Do Duckweeds Adapt to Water and Microbes?

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DO DUCKWEEDS ADAPT TO WATER AND MICROBES?

BY
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THESIS

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ABSTRACT

DO DUCKWEEDS ADAPT TO WATER AND MICROBES?

by

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Organisms in their home environment sometimes outperform transplanted individuals native to other areas, in a phenomenon termed local adaptation. While local adaptation is traditionally considered to be driven by the abiotic factors of an environment, scientists have recently increased consideration of biotic factors. Specifically, of interest to many is host-associated microbiomes, which can alter host trait expression. As the desire to commercialize microbiome treatments for agriculture and medicine grows, it is important to analyze the potential value of local microbiomes, which may be adapted to their hosts, or to which local hosts may have adapted. Using *Lemna minor* (duckweed) as a model organism, we examined differences in host fitness when grown in local and foreign microbiomes and in local and foreign water. We hypothesized that duckweeds grown with their local microbiome and water would display higher fitness than those grown in different contexts. For the study, we collected duckweeds, microbes, and water from 4 different sites in Durham, New Hampshire. Water, duckweeds, and microbes were crossed and assembled as microcosms in 96-well plates. After 2 weeks, we measured frond area as a proxy of duckweed fitness and optical density, as a measure of microbial cell growth across the microbiome. We found that duckweeds grown in their local water and microbiome had higher fitness. We also found that microbe fitness was not dependent on their local water or plant. Therefore, local adaptation to a host’s microbiome
conveys benefits to the host, and inocula with similar compositions as the local microbiome may be more beneficial than generic compositions.
INTRODUCTION

Microorganisms are found ubiquitously throughout the earth, including associated with a variety of host organisms (Berg et al., 2020). These host-associated microbiomes have been shown to alter host trait expression in various studies (Gould et al., 2018). Extensive research on the gut-associated microbiome has found that it alters host trait expression in various capacities (Turnbaugh et al., 2008; Kaiko & Stappenbeck, 2014). For example, changes in the human gut microbiome composition have been linked to health issues like obesity and Irritable Bowel Syndrome (IBS) (Mondot et al., 2013). Plant-associated microbiomes have similar effects on their hosts, mediating responses to biotic and abiotic factors (Trivedi et al., 2022). For instance, microbial influence on flowering time has been observed in numerous plant species (Lu et al., 2018; O’Brien et al., 2021).

As our knowledge of how microbiomes can affect host trait expression grows, so does our desire to use this to our advantage. The arrival of effective microbiome treatments is anticipated in many different fields. Benefits ranging from fostering better drug responses (Lam et al., 2019) to overall improvements in health through manipulation of the gut microbiome (Schupack et al., 2022) have been theorized. In the world of agriculture, we hope engineering microbial treatments could increase crop yields and overall productivity (Qiu et al., 2019).

Some microbiomes may be better sources of beneficial effects than others. Local adaptation refers to the idea that organisms native to an environment will perform better than those from different habitats (Blanquart et al., 2013). Many organisms have been shown to adapt to their native microbiome, including plants (Rúa et al., 2016). In addition, microbes have exhibited adaptation to their native hosts (Batstone et al., 2020). Therefore, local microbiomes might be better sources of beneficial microbes because the hosts have adapted to the specific...
species and strains they contain. Conflicting data surrounding the impacts of biotic factors on local adaptation has been obtained, with some studies finding more local adaptation in the presence of biotic factors and others seeing no influence (Briscoe et al., 2020; Hargreaves et al., 2020). Here, we tested the idea that local microbiomes may convey more benefits to their hosts than foreign ones.

We tested our hypothesis using *Lemna minor* and its associated microbiome as a model. The common pond plant *Lemna minor*, also referred to as duckweed, has been used as a model for plant biology since the 1950s (Acosta et al., 2021). This organism’s rapid doubling time and small size make it easy to manipulate and ideal for experiments (Zhang et al., 2010). Previous experiments have cited duckweed as a good model organism for host-microbiome interaction experiments (Jewell et al., 2023). While other studies have researched local adaptation in duckweed to their microbiome, few have used microbes directly from the source. Culturing microbes, instead of taking them directly from the source, was cited in one study as potentially limiting to our understanding of local microbiome benefits in duckweeds (O’Brien et al., 2023).

