograms of strain winning probability for Front, Core and Control treatment with a quasi-extinction threshold 0.5.

**S9 Genotyping and molecular analysis details**

To characterise the COI genotype of the strains, DNA was extracted from 10 cells per strain using the Chelex®100 (Bio-Rad Laboratories GmbH, Germany) method (Barth et al. 2006) and used as template for the amplification of the COI gene with primers F199dT-B and R1143dT (Strüder-Kypke & Lynn 2010). PCR products were sequenced in both directions with primers M13uni(-21) and M13reverse (Messing 1983) performed by Eurofins Genomics GmbH (Germany). Strain-specific sequence data were compared with the non-redundant sequence database using NCBI-BLAST and an in-house database to infer COI genotype affiliation.

The evolutionary experiment was started from a mix of the 20 strains, and at cycle 30 we assayed...
the genetic diversity of the long-term populations by looking at the COI marker signals. DNA was extracted as described above. Then a simple RFLP (Restriction Fragment Length Polymorphism) method was applied by 1) amplification of COI gene fragments with primers CoxL11058 and CoxH10176 (Barth et al. 2006) using DNA from the selection lines, 2) performing a double-digest with restriction enzymes HindIII and PvuII (New England BioLabs GmbH, Germany) of the selection-line specific PCR products, followed by 3) agarose gel-electrophoresis to visualize the different characteristic restriction pattern for the detected COI genotypes of founder strains.

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Table S1. Details and trait values for each of the 20 strains of the founder population.

| Strain   | Origin (provided by) | In OK lab since | COI genotype | COI genotype detected at cycle 30 | Median dispersal | 95% CI dispersal | Median r₀ | 95% CI r₀ | Median N   | 95% CI N   |
|----------|----------------------|-----------------|---------------|----------------------------------|------------------|------------------|----------|-----------|-------------|-------------|
| AMF_11_1A | Russia (A. Potekhin) | 2014            | b05           | range core and control           | 0.060            | 0.044; 0.141     | 0.162    | 0.075; 0.479 | 263.5       | 204.0; 357.4 |
| AMF_11_1B | Russia (A. Potekhin) | 2014            | b04           | not detected                     | 0.068            | 0.052; 0.144     | 0.093    | 0.063; 0.391 | 299.5       | 229.7; 386.2 |
| AMF_11_5A | Russia (A. Potekhin) | 2014            | *             | not detected                     | 0.311            | 0.275; 0.424     | 0.093    | 0.074; 0.275 | 318.3       | 270.1; 368.4 |
| AMF_11_5B | Russia (A. Potekhin) | 2014            | b07           | range front                      | 0.124            | 0.088; 0.292     | 0.042    | 0.026; 0.219 | 183.3       | 116.7; 399.1 |
| c109     | Unknown              | 2014            | b01           | not detected                     | 0.185            | 0.159; 0.285     | 0.096    | 0.061; 0.353 | 288.0       | 223.3; 371.7 |
| c2       | Isolated from commercial mix | 2004       | b07           | range front                      | 0.069            | 0.049; 0.176     | 0.065    | 0.033; 0.393 | 162.0       | 106.5; 393.3 |
| cra      | Kraków, Poland      | 2006            | b07           | range front                      | 0.095            | 0.060; 0.320     | 0.039    | 0.008; 0.213 | 148.2       | 44.2; 830.9  |
| cyp      | Cyprus               | 2003            | b07           | range front                      | 0.148            | 0.111; 0.319     | 0.034    | 0.016; 0.279 | 165.6       | 87.3; 620.0  |
| goe_1    | Stuttgart, Germany  | 2008            | a18**         | not detected                     | 0.248            | 0.212; 0.373     | 0.056    | 0.037; 0.239 | 277.3       | 196.7; 415.2 |
| goe_11   | Stuttgart, Germany  | 2007            | b01           | not detected                     | 0.202            | 0.174; 0.302     | 0.075    | 0.049; 0.358 | 279.3       | 199.5; 403.2 |
| goe_14   | Stuttgart, Germany  | 2007            | b07           | range front                      | 0.272            | 0.235; 0.397     | 0.100    | 0.068; 0.405 | 251.0       | 203.7; 309.2 |
| k2_41c   | Japan, parents KNZ 5 & KNZ 2 | 2001 | b07           | range front                      | 0.123            | 0.090; 0.270     | 0.042    | 0.030; 0.102 | 251.9       | 181.6; 403.3 |
| K4_12    | Japan, parents KNZ 5 & KNZ 2 | 2001 | b07           | range front                      | 0.057            | 0.038; 0.171     | 0.044    | 0.019; 0.286 | 201.3       | 116.6; 667.8 |
| K6_14    | Japan, parents KNZ 5 & KNZ 2 | 2001 | b07           | range front                      | 0.207            | 0.169; 0.355     | 0.079    | 0.060; 0.272 | 348.3       | 273.9; 432.9 |
| k7       | Japan, parents KNZ 5 & KNZ 2 | 2001 | b05           | range core and control           | 0.051            | 0.037; 0.121     | 0.135    | 0.084; 0.330 | 293.4       | 256.0; 334.2 |
| k8       | Japan, parents KNZ 5 & KNZ 2 | 2001 | b07           | range front                      | 0.411            | 0.370; 0.537     | 0.073    | 0.058; 0.239 | 249.1       | 204.4; 298.5 |
| k9_48c   | Japan, parents KNZ 5 & KNZ 2 | 2001 | b07           | range front                      | 0.137            | 0.114; 0.224     | 0.063    | 0.048; 0.202 | 329.1       | 239.9; 446.2 |
| m3       | Isolated from commercial mix | 2004       | b07           | range front                      | 0.361            | 0.319; 0.495     | 0.059    | 0.045; 0.204 | 309.0       | 231.1; 427.8 |
| tueb     | Tübingen, Germany (H-D Görtz) | 2001 | b07           | range front                      | 0.082            | 0.051; 0.282     | 0.032    | 0.018; 0.216 | 223.0       | 82.9; 662.9  |
| ven      | Venice, Italy        | 2006            | a01**         | not detected                     | 0.253            | 0.220; 0.363     | 0.083    | 0.063; 0.286 | 353.7       | 276.8; 446.0 |

