Use and production of miniature landscapes to study species distributions

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Abstract

Species geographic distributions combine to create past, present, and future ecosystems. The boundaries of these distributions are created by ecological, evolutionary, and biogeographical forces operating on diverse spatial and temporal scales. Understanding how these forces shape distributions has long been a goal of ecology, a goal which is becoming more pressing in our rapidly changing world. However, geographic distributions are challenging to study owing to issues of space, time, and replication. An increasingly popular approach to studying species distributions are small, replicable experimental arenas ('micro-landscapes') which circumvent many of the issues facing field studies. Micro-landscapes, while promising, have only been applied to a limited range of questions regarding species distributions, possibly due in part to design limitations. One potential source of novel designs is 3D printing, but its practicality for bioresearch remains uncertain. In this thesis, we first review the growing use of micro-landscapes to test biogeographic theory, then explore 3D printing as an intriguing source of novel landscape designs. Our review identified areas where micro-landscapes have been well used (e.g. studying range expansions) and new areas where they could be applied (e.g. range contractions), as well as some of the challenges inherent in studying ecology in these simplified systems. Our tests of 3D printing showed that printed items are difficult to sterilize, such that non-autoclavable plastics are likely poor choices for any work which requires sterility. Autoclavable plastics have cost and production limitations which likely restricts them to a narrow range of applications, at least for the time being. Despite these limitations, 3D printing may still be useful for certain micro-landscape applications. Micro-landscapes thus represent an exciting interdisciplinary platform which enables innovative tests of theory and equipment design, making them fertile grounds for collaboration between the field and the laboratory.

Resumé

Les distributions géographiques des espèces se combinent pour créer des écosystèmes passés, présents et futurs. Les limites de ces distributions sont créées par des forces écologiques, évolutives et biogéographiques opérant à diverses échelles spatiales et temporelles. Comprendre comment ces forces façonnent les distributions est depuis longtemps un objectif de l'écologie, un objectif qui devient de plus en plus pressant dans notre monde en évolution rapide. Cependant, les distributions géographiques sont difficiles à étudier en raison de problèmes d'espace, de temps et de réplication. Une approche de plus en plus populaire pour étudier la répartition des espèces consiste en de petites arènes expérimentales reproductibles («micro-paysages») qui contournent nombre des problèmes auxquels sont confrontées les études de terrain. Les micro-paysages, bien que prometteurs, n'ont été appliqués qu'à une gamme limitée de questions concernant la répartition des espèces, peut-être en partie en raison des limites de la conception. L'impression 3D est une source potentielle de nouvelles conceptions, mais son caractère pratique pour la recherche biologique reste incertain. Dans cette thèse, nous passons d'abord en revue l'utilisation croissante des micro-paysages pour tester la théorie biogéographique, puis nous explorons l'impression 3D en tant que source intrigante de nouveaux designs de paysages. Notre examen a identifié des domaines dans lesquels les micro-paysages ont été bien utilisés (par exemple, l'étude de l'expansion des aires de répartition) et de nouveaux domaines où ils pourraient être appliqués (par exemple, les contractions des aires de répartition), ainsi que certains des défis inhérents à l'étude de l'écologie dans ces systèmes simplifiés. Nos tests d'impression 3D ont montré que les articles imprimés sont difficiles à stériliser, de sorte que les plastiques non autoclavables sont probablement de mauvais choix pour tout travail nécessitant une stérilité. Les plastiques autoclavables ont des limitations de coût et de production qui les restreignent probablement à une gamme étroite d'applications, du moins pour le moment. Malgré ces limitations, l'impression 3D peut toujours être utile pour certaines applications de micro-paysage. Les micro-paysages représentent ainsi une plate-forme interdisciplinaire passionnante qui permet des tests innovants de théorie et de conception d'équipements, ce qui en fait un terrain fertile pour la collaboration entre le terrain et le laboratoire.

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This research truly was an adventure across far-flung fields ranging from glass blowing to macroecology, and I'm lucky to have had Dr. Anna Hargreaves as my supervisor throughout it all. I am thankful for her patience and thoughtfulness; she always strived to account for my changing goals and wellbeing. I'm most grateful for the lessons she taught me which transcend a thesis or a degree, namely, how to write, manage my time, and advocate for my needs.

This project was always going to be a wild ride as I was scaling up from DNA and Anna was scaling down from mountains. Luckily, we had committee members Dr. Graham Bell and Dr. Susan Bailey to meet us in the middle, and I am grateful for their willingness to help me troubleshoot the growing pains of establishing a new lab system. I appreciate Dr. Bell letting me use his lab space and glassware as we got ours set up, and Dr. Bailey Skyping in to help me tackle some particularly vexing contamination. I would also like to thank Kris Krebs from the Bell Lab for teaching me the lab skills I would use for the rest of my thesis.

My work would have been impossible without the wonderful support of my lab mates. At least half of the ideas in this thesis were verbally run by Shannon Meadley Dunphy, who was always willing to hear me out no matter how zany the proposal. Ella Martin added some much-needed equanimity, helping me put everything in perspective when I started to feel overwhelmed. I want to especially thank Josh Persi for his constant support and near-daily check-ins; his confidence in me was always appreciated and made my time at McGill a bit easier and a lot more fun.

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Preface & Author Contributions

All tables and figures will be presented at the end of each chapter, with the exception of Table 1 in Chapter 2 which is imbedded in the text.

This thesis is presented in manuscript format.

Chapter 1: Cole Larsen performed the literature search, identified and extracted information from the reviewed papers, and organized them into conceptual blocks for the manuscript. Cole Larsen and Dr. Anna Hargreaves worked together on the outline and final manuscript.

Chapter 2: Cole Larsen designed the study, performed all experiments, and wrote the manuscript, all with input from Dr. Anna Hargreaves.

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Introduction

Species geographic distributions and the ecosystems they create are the products of complex feedbacks between ecology, evolution, and biogeography (Sexton et al. 2009). How these forces interact to structure and change distributions has long been a key focus of biological theory, macroecology, and field studies (Merriam 1894, Clausen et al. 1940, MacArthur 1984). However, the large spatial and temporal scales over which distributions occur and change makes it challenging to directly study processes of interest. As such, many key theories and predictions remain untested, or undertested, in the field. Filling these knowledge gaps becomes increasingly pressing in the face of global environmental change as many species distributions will be under increased pressure from climate change, habitat modification, and species invasions (McCarty 2001, Massot et al. 2008, Freeman et al. 2018). Testing relevant theory thus requires creative solutions to circumvent the inherent limitations of studying natural systems.

Species distributions and landscape-level processes such as dispersal are difficult to study in the field owing to issues of space, time, and replication. While field experiments are unquestionably important because of their ability to directly observe species in situ, these limitations often restrict the scope of studies. The vast distance over which geographic dynamics occur makes it challenging to ever characterize more than a local snapshot (Brown 1995). Furthermore, this snapshot only captures a brief moment in time; many processes play out over long ecological or evolutionary timescales and are thus experimentally intractable regardless of spatial scale. As such, most field studies of species distributions occur over a limited area and a few field seasons (often less than one generation of the organism in question), making it challenging to determine if observed trends hold across space and time (Hargreaves et al. 2014, Pironon et al. 2017). These spatial and temporal limitations often lead to low replication, power,

and reproducibility, even for the most ambitious field studies (Sexton et al. 2009, Lemoine et al. 2016).

Laboratory systems have long been used to circumvent many of the issues facing ecological and evolutionary field studies (Jessup et al. 2004, O'Malley et al. 2015). The small size, quick generation times, and often tractable genetics of laboratory organisms directly address many of field ecology's key limitations. Historically, lab systems have been especially attractive for studying community ecology because communities can be precisely constructed and interactions of interest easily isolated, and real-time evolution because small organisms reproduce rapidly (e.g. bacteria, protists; Holyoak and Lawler 2005, Jessup et al. 2005, Altermatt et al. 2015). Often these studies occur in extremely streamlined environments (e.g. wells in a plate or test tubes; Kassen and Bell 1998, Barrett et al. 2005), which can be built upon to increase spatial complexity if desired (e.g. stratified habitat [Rainey and Travisano 1998, Tan et al. 2017], linked patches [Holyoak and Lawler 1996, Tekwa et al. 2015]). Recently, this focus on spatial dynamics and landscape complexity has led to microcosm and mesocosm studies specifically designed to test theory regarding species distributions and the forces which shape them, such as landscape structure and dispersal (hereafter referred to as "micro-landscapes").

Micro-landscapes are a rapidly growing approach to studying the processes which influence species distributions. The systems are small, replicable arenas which can isolate key landscape-level processes or properties on convenient scales, such as range expansions, habitat fragmentation, and environmental gradients (reviewed in Chapter 1; Larsen and Hargreaves 2020). These systems, when paired with fast-reproducing organisms, allow for multi-generational manipulative eco-evo studies otherwise impossible at larger spatial scales. Thus, micro-landscapes are a useful middle ground between theory and field studies, making them an

exciting platform for multidisciplinary collaboration between ecologists and laboratory-based researchers. However, these two worlds do not always overlap organically. Presenting micro-landscape findings and discussing their potential in a way accessible to researchers with diverse backgrounds is an important step forward in increasing discourse between the laboratory and the field. As such, we reviewed the micro-landscape literature to showcase how micro-landscapes contribute to theory regarding species distributions and provide a buffet of ideas for researchers interested in using micro-landscapes, regardless of their research backgrounds.

Despite their rapidly growing diversity, the majority of micro-landscape studies explore a few core theories and iterate a few core designs (e.g. Altermatt et al. 2015). This focus is probably because the simplest and most modular micro-landscapes are best suited for testing certain theories (e.g. simple expansion/metapopulation dynamics; Warren 1996, Fronhofer and Altermatt 2015). There are many theories and dynamics ripe for testing simply awaiting the appropriate experimental design. Several pioneering studies have shown the potential for ground-up customization but have used systems which are difficult to replicate (e.g. very large; Baym et al. 2016), or present limited room for extensive modification (e.g. can only change connectivity; Kurkjian 2018). As such, increasing design accessibility and flexibility could expand the scope of micro-landscape studies by making novel designs easier to produce, share, and iterate.

A potential source of novel micro-landscapes, and custom lab equipment in general, is 3D printing. 3D-printing offers unprecedented levels of customization and is increasingly accessible and affordable (Ngo et al. 2018). Few studies have used 3D printing to produce laboratory equipment (but see Kadilak et al. 2017), leaving open many important questions such as item sterility and durability. Pioneering studies have tested initial sterility, but we have found none to date which have tested whether items can be repeatedly sterilized (Neches et al. 2016,

Guerra et al. 2018). Sterility is important for labware as many model systems (e.g. bacteria, protists) are susceptible to contamination which can thwart entire projects. Sterility is particularly important for micro-landscapes used for multi-generational studies, as they are acutely vulnerable to contamination (e.g. items are reused, kept "active" for long periods of time, or are periodically disturbed by adding nutrients). Sterilization should also be repeatable to maximize the lifespan of an item. However, not all plastics can be sterilized in the same way (e.g. some are autoclavable, some not), meaning a plastic's physical properties can directly influence its practicality. As such, we tested whether items printed from plastics which varied in cost and performance could be repeatedly sterilized, a key barrier to clear before printed items are considered for more complex projects.

The ultimate goal of my investigations in micro-landscape uses and development was to make a new system to study dispersal evolution. The most promising version of this project was to study range expansions using the nematode *Caenorhabditis elegans*. I would build upon early *C. elegans* micro-landscape studies (Friedenberg 2003a, b) to create a system capable of simulating range expansions across varying types of habitat (e.g. fragmented, varying resource availability). Landscape-level processes like range expansions and features like fragmentation and resource availability are some of the most difficult to control for in the field, making them attractive targets for micro-landscapes. These properties are tractable at the micro-landscape level, and it is surprising few studies have looked at their interacting effects. *C. elegans* is a promising organism for this new system because it has well-characterized foraging and dispersal behavior and a diverse range of dispersal phenotypes (e.g. Gray et al. 2005, Pradhan et al. 2019); it is also small, quick reproducing, and feeds on resources easy to control spatially (e.g. bacteria slurry). My relatively simple landscapes could then be iterated to test increasingly complex

theory, laying the foundation for a more extensive micro-landscape program. However, this project was put on hold indefinitely due to the global COVID-19 pandemic and 2020 research shutdown.

Chapter 1: Miniaturizing landscapes to understand species distributions

Cole Larsen & Anna Hargreaves
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Abstract

Species' geographic distributions shape global patterns of biodiversity and therefore have long been of interest to ecology and conservation. Theory has generated valuable hypotheses about how landscape structure, dispersal, biotic interactions and evolution shape range dynamics, but most predictions have not been tested on real organisms because key variables are difficult to isolate, replicate, or manipulate in natural ecosystems. An exciting and rapidly emerging approach is to extend classical microcosm and mesocosm systems to create experimental 'micro-landscapes'. By enabling researchers to manipulate geographic features of interest, replicate landscapes, control colonization, and follow dynamics across evolutionary timescales, micro-landscapes allow explicit tests of the ecological and evolutionary underpinnings of species distributions. Here we review the micro-landscape systems being used to advance biogeography, the major insights they have generated thus far, and the features that limit their application to some scenarios. We end by highlighting important questions about species' biogeography that are ripe for testing with experimental micro-landscapes, particularly those of immediate concern given rapid global change, such as range contractions and constraints to range expansion.

Introduction

Species' geographic distributions combine to form the ecosystems we see today, and their stability through time will govern the future distribution of global biodiversity, including pests and invasive species. Understanding the ecological, demographic, and evolutionary processes that shape species distributions are therefore fundamental goals of ecological, evolutionary and conservation biology (Sexton et al. 2009), with a resurgence of interest as native and exotic species expand, shrink, or shift their ranges in response to anthropogenic change (Massot et al. 2008, Freeman et al. 2018). Decades of theory (Janzen 1967, MacArthur 1972), macroecology (Merriam 1894), and field experiments (Clausen et al. 1940) have made great progress in understanding the ecological and evolutionary determinants of range edges. Nevertheless, many key predictions have proven difficult to test in natural settings.

An obvious challenge to testing theory in nature is the spatial scale over which range dynamics play out. Models predict that for a given species, the landscape arrangement of habitat quality and patchiness govern occupancy and therefore the species' distribution (Holt and Keitt 2000, Holt et al. 2005). Empirical tests have confirmed some core predictions, for example that declining habitat quality is globally important in determining high-elevation range edges (Körner and Paulsen 2004, Halbritter et al. 2013, Hargreaves et al. 2014). But even ambitious field experiments rarely include replicate range edges, and biologists seldom have independently varying landscape configurations with which to test their effects. Not surprisingly, predictions involving landscape-scale attributes, e.g. habitat patchiness (Pachepsky and Levine 2011), or range edges that are hard to delineate in the field, e.g. highly fragmented edges (Holt and Keitt 2000), remain poorly tested in natural systems.

A second challenge is the long timescale over which landscapes and evolution shape species distributions. Models predict that temporal processes strongly influence range dynamics,

but aside from rare exceptions where processes are observed in real time, biologists must infer past processes from present signatures. For example, theory predicts that increasing habitat turnover can limit species' ranges ('metapopulation range limits'; Holt and Keitt 2000; see Glossary). Yet empirical examples of turnover-driven range limits (e.g. Sjögren 1991) remain rare due to the difficulty of tracking occupancy long enough to detect colonization and extinction events. Thus meta-population dynamics at range edges are generally inferred from population traits (Darling et al. 2008) or structure (Bay et al. 2008). Evolution is particularly difficult to observe at natural range edges. For example, models show that local adaptation at range-edges can promote range expansion, but can be easily thwarted by too much or too little gene flow from core populations (Alleaume-Benharira et al. 2006). Case studies confirm adaptation can lead to range expansion (e.g. Pateman et al. 2012), but genetic differences across species ranges are often small and the prevalence of adaptation at range edges remains unclear (Eckert et al. 2008).

A third, more specific challenge is the role of dispersal. Dispersal is critical for propelling range expansions and sustaining ranges across fragmented or patchy environments (Sjögren 1991, Phillips et al. 2006). Further, dispersal can evolve quickly in response to range expansion or fragmentation (Cheptou et al. 2017), and models predict complex feedbacks between dispersal evolution and range dynamics (Travis and Dytham 1999, Shine et al. 2011). Empirical studies confirm that fragmentation selects against dispersal (Cheptou et al. 2017) while range expansion selects for dispersal (Phillips et al. 2006), but most studies are space-for-time substitutions assessing dispersal traits. The shape of dispersal kernels, frequency of long-distance dispersal, and dispersal evolution are key parameters in expansion models (Travis 2009, Gilbert et al. 2017) and notoriously difficult to quantify in nature (Wang and Smith 2002).

One way to tackle biogeographic questions that are hard to test in natural systems is to miniaturize landscapes using small 'model' organisms (Fig. 1). Microcosms and mesocosms have been used for more than a century to test ecological and evolutionary theory (Jessup et al. 2005), but have only been extended relatively recently into 'micro-landscapes' that test theory about species' geographic distributions (Fig. 2). We use 'micro-landscape' to refer to experimental arenas created to explicitly test processes operating at geographic scales, where habitat can be moved or manipulated between replicate experiments and organisms occupy the landscape for multiple generations (Table 1); we apply this term for our review rather than as a formal definition. Two-dimensional systems best simulate natural landscapes, but we also consider linear and 2-patch systems, consistent with influential models of species range limits (e.g. Kirkpatrick and Barton 1997, Gomulkiewicz et al. 1999). We argue that micro-landscape experiments are a powerful tool that can bridge theory and nature, enabling controlled and targeted tests of model predictions with living organisms (Srivastava et al. 2004, Jessup et al. 2004, Benton et al. 2007, O'Malley et al. 2015).

Here we review how experimental micro-landscapes have been used to test theory related to the proximate (ecological) or ultimate (evolutionary) limits to species distributions. We include direct simulations of range edges but also tests of landscape effects on dispersal evolution, local adaptation to low-quality habitat, meta-population dynamics (related to meta-population range limits), and genetic rescue (related to range contractions). As many processes bear on species distributions, applying these criteria involves some subjectivity. We include micro-landscape studies that test more general biogeographic hypotheses using species richness as the response (e.g. community examples in Table 2), but do not review community ecology studies *per se* as these have been reviewed elsewhere (Srivastava et al. 2004, Jessup et al. 2005,

O'Malley et al. 2015). We highlight micro-landscapes' potential to complement existing approaches (models, macroecology, field studies) by reducing the challenges of space and time. We describe the diversity of micro-landscapes in use, showcase some of their unique contributions to biogeography, and highlight areas where they could make substantial future advances.

Literature search

Glossary
Allee effect: Positive relationship between population size/density and performance at low densities, such that populations below a threshold density cannot maintain positive growth rates.
Expansion front: the leading edge of a range expansion
Gene-surfing: spatial spread and increase in frequency of alleles due to genetic drift at the edge of an expanding range, particularly of mutations that arise at expanding fronts
Genetic drift: change in allele frequencies due to random sampling between generations; effects are greater in small populations.
Habitat turnover: when habitat switches from suitable to unsuitable (and back) through time. Higher turnover results in more frequent population crashes and local extinctions.
Metapopulation range limits: range edge imposed by declining habitat availability (e.g. habitat patches become smaller, fewer, more isolated, or experience higher turnover), such that patch colonization does not keep pace with patch extinction. Can arise from increased habitat fragmentation or turnover without a decline in habitat quality *per se*.
Negative density-dependent dispersal: dispersal is more common at low population densities, e.g. to escape Allee effects.
Positive density-dependent dispersal: dispersal is more common at high population densities, e.g. to escape overcrowding.
Pulled expansion: expansion rate determined by growth rate at low-densities (with negligible Allee effects) and dispersal. Successive founder events at the expansion front can cause rapid loss of genetic diversity.
Pushed expansion: expansion rate determined by growth rates at all densities and dispersal. Requires population build up after dispersal events due to stronger Allee effects.
Spatial sorting: fast-dispersing individuals accumulate at expansion fronts (they arrive there first) and mate with each other (slow potential mates have been left behind), such that the next generation has even higher mean dispersal. Selects for increased dispersal at expanding edges even if dispersers do not have higher fitness than non-dispersers.

We searched for studies that used micro-landscapes to study species distributions. Our intention was not a comprehensive list of all such studies, but to explore the questions being tested and

systems in use. To this end, we searched Web of Science using a term with one part to identify relevant topics and two to identify micro-landscape approaches: (**TOPIC:** ("range limit*" or ("species" NEAR/3 "distribution*") or “range expansion” or (“range*” NEAR/3 “expanding”) or "biological invasion" or (“range” NEAR/3 “contract*”) or (“density” NEAR/3 “dependen*”) or metapopulation* or "environmental gradient*" or ("habitat" NEAR/3 "patch*") or ("landscape" NEAR/3 "fragment*") or ("landscape" NEAR/3 "patch*") or (“dispersal” NEAR/3 “evolution*”) or "habitat corridor*" or "habitat connectivity*" or ("spat*" NEAR/3 "structure*") or ("landscape" NEAR/3 "spat*")) *AND* **TOPIC:** (experiment* or laboratory or greenhouse) *AND* **TOPIC:** (microcosm* or mesocosm* or microlandscape* or petri* or "experimental landscape*" or “artificial metapopulation*” or bacteria* or *Escherichia* or microbial or protist* or protozoa* or ciliate* or yeast or *Saccharomyces* or *Arabidopsis* or beetle* or *Tribolium* or microarthropod* or *Caenorhabditis* or nematode*; final search date = Dec 2019). This yielded >2000 studies, of which ~60 were relevant. We then added studies from our personal libraries or recommended by colleagues, searched the references of papers from steps 1 and 2; and searched the papers citing particularly relevant studies, yielding ~10 more studies. In total, we found 72 studies published from 1980 to 2019, mostly from the past decade (Fig. 2).

Overview of micro-landscape systems

The central value of micro-landscape experiments is that researchers can recreate key axes of landscape variation and allow ecological processes (e.g. dispersal, competition) and/or evolutionary processes (e.g. selection, mutation, drift) to play out across them. Diverse designs enable researchers to isolate parameters that are difficult to standardize or manipulate across large, natural landscapes, such as habitat turnover, patchiness, connectivity and quality (Table 1). Populations can persist, grow, and potentially disperse or interact in controlled and repeatable

environments, while researchers track responses (e.g. population size, density, traits) across space and time. Micro-landscapes are generally orders of magnitude bigger than the organisms inhabiting them (Fig 3). As with any micro/mesocosm experiment, their simplicity is both their strength, enabling targeted tests of model predictions, and weakness, as they will always be extreme simplifications of natural landscapes (see *Challenges & Limitations* below).

Micro-landscapes themselves range from standard laboratory items to highly custom equipment (Fig 1). While landscape configurations are limitless, setups differ in three important ways. Suitable habitat can be continuous or patchy, and habitat quality can be uniform or variable (Table 1). As variation in habitat patchiness and quality are key parameters in most range-dynamic models (Sexton et al. 2009, Hargreaves and Eckert 2014), this enables targeted tests of theory. A third important feature is how dispersal occurs, either experimenter-mediated (e.g. transfers among wells in a 96-well plate) or organism-driven (e.g. free dispersal across continuous agar; Table 1). The former enables explicit tests of dispersal rates on eco-evolutionary processes (Bell and Gonzalez 2011), while the latter enables tests of dispersal evolution and its feedbacks (Weiss-Lehman et al. 2017). Designs are flexible and customizable, such that core designs can be modified to create many landscapes types.

Small organisms offer advantages beyond enabling the miniaturization of landscapes. Small size allows control over population size, genetic diversity, and species assemblages (Jessup et al. 2005, Altermatt et al. 2015). Multiple species can be combined to test interactions like competition and predation. One of the biggest advantages of small organisms is their rapid generation time. Evolution can occur in a few generations if starting populations have enough standing genetic variation in the trait of interest (e.g. Wiess-Lehman et al. 2017), or if evolution is measured as changing frequency of divergent clones (e.g. Friedenberg 2003b). For truly

'micro' organisms such as bacteria, *de novo* evolution can be observed on reasonable time scales (Baym et al. 2016). Many lab organisms have well-characterized genomes and established genetic tools, such that specific phenotypes can be created or labelled and evolution can be studied at the phenotypic and molecular scale.

Questions tested using micro-landscapes

Micro-landscape experiments have been used to explore many of the complex processes that shape how species occupy landscapes. We highlight their most important contributions to studying species distributions below, and provide more detail on individual studies in Table 2.

Range expansions into high-quality habitat (invasions)

Range expansions are central to biogeography, enabling newly evolved species to colonize suitable habitat, existing species to track their niche during climatic change, and exotic species to become invasive. Expansions depend on ecological and evolutionary processes (Chuang and Peterson 2016), making their dynamics complex. As it is relatively easy to create lab systems where organisms spread into new habitat for distances 1000-fold longer than their body size (Fig 3), range expansions have been particularly well-studied using micro-landscapes (Table 2a).

Predicting how ranges expand is important for managing invasions, but while observed expansions can be retroactively modelled it can be hard to disentangle the effects of stochasticity or test mechanisms (Chuang and Peterson 2016). By enabling replicated expansions of known genotypes, micro-landscapes provide controlled tests of expansion-model predictions (Table 2a.*i* & *ii*). Ciliate expansions illustrated the importance of estimating and modeling stochasticity; Fisher wave models predicted mean expansion speed across replicates, but incorporating demographic stochasticity better captured the dynamics of individual expansions (Giometto et al.

2014). Yeast studies demonstrated the utility of 'pulled' vs 'pushed' expansion models and the importance of low-density growth rates in distinguishing them (see Glossary). Expansions on media that favoured low-density growth were well-predicted by pulled models, whereas expansions on media with slower low-density growth were pushed (Gandhi et al. 2016). Finally, by carefully controlling immigration to new patches, insect studies have confirmed that colonization and subsequent expansion are strongly influenced by the size and genetic diversity of founder populations (Table 2a.*ii*).

One of the most consistent predictions of evolutionary expansion models is that expansion into uniformly good and empty habitat (e.g. invasions) should select for increased dispersal at the expansion front, accelerating expansion (reviewed in Hargreaves and Eckert 2014). Theory suggests increased dispersal at expansion fronts can evolve via spatial sorting of better dispersers to the front or if dispersal increases fitness, e.g. by escaping competition (Shine et al. 2011). Empirical support has come mostly from space-for-time substitutions or comparing dispersal traits of historic vs. modern specimens (Hargreaves and Eckert 2014, but see Phillips 2006), while mechanisms have remained elusive. A pioneering micro-landscape study found evolution of increased dispersal ability at an expanding bacterial front (Taylor and Buckling 2011), a result since confirmed in ciliates, beetles, and plants (Table 2a.*iii*). Moreover, micro-landscapes studies confirmed that increased dispersal can arise from *de novo* mutation (bacteria) or spatial sorting [plants (Williams et al. 2016), beetles (Ochocki and Miller 2017)] and depends strongly on landscape structure (Williams et al. 2016). Thus, micro-landscape studies not only corroborated field observations but illuminated mechanisms.

In addition to selection and spatial sorting, theory predicts stochastic processes such as drift and mutation influence expansion dynamics (Gilbert et al. 2017). In models, neutral or

deleterious alleles can increase in frequency at expansion fronts due to drift during successive founder events ('gene surfing'; Klopfstein et al. 2006) and alter expansion dynamics. Gene-surfing has been inferred using population genetics in natural, post-expansion populations, but micro-landscape experiments have shown it definitively for both neutral and deleterious alleles (Table 2a.*iv*). Micro-landscapes have also demonstrated the effects of mutation and drift at expanding fronts. Drift significantly reduced genetic diversity and increased genetic variance after a few generations of flour-beetle expansion (Weiss-Lehman et al. 2019). Gene-surfing can enable less competitive genotypes to persist at expanding fronts by 'outrunning' selective sweeps (Korolev et al. 2012), and allow harmful mutations to accumulate to the point where they collapse expansions (Song et al. 2016).

Micro-landscape studies have also shown how expansion dynamics can be affected by biotic interactions (Table 2a.*v*) Yeast experiments confirmed theory that the strength of Allee effects determine whether expansions are pushed or pulled (Gandhi et al. 2016). They also showed that cooperation can accelerate expansion, if cooperators accumulate at the expansion front to avoid defectors and then have higher fecundity as a result of cooperation (Datta et al. 2013, Van Dyken et al. 2013). Micro-landscapes have also helped validate theory (Case et al 2005) that expansion can stall if expanders run out of species or morphs they rely on (e.g. hosts, mutualists, mates), until the required partner expands or the dependent one evolves its way out of the interaction. Using two bacteria morphs engineered so one depended on the other for a key metabolite, researchers demonstrated that expansion of the reliant strain stalled until its partner expanded (Goldschmidt et al. 2017). Beetle expansions have shown how escaping a required interaction can accelerate expansion; female-biased dispersal yielded faster expansions as pregnant dispersers founded new populations without waiting for mates (Miller and Inouye

2013). To our knowledge micro-landscapes have not yet tested how species adapt their way out of an interaction on a more permanent basis, e.g. evolution of selfing.

Range dynamics along environmental gradients: adaptation

While expansion into good habitat is useful for testing many theoretical predictions, natural ranges are often limited by declining habitat quality (Hargreaves et al. 2014), such that range expansion requires niche expansion or amelioration of the limiting gradient. Much theory has been devoted to understanding when local adaptation to range-edge conditions will enable expansion into previously uninhabitable habitat (Antonovics 1976, Gomulkiewicz et al. 1999, Gilbert et al. 2017), but local adaptation at range edges and its role in range expansion are still rarely tested in nature (but see Pateman et al. 2012, Hargreaves et al. 2019). Creating an environmental gradient that temporarily limits expansion across an experimental landscape can be challenging; pioneering studies have done so in several systems (Table S2) but they await broad uptake. Recent micro-landscape studies using microbes have elegantly demonstrated such adaptation-expansion cycles, quantifying the 'wait times' between adaptations, genomic architecture of adaptation, and conditions (dispersal rate, gradient steepness) that facilitate adaptation along environmental gradients (Low-Décarie et al. 2015, Baym et al. 2016; Table 2b).

Adaptation to poor-quality, range-edge habitat can not only facilitate expansion into even more hostile habitat, but can rescue stationary populations from habitat deterioration. Genetic rescue at range edges is of wide conservation interest as species' warm range edges grow even hotter under climate change. A seminal experiment established a yeast range margin across a gradient of increasing salinity, then increased the salinity of the entire gradient for several generations with varying dispersal levels (Bell and Gonzalez 2011). The initial gradient confirmed long-standing predictions (Antonovics 1976) that intermediate levels of gene flow

should maximize local adaptation at range edges (Table 2b). Genetic rescue from habitat degradation at range edges required dispersal and was facilitated by prior adaptation (Bell and Gonzalez 2011, Low-Décarie et al. 2015), important empirical demonstration that range edges can be reservoirs of adaptive diversity to counter rapid environmental change (Hampe and Petit 2005).

Range dynamics along environmental gradients: species interactions

Biologists have long deduced that antagonistic interactions (e.g. competition, parasitism) affect species' ranges (Darwin 1859), and models show that biotic interactions can create range edges with or without abiotic gradients (Case et al 2005). While field experiments confirm the role of interactions in particular cases (Brown and Vellend 2014, Alexander et al 2017), biotic and abiotic factors are hard to manipulate independently and their relative importance in limiting species' ranges is still debated. Micro-landscapes have not been used extensively to test how interactions limit ranges (Table 2c), but a seminal study shows their potential power. Three fruit fly species were exposed to a temperature gradient either as single species without the ability to disperse among temperatures (measuring their innate thermal tolerance), as single species with dispersal allowed, and as an assemblage of competing and dispersing species (Davis et al 1998). Dispersal without competition extended fly ranges beyond their innate temperature tolerance by maintaining sink populations, while competition excluded species from areas they could tolerate climatically, significantly limiting their distributions (Davis et al 1998).

Dynamics within patchy ranges: effects of landscape structure & dispersal

Landscape structure and dispersal affect recolonization and population stability, with implications for meta-population range limits, range expansion through fragmented habitat, and range contraction via increasing patch isolation or turnover (Fahrig and Merriam 1994, Howell et

al. 2018). However, such effects are especially challenging to study in nature due to low replication of landscape configurations, difficulty tracking dispersal, and difficulty disentangling general principles from biogeographic accidents–constraints that can be overcome using micro-landscape experiments (Table 2d).

Micro-landscapes have been used to test how habitat configuration influences colonization (Table 2d.*i*). They have confirmed core predictions of island biogeography theory, that population density and species richness are generally lower on 'island' vs. 'mainland' habitat and decline with increasing distance from the mainland (Henebry & Cairns 1980). Similarly, in patchy habitat greater distance among patches makes dispersal positively density dependent and slows colonization (Williams and Levine 2018; Fig. 1), as predicted by theory (Pachepsky and Levine 2011). Micro-landscapes have also tested theory regarding linear habitat, confirming that branching habitat (e.g. rivers) is more quickly colonized than unbranched habitat and maintains greater diversity within and among communities (Table 2d.*i*).

Micro-landscape experiments have provided some of the strongest tests of how dispersal mitigates habitat fragmentation, relevant to species whose range edges coincide with increasingly fragmented habitat. Using natural dispersal variation among species or by manipulating dispersal rates, micro-landscape experiments have generally supported theory (Fahrig and Merriam 1994) that higher dispersal rates reduce local extinctions and buffer population densities (Table 2d.*ii*). Micro-landscapes have validated conservation theory that dispersal corridors among remaining patches mitigate species loss after fragmentation, also relevant to species undergoing range contraction via fragmentation (Table 2d.*ii*). They can also test the effect of corridor geometry, yielding sometimes non-intuitive results, including unpredictable variation among configurations

(Chisholm et al. 2011) and that high dispersal can hasten metapopulation extinction if it synchronizes population dynamics, and therefore local extinction, across patches (Burkey 1997).

Landscape structure and dispersal are not independent over evolutionary timescales; landscapes select on dispersal and increases or decreases in dispersal feed back to affect perceived landscape structure (e.g. fragmentation; Cheptou et al. 2017). Whereas several micro-landscape studies have examined dispersal evolution at expanding range edges (Table 2a), few have tested dispersal evolution within ranges. Notable exceptions confirmed predictions from evolutionary models (reviewed in Hargreaves and Eckert 2014) that increasing patch isolation selects against dispersal, whereas increased turnover selects for increased dispersal as a form of bet hedging (Table 2d.*iii*), a phenomenon still rarely shown in nature. Incorporating dispersal evolution into experiments on landscape connectivity is an obvious and exciting avenue for future work.

Conditional dispersal in equilibrium and non-equilibrium ranges

While inherent dispersal propensity and ability are fundamental to much of the theory described above, individual dispersal is often influenced by external factors both demographic (e.g. population density) and environmental (e.g. matrix inhospitability). These cues can be challenging to manipulate in the field, making micro-landscapes an attractive option for testing conditional dispersal (Table 2d.*iv*).

The most commonly modelled influence on dispersal is density-dependence (Hargreaves and Eckert 2014). While the nature of density dependence (negative, neutral, positive; see Glossary) can strongly affect connectivity and range dynamics, empirical evidence is ambiguous, showing all types of dependence in diverse taxa (Fronhofer et al. 2015b). By manipulating dispersal cues (e.g. perceived density via chemical cues) independently of demography (e.g.

actual density) and landscape structure, micro-landscape experiments have shown that density dependent dispersal can