**Thesis Objectives:**

This project aimed to analyze the effects of local adaptation to the microbiome on host fitness in duckweeds. It was hypothesized that duckweeds grown in their home water and microbiome would display higher fitness than those grown in different contexts. In addition, we hypothesized that microbes grown with their home water and duckweeds would display higher cell density than those grown in different contexts.
MATERIALS AND METHODS

Experimental Design:

To evaluate the impacts of local and nonlocal microbiomes and water on duckweed fitness, duckweeds, water, and microbes were collected from four sites around Durham, New Hampshire. Randomized crosses of sterile duckweeds were loaded into 96-well plates along with microbes and sterile water from each site. Frond area and optical density were analyzed after 14 days. A graphical representation of this process can be found in Figure 1.

![Figure 1](image)

**Figure 1:** Experimental design for this project, depicting collection sites and the flow of work. Duckweeds, water, and microbes were collected from the four sites, covering a spatial range that is detailed in Figure 2. These sites are depicted by the green, orange, blue, and purple dots (Durham Reservoir, Woodman, Mill, and Upper Mill, respectively). Randomized crosses accounting for all combinations of plants, water, and microbes (and a no-microbe treatment) were made in 96-well plates.

Collection Sites, Samples, and Storage:

Microbes, water, and duckweeds were collected from four locations across Durham, New Hampshire: Mill (M), Upper Mill (UM), Woodman (W), and the Durham Reservoir (DR). Gloves were rinsed with 70% ethanol and worn for the entire collection process. Duckweeds were collected using pre-sterilized spatulas. All tubes used for collection were sterile. Each tube used was rinsed 3 times in the source water prior to collection. A map of the collection sites can be found below in Figure 2.
Figure 2: Map of sites for water, microbes, and duckweeds from around Durham, New Hampshire. The four sites covered a spatial range between 43.12248009 and 43.14755972 degrees latitude and -70.91917424 to -70.9441286 degrees longitude. Sampling sites are denoted with colored circles (green, orange, blue, and purple).

Microbes were collected from each of the four sites between September-October 2023. To obtain the microbes, three 45 mL tubes of water were collected at each site. We centrifuged the collected water for 15 minutes at 4,000 rpm and 23°C. The supernatant was poured off, and 2mL of ~25% sterile glycerol was added to the pellets. The pellets were mixed and stored at -80°C until needed. Before experimentation, the microbes were diluted in 2 mL of DI water. Sterile ~25% glycerol was diluted by half in DI water for control treatments.

Water samples were collected from each of the four sites from September-October 2023. Three 40 mL samples of water were collected at each site. We placed the samples directly into a -20°C freezer and stored them until needed. Before experimentation, we removed excess dirt and debris using gravity filtration and paper filters. We filter-sterilized water samples by passing them through a 0.2 µm filter, 60 mL at a time.
Duckweed samples were collected from each of the four sites during the spring of 2022. We sterilized all duckweed samples in 1% bleach for 1 to 1.5 minutes, and then rinsed in sterile water three times; the first rinse was 45 seconds long, and the second two were 5-10 minutes. The plants were transferred to sterile 0.5x Krajnčič’s media and were allowed to grow. Enrichment in Krajnčič’s with yeast and mannitol was done to check for failure of the sterilization procedure. Lines were grown in this media until they were needed for experimentation.

**Experimental Set-Up:**

To simultaneously test if duckweeds and microbes were adapted to their local water, crosses of duckweeds, microbes, and water were made in 96-well plates. 5x4 well blocks for each duckweed genotype were plotted, with the location of the field-collected water, cryo-preserved microbes, and gnotobiotic plants being completely randomized within the block, accounting for all possible combinations. Four of these smaller blocks, one for each duckweed genotype, made up one replicate. Separate maps of the randomized locations for duckweeds, water, and microbe treatments were made as references for plating. 10 flat-bottom 96 well plates were used for this experiment, for a total of 12 replicates.

We added 205 uL of field-collected water to the plates according to the randomized map. Attached units of sterile duckweed fronds (ranging from 1-4 individual fronds) were added to the plates, with the added genotype corresponding to the randomized map. The plates were sealed with Breatheasier seals. It was calculated that adding 9.1 µL of microbes to the 205 µL of water would bring the cells back to their original concentration at the time of collection. 9.1 uL of resuspended microbes, or control treatment, were added to each well by punching through the
seals, with the microbe treatment according to the map. The plates were then sealed with Breatheasy seals and the plate’s lid.

The plates were placed in a Percival® model AR-75L3 growth chamber to stimulate growth. Lights at an intensity of 350 µmoles/m²/s were on from 6:00 am to 10:00 pm at 23°C, and then turned off and remained off until 6:00 am the next day, resting at 18°C. The plates were left in the chamber for 14 days. Images of the plates were taken throughout this time. The plates were placed on a stage above a set of 4 cameras, each connected to a Raspberry Pi that the images could be saved to (Kose et al., 2023). The images taken were analyzed with DuckPlate software. This software divided plate images into individual wells, identified the duckweed from the background, and measured the area of the corresponding fronds (Kose et al., 2023). Frond area was correlated to the fitness of the plants (O’Brien et al., 2020). After day 14, 200 µL of the remaining water was removed and replated. OD_{600} and OD_{420} readings were taken for each well with a BioTek Cytation 5 plate reader to analyze cell density.

Data analysis:

Statistical analysis on the duckweed frond area was performed using R statistical software (R Core Team, 2021). Five different treatment combinations were analyzed: duckweeds, water, and microbes from different sites (all different), water and microbes from the same site, but non-local to the duckweeds (water and microbe same), duckweeds with local water but non-local microbes (plant and water same), duckweeds with local microbes but non-local water (plant and microbe same), and duckweeds, water, and microbes from the same site (all same). Differences in mean cell density for OD_{600} and OD_{420} readings were also quantified using
R statistical software. The same five treatment combinations were analyzed as for duckweed frond area.

Significance differences between treatments were analyzed using the MCMCglmm package in R (Hadfield, 2010). Quantitative levels of site matching to duckweeds and microbes were performed. For example, in the “all different” and “water and microbes same” treatments, zero sources are identical to the duckweeds. In the “plant and water same” and “plant and microbe same” treatments, one source is identical to the duckweeds. In the “all same” treatment, two sources are identical to the duckweeds. The quantitative levels were different when considering microbial growth: zero sources are the same as the microbes in the “all different” and “plant and water same” treatments, and one source is the same as the microbes in the “plant and microbe same” and “water and microbes same” treatments. Models comparing the “all different” treatment to the “water and microbes same”, “plant and water same”, “plant and microbe same”, and “all same” treatments were made for both average frond area and cell densities (OD$_{600}$ and OD$_{420}$). This was done to see if any treatment conveyed more benefits (higher fitness) to the duckweeds or microbes than others.
RESULTS

Duckweed Fitness:

Duckweeds grown with both their home water and microbiome overall had higher final frond area than those grown in treatments that were quantitatively less similar (0 or 1 identical sources) to their home conditions. Significantly higher frond area (fitness) was seen in the quantitative model between plants grown in both their home water and microbes and plants grown with no local sources of water or microbes (p=0.0307). No statistically significant difference was seen in the quantitative model between the frond area of duckweeds grown with at least one local source of microbes or water compared to those with no local sources of water or microbes (p=0.098). No significantly different frond area was seen between duckweeds grown in non-local water and non-local microbes from the same site than in plants grown in no local sources of microbes and water (p=0.824, Figure 3). No significantly different frond area was seen between plants grown in local water and non-local microbes and plants grown with non-local water and microbes (p=0.248, Figure 3). No statistically significant difference in frond area was seen between plants grown with local microbes and non-local water and those grown in no local sources (p=0.13, Figure 3). No significant difference was seen in the non-quantitative model between plants grown with both local microbes and water and those grown with no local sources of water or microbes (p=0.171, Figure 3). This pairwise comparison does not take into consideration the intermediate fitness held by duckweeds when grown with local microbes or water that can be predicted by the quantitative models, hence the differences in significance.
Figure 3: Average final area of duckweed fronds in pixels for varying treatments. Points represent the average pixel area of the duckweed frond in each well on day 14 of the experiment for each treatment category. Bars represent standard error. Data from 960 wells was analyzed. The number of unique datapoints in each replicate varied by treatment category: “all different” had 36 different treatments, “water and microbe same” had 12 unique treatments, “plant and water same” had 16 unique treatments, “plant and microbe same” had 12 unique treatments, and “all same” had 4 unique treatments. Each treatment itself was replicated 12 times.

