Progress report for project 1.02 and 2.02 (as of October 2021)

The projects ‘Impact of the trade-offs on eco-evolutionary feedbacks in predator-prey systems’ and ‘Indirect response to external drivers through trait variation in predator-prey systems‘ aimed to gain important new insights into the reciprocal interaction between trait and population dynamics in predator-prey systems. Within the projects, we quantified the distribution of several traits within green algae *Chlamydomonas reinhardtii*, the potential for trade-offs as well as the consequences of the trait variation and trade-offs for population dynamics when the green algae grow together with a rotifer predator (*Brachionus calyciflorus*) and how population dynamics contribute to the maintenance of diversity with the prey population. During the second phase of DynaTrait, we focused on the importance of the trait variation for buffering external perturbations. We used for our projects a predator-prey systems where a rotifer predator feeds on the green-algae *Chlamydomonas reinhardtii* and we focused on trait variation within *C. reinhardtii*. We used a set of previously collected *C. reinhardtii* lines from a selection experiment where different *C. reinhardtii* strains evolved with and without predation by a rotifer for 6 months. In collaboration with the groups of Ursula Gaedke (University of Potsdam) and Masato Yamamichi (University of Queensland, Australia), we combined experimental work with modelling. Together with Uwe John (AWI Bremerhaven), we complemented trait measures by sequence analyses.

1. Traits and trade-offs

Shape of defence-competition trade-off: We examined the trade-off between defence and competition using isolated *Chlamydomonas reinhardtii* genotypes from a selection experiment where different Chlamydomonas strains (i.e., genetic background) evolved with and without predation by a rotifer for 6 months. Examining the distribution of defence against rotifer predation and maximum growth rates in trait space, we found convex trade-off curves for the isolates from the selection lines with and without rotifer predation. The trade-off curves differed, however, between the two treatments in two important ways. First, we found a significant difference in the curvature of the trade-off curve, with a stronger convex curve for the isolates for the selection lines which evolved in the presence of the rotifer predator. This more convex trade-off curve indicates the evolution of a more costly defence in the presence of the rotifer predator. Second, we found morphological differences in the evolved isolates. Isolates from the treatment evolving in the presence of the rotifer predator were growing significantly more often in aggregates of several algal cells, whereas isolates from the treatment evolving in the absence of the rotifer predator grew mostly as single cells. Additional transcriptome analyses confirmed the heritability of the phenotype. Whole genome sequencing of a subset of isolates revealed several mutations that were unique to all isolates from the selection treatment with rotifer predators but absent from the isolates from the selection treatments without rotifers. The observation of the repeated mutations suggests strong selection (Bernardes et al. 2021 *Nature Communications*).

Trait variation: In addition to defence and growth rates, we measured several additional traits to develop a better understanding of the trait variation across *C. reinhardtii* isolates. Specifically, we measured growth parameters (carrying capacity, area under the curve), morphology and the cells' ratio of carbon to nitrogen. These data haven been collected but have not been analysed yet.

Trait variation and fitness: Going one step further and aiming to link fitness with morphological and trophic traits, we measured functional response curves, half-saturation constants and morphological traits for six *C. reinhardtii* isolates. The functional response curves were used to estimate attack rates, handling time as well as pre- and post-ingestion defences (Ehrlich & Gaedke 2018 Ecol. Evol). We found that isolates can be grouped into *C. reinhardtii* with pre- and post-attack defences against consumption by the rotifer predator. Information on the morphological traits was collected by image flow cytometry (Amnis®ImageStream) and we found that size, aggregation, and circularity separated the isolates into well and less defended prey. Finally, linking the traits to fitness in the presence and absence of the rotifer predator, we found that cell size and carrying capacity explained most variation in fitness under these conditions. In addition, we used the data to test how the trade-off changes with respect to the shape of the trade-off curve (i.e., costly defence or cheap defence) and the ranking of the isolates when focusing on different traits representing defence and competitive ability. We found that the shape of the trade-off changes from between convex and linear without changing the ranking of the isolates (i.e., from most defended to least defended).

2. Trade-offs and eco-evolutionary dynamics

Based on theoretical work (Ehrlich et al. 2016) we designed experiments to test the invasion capability of a third algal genotype into a system with a rotifer predator and two prey genotypes. The latter are a defence or a competition specialist. The model with a two-dimensional trade-off assumes only two niches (defence and growth specialist) and the model analyse revealed that more than two types can only coexist under the assumption of fitness equality in this system. Thus, when adding a third prey genotype at low frequency to the system with the two specialists present, the possible outcomes are that the type invades and replaces one or both resident prey types or fails to invade. Whether invasion is possible or not depends on the traits of the third genotype. Initial experiments with *C. reinhardtii* and the rotifer confirmed the predictions from the model that a third type can or cannot invade depending on the trait combinations. The results of the full experiments were, however, less clear as invasion success of a third genotype differed across replicates and seemed independent of the position in trait space of the third genotype. Applying Chesson's Modern theory of Coexistence, we ran additional experimental tests with the *C. reinhardtii* isolates used in this experiment testing whether fitness and niche differences measurements were better predictors for the invasion capability than the positions in trait space. We found for most combinations of *C. reinhardtii* isolates a priority effect. As the algal and rotifer population size and the frequencies of the defended and undefended prey differed at the time point of the addition of the third genotype, it is possible that the priority effect played an important role in this experiment. We currently try to match the predictions from the fitness and niche differences to the outcome of the invasion experiments.