*Identified as Paramecium multinucleatum

** determined in Killeen, J., Gougat-Barbera, C., Krenek, S. & Kaltz, O. (2017). Evolutionary rescue and local adaptation under different rates of temperature increase: a combined analysis of changes in phenotype expression and genotype frequency in Paramecium microcosms. Molecular Ecology, 26, 1734-1746.
Table S2. Results of the multiple regression between strains’ traits (dispersal, growth rate and equilibrium density medians) and winning probability across treatments.

|         | Dispersal | r₀      | $\bar{N}$ |
|---------|-----------|---------|-----------|
|         | $F_{1,16} = 8.00$ | $F_{1,16} = 9.85$ | $F_{1,16} = 0.87$ |
|         | $p = 0.0121$ | $p = 0.0064$ | $p = 0.3652$ |
|         | $\beta$ coefficient = 0.55 | $\beta$ coefficient = 0.64 | $\beta$ coefficient = -0.21 |
| Front   | $F_{1,16} = 21.68$ | $F_{1,16} = 199.5$ | $F_{1,16} = 19.3$ |
|         | $p = 0.0003$ | $p < 0.0001$ | $p = 0.0005$ |
|         | $\beta$ coefficient = -0.31 | $\beta$ coefficients = 0.97 | $\beta$ coefficient = -0.33 |
| Core    | $F_{1,16} = 7.66$ | $F_{1,16} = 92.3$ | $F_{1,16} = 9.43$ |
|         | $p = 0.0137$ | $p < 0.0001$ | $p = 0.0073$ |
|         | $\beta$ coefficient = -0.27 | $\beta$ coefficient = 0.96 | $\beta$ coefficient = -0.33 |