Microbial Cell Density:

Microbes grown with both their home water and duckweeds overall did not have higher cell density than those grown in treatments that were quantitatively less similar (0 or 1 identical sources) to their home conditions (p=0.946). The microbial communities grown with both their local duckweed and local water did not have higher total cell densities than those grown with non-local sources of plant hosts and field water (p=0.944, Figure 4). No significant difference was seen in cell density for microbes grown with non-local plants and non-local water from the same site compared to those grown with no local sources of plant or water (p=0.608, Figure 4).
Cell density for microbes grown with their local plant and water from a non-local site was not significantly different than microbes grown with no local sources of plants or microbes \((p=0.911, \text{ Figure } 4)\). No significant difference in cell density was observed between microbes grown with their local water and a non-local plant and those grown with no local sources of plant or water \((p=0.384, \text{ Figure } 4)\). The highest variance in cell density was seen in microbes grown when the duckweed and water were from the same source but not from the same source as the microbes.

![Figure 4: Average recorded optical density at 600 nm for varying treatments, obtained from R statistical software. Optical density at 600 nm is a measure of cell density at a wavelength not expected to be affected by microbes with photosynthetic pigments. Points represent the average optical density at 600 nm for each well on day 14 of the experiment, for each treatment category. Bars represent the standard error. Data from 960 wells was analyzed. The number of unique datapoints in each replicate varied by treatment category: “all different” had 36 different treatments, “water and microbe same” had 12 unique treatments, “plant and water same” had 16 unique treatments, “plant and microbe same” had 12 unique treatments, and “all same” had 4 unique treatments. Each treatment itself was replicated 12 times.](image)
The optical density at 420 nanometers was also analyzed, which is a biased measure of cell density that will have the highest absorbance when photosynthetic microbes are more abundant. These microbes grown with both their home water and duckweeds overall did not have higher cell density than those grown in treatments that were quantitatively less similar (0 or 1 identical sources) to their home conditions \((p=0.604)\). Microbial communities grown in their local water but with a non-local plant did not display significantly higher cell density than those grown with no local sources of plant or water \((p=0.266, \text{ Figure 5})\). There was no statistically significant difference in cell density between microbes grown with non-local plants and non-local water from the same site and microbes grown with no local sources of plants or water \((p=0.523, \text{ Figure 5})\). The cell density in microbes grown with their local plant and non-local water was not significantly different than the density of microbes grown with no local sources of plants or water \((p=0.849, \text{ Figure 5})\). No statistically significant difference was seen between microbes grown with their local water and plants and those grown with no local sources of plants or water \((p=0.831, \text{ Figure 5})\). The highest variance in \(\text{OD}_{420}\) was seen in experiments where the duckweed and water were from the same source, but not the same source as the microbes. The average cell density \((\text{OD}_{420} \text{ reading})\) and variance between treatments were similar to the trends seen in the \(\text{OD}_{600}\) data.
Figure 5: Average recorded optical density at 420 nm for varying treatments, obtained from R statistical software. Optical density at 420 nm is a measure of cell density at a wavelength expected to be affected by microbes with photosynthetic pigments. Points represent the average optical density at 420 nm for each well on day 14 of the experiment. Bars represent the standard error. Data from 960 wells was analyzed. The number of unique datapoints in each replicate varied by treatment category: “all different” had 36 different treatments, “water and microbe same” had 12 unique treatments, “plant and water same” had 16 unique treatments, “plant and microbe same” had 12 unique treatments, and “all same” had 4 unique treatments. Each treatment itself was replicated 12 times.
DISCUSSION

Organisms in a population undergo local adaptation when alleles beneficial to particular environments become fixed. Local adaptation often results in organisms in their native environment outperforming non-native organisms. Adaptation to an organism’s local microbiome may result in higher fitness when paired with local versus non-local microbes, which is important to consider in the development of inoculation products for agriculture and other industries. We hypothesized that duckweeds grown with their home water and microbiome would have higher fitness than those grown in different contexts. We found significantly higher frond area, and therefore higher fitness, when duckweeds were grown in both their home water and microbiome as compared to duckweeds grown with no local sources of microbes or water, suggesting local adaptation to both abiotic and biotic environments. These findings imply that microbe compositions similar to those found in nature would be a more beneficial source for inocula development.

Host-microbiome interaction studies have been searching for ways to construct ideal inocula treatments. Studies focusing on the gut microbiome have used modeling to select microbes with identified involvement in certain biochemical pathways for synthetic, defined treatments (Clark et al., 2021). Other gut microbiome studies focus on selecting microbes that have known, beneficial effects on hosts that can aid recovery from gastrointestinal diseases, such as Clostridium difficile (C. diff.) infections (Mueller et al., 2022). Another recent study investigated the inconsistent benefits of artificial microbiome inocula, finding that the benefits they were expecting only occurred after selecting on microbiome effects for multiple plant generations (Jacquiod et al., 2022). While this research is promising, discussions of local adaptation have not been cited by many of these studies.
Our study found that higher fitness was seen in duckweeds grown in both their local water and microbiome versus those grown with just one local source. A study performed by Briscoe et al. (2020) found similar results, stating that local adaptation in plants may increase when both abiotic and biotic factors are present. Other studies have found the opposite, stating that local adaptation is not affected by the presence of both biotic and abiotic factors (Hargreaves et al., 2020). The inconsistency of these findings suggests that the impact of biotic and abiotic factors on local adaptation may be dependent on the organism.

We measured total cell density as a proxy for the average fitness of all microbes in the community. However, individual microbes could have displayed very different trends in cell density from the total cell density. It was hypothesized that microbes grown with their home water and home duckweed would display higher fitness than those grown in different contexts, but we did not find any significant positive effect of plants, water, or a combination of both from the same source on total microbe cell density. Overall, the highest cell density was seen in microbes grown in their home water with a non-local plant, which is suggestive, but not indicative of some local adaptation. However, this approach is not without limitations.

Measuring optical density in this way is complex, as it simultaneously measures the light reflected by all cells. Because all cells are measured at the same time, it is not known how each subset of microorganisms varies in growth. While as a whole the microbiome did not display higher fitness with their home water and plants, it is possible that individual components of these microbiomes did.

Other studies looking into whether microbes locally adapt to their native environments have come back with positive results. One study found that mycorrhizal fungi were able to take in a higher amount of carbon from their local soil than non-local fungi (Johnson et al., 2010).
Another study observed local adaptation in *Saccharomyces paradoxus* to climate conditions (Leducq et al., 2014).

Some microbial cells absorb more light at 420 nm rather than 600 nm because of the pigments that they produce. This gave us the opportunity to test if the cell density patterns observed for photosynthetic microbes provided a different result with respect to local adaptation. We hypothesized that microbes grown with their home duckweed and home plant would display higher fitness than those grown in different contexts. However, we did not find any significant positive effect of plants, water, or a combination of both from the same source on total photosynthetic microbe cell density. Overall, the highest difference in OD$_{420}$ readings was seen in microbes grown in non-local plants and water from the same site. The trends seen in the mean cell density and variance between treatments were similar to those from the OD$_{600}$ readings, but averages trended higher. This suggests that many duckweed-associated microbes are able to produce pigments, but that these pigment-producing microbes do not on average have more or less local adaptation than non-pigment-producing microbes.

In this study, we sampled two pairs of sites that were within the same watershed: Mill and Upper Mill, and Durham Reservoir and Woodman. Watersheds are defined as areas of land that collect precipitation and drain to a common body of water (McGinnis, 1999). The movement of water down a watershed contributes to the transport of sediment, organisms, and nutrients (Najafi et al., 2021; McKay et al., 2017). Various studies have found that sites within the same watershed share some commonality in their microbiomes (URycki et al., 2022; Comte et al., 2018). Because of this, the microbiomes sampled from the same watershed may be more similar to each other than those from different watersheds. If so, we would predict that microbiomes from the same watershed, but not the same site, may benefit duckweeds more than microbiomes from different
watersheds. Re-analyzing our data with this fact in mind could provide further insight into this theory.

**Conclusions:**

Overall, we found higher fitness in duckweeds grown in both their local water and microbes as opposed to those grown in one or no local sources. We also found that microbes had non-significantly higher fitness when grown with their local water and a non-local plant. This study was one of the first in duckweed to skip the culturing step and instead took and used microbes directly from the source environment. The elimination of this culturing step could improve our understanding of the benefits of local microbes to the host. For example, this research has expanded the knowledge surrounding the importance of local adaptation in inocula development and suggests that future experimentation could generate better microbial treatments by incorporating more local microbes.
REFERENCES