3. Trait variation and buffering of external perturbations

We tested whether and how trait variation in the prey population can buffer external perturbations to the predator using model analyses and experiments.

Indirect evolutionary rescue: Indirect evolutionary rescue (IER) is a mechanism where a non-evolving population is saved from extinction in an otherwise lethal environment by evolution in an interacting population (Yamamichi & Miner 2017). This process has been initially described for predator-prey models, where extinction of the predator is prevented by a shift in the frequency of defended towards undefended prey when reduced predator densities lower selection for defended prey. We test how increased mortality, and the initial frequencies of the prey types affect the likelihood of IER. Combining the analyses of model simulations and experiments with rotifers feeding on an algal population we show IER in the presence of increased predator mortality. We found that IER was depending on the possibility of the prey population to evolve as well as on the frequency of the defended prey, with high frequencies of defended prey resulting in extinction because prey evolution was too slow. This frequency dependency for IER was more pronounced for higher predator mortalities. (Hermann & Becks, *under review*).

Indirect evolutionary rescue and trait range: Building on the results of IER described above, we ran another set of experiments, where we manipulated the trait range for defence and growth rate as well as the mortality level of the predator. Differences in the minimum and maximum trait values of the two prey types are predicted to affect the likelihood of observing IER because the rate of change in the two prey types in response to reduced predation depends on the trait differences. When changes in the average defence level in the prey population are too slow to allow positive growth of the predator in the presence of an increased mortality, IER will not occur. This work is done in collaboration with Masato Yamamichi who focuses on the analyses of a mathematical model. The works is currently ongoing.

Indirect evolutionary rescue, predator diversity and competition: We further run a IER experiment with a different predator (the ciliate *Tetrahymena thermophila*) with the aim to test how the likelihood of IER is affected by the predator traits. We use the same set up as in Hermann & Becks (under review) and will compare how the likelihood of IER changes with different predators. As a potential extension of this, we will investigate the role of IER on coexistence of the two predators. For this we plan to collaborate with Ellen van Velzen (University of Potsdam) on the modelling part.

Microplastic and trophic interactions: While many environmental perturbations lead to the extinction of a species, some perturbations affect the trophic interactions between consumer and its resources. Microplastic affects the uptake of *C. reinhardtii* by the rotifer *B. calyciflorus* and can thus affect the trophic interaction between the two trophic levels. We tested how functional response curves for six *C. reinhardtii* isolates differing in their levels of defence and competitiveness are affected by the presence of microplastics in two different sizes (i.e., one can be ingested but not digested, the other cannot be ingested) and at different concentrations. We found that ingestion rates are affected, but that the effect was depending on the *C. reinhardtii* isolates and the microplastics sizes and concentrations. For some of the *C. reinhardtii* isolates, we found an overall reduction in the ingestion rates for all *C. reinhardtii* densities offered in the experiment, while we observed for other isolates differences only at high *C. reinhardtii* densities. The data also suggest that for some *C. reinhardtii* isolates, the ingestion rates were increased in the presence of the microplastics. In additional experiments, we found that differences in functional response curves reduced rotifer growth rates (numerical response).

Indirect evolutionary facilitation I: Building on the observations that the presence of microplastic affects the trophic interaction between prey and predator, we analysed a chemostat predator-prey model asking under which conditions (concentration of plastic beads and differences in the defence level of the defended prey) a shift in the prey defence level (i.e., a shift in the frequency of the defended and undefended prey) can cancel the effect the microplastics have on the predator growth rates (= indirect evolutionary facilitation (IEF)). We found that IEF occurs at high concentrations of microplastics and when the trait differences in the defence levels of the undefended and defended prey are large. For some combinations of differences in defence trait and microplastic concentrations, we observed higher predator densities in the presence of microplastics than in in the absence of microplastics. The model analyses will be extended to examine how the consequences of the microplastics on the functional and numerical responses alter the dynamical behaviour of the system (e.g., shift from cycles to steady state dynamics).

Indirect evolutionary facilitation II: We will run chemostat experiments to test predictions from the model predator-prey model described above.

Development of the projects:

Noemi Woltermann joined the project ‘Impact of the trade-offs on eco-evolutionary feedbacks in predator-prey systems’ as PhD student. Noemi Woltermann did however not complete her PhD thesis after becoming mother in the 2nd year of her PhD. This has slowed down the progress, but we plan to finish some of the projects with the help of Master students.

References

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