Original data for manuscript:

Invasion triple trouble: environmental fluctuations, fluctuation-adapted invaders and fluctuation-mal-adapted communities all govern invasion success

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Abstract

Background

It has been suggested that climate change will lead to increased environmental fluctuations, which will undoubtedly have evolutionary consequences for all biota. For instance, fluctuations can directly increase the risk of invasions of alien species into new areas, as these species have repeatedly been proposed to benefit from disturbances. At the same time increased environmental fluctuations may also select for better invaders. However, selection by fluctuations may also influence the resistance of communities to invasions, which has rarely been tested. We tested eco-evolutionary dynamics of invasion with bacterial clones, evolved either in constant or fluctuating temperatures, and conducted experimental invasions in both conditions.

Results

We found clear evidence that ecological fluctuations, as well as adaptation to fluctuations by both the invader and community, all affected invasions, but played different roles at different stages of invasion. Ecological fluctuations clearly promoted invasions, especially into fluctuation mal-adapted communities. The evolutionary background of the invader played a smaller role.

Conclusions

Our results indicate that climate change associated disturbances can directly increase the risk of invasions by altering ecological conditions during invasions, as well as via the evolution of both the invader and communities. Our experiment provides novel information on the complex consequences of climate change on invasions in general, and also charts risk factors associated with the spread of environmentally growing opportunistic pathogens.

Data description:

Data represent time of sampling (1,2,3,4) time points, equaling 3,6,9,12 days after the initiation of the experiment. Experiment consisted of two invasion environments (constant and fluctuating) in which invader that had evolved in constant or fluctuating environment, competed against community that had evolved in constant or fluctuating environment (in all possible combinations) This experiment was repeated 10 times with independently evolved invader clones (invader clone ID). All communities in experiment had evolved also independently. As an indicator of invasion success we calculated the number of colonies of invader, and other species, from -5 diluted samples plated on DNASE agar plates.

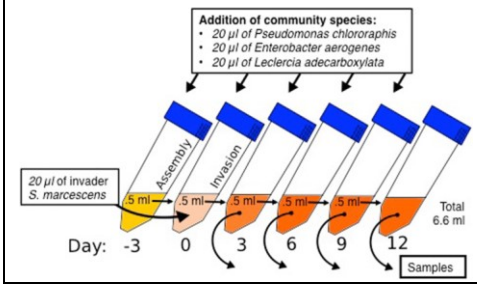
The more detailed information of conduction of the experiment can be found from original publication (same information copied below)

Invasion experiment

As we were interested in the effects of invasion environment (constant vs. fluctuating temperature), evolutionary background of the invader (evolved in constant vs. fluctuating temperature) and evolutionary background of the community species (evolved in constant vs. fluctuating temperature) on the invasion success of *S. marcescens*, we set up an experiment testing all 3 effects together. We had 2 different thermal environments where the invasion took place: constant (30 °C) and rapidly fluctuating (2 h 20 °C - 2 h 30 °C - 2 h 40 °C) and community members had either evolved in constant environment or in fluctuating environment. Moreover, in these very same conditions we tested how clones of *S. marcescens,* that had evolved in independent replicate populations ($n = 10$), either in fluctuating or constant temperatures, were able to successfully invade. The experiment was initiated by allowing the community species an assembly / establishment period prior to invasion (Fig. 1). Community species were mixtures of clones from 10 independently evolved populations evolved either in constant or in fluctuating environments (previously mixed and frozen at − 80 °C (1:1 in 80% glycerol)). After thawing, 20 µl of each species clone mix (totalling 60 µl) was pipetted into experimental 15 ml centrifuge tubes (Sarstedt, Numbrecht, Germany) filled with 6 ml of NB, and tubes were placed in thermal treatments: at constant 30 °C or at fluctuating 2 h 20 °C - 2 h 30 °C - 2 h 40 °C (thermal cabinets: ILP-12, Jeio Tech, Seoul, Korea). Centrifuge tube caps were left loose to allow airflow. Throughout the experiment the cultures were stationary.

After the three-day community assembly period we renewed the resources by pipetting a 500 µl sample of each community into new tubes filled with 5.5 ml of NB. To maintain all community species in the community we supplemented cultures with 20 µl of each community species clone mix (the clones, before mixing, were also individually grown beforehand for 3 days at 30 °C, 60 µl of bacteria in 6 ml of NB in 15 ml centrifuge tubes). The added clone mixes had always the same evolutionary background as the community species (Fig. 1).

Fig. 1
Overview of the setup of the invasion experiment. Invasions started with the three-day "assembly" period for the community. Invasion occurred three days later. Renewal to new tubes and sampling occurred every three days. This procedure was repeated with invaders adapted to fluctuating or constant temperature making invasion against community adapted to fluctuating or constant temperature in constant or fluctuating environment. Each of the eight combinations was replicated 10 times



After renewal and species supply, invasions were initiated to create the 8 different environment - evolutionary background combinations. The 20 (10 from fluctuating and 10 from constant environment) *S. marcescens* invader clones had been propagated beforehand for 2 days at 30 °C (60 µl of bacteria in 6 ml of NB in 15 ml centrifuge tubes). To each community, we added 20 µl of *S. marcescens* invader clones, which corresponds to 4% of the renewed community (500 µl) and is equivalent with the amount of external gene flow from each community species (see above). After which the communities, now with an invader, were placed in thermal chambers. The rationale for species supplementation is the very effective invasion by *S. marcescens.* In un-supplemented system *S. marcescens* dominate the whole community within few days. Unfortunately, the supplementation renders detailed work on community's responses to invasion useless.
The communities were propagated for 12 days after the invasion, and we renewed the communities (as above), added the gene flow from the community species (as above) and froze samples of each community to − 80 °C (final concentration of 40% glycerol) every 3 days (3, 6, 9 and 12 days after the invasion). After the experiment we plated all the community samples from all the time points (3, 6, 9 and 12 days after invasion, altogether 320 samples). We used a standard dilution series technique to achieve a 105-fold dilution series that allowed the counting of separate colonies on agar plates. We plated the samples on DNase test agar with methyl green (Becton and Dickinson and Company, Sparks, MD; premade at Tammer-tutkan maljat, Tampere, Finland) that enabled the separation of *S. marcescens* from the community species (see: [3334]). The fate of specific community species was not monitored during the invasion experiment, due to frequent supplementation of species (see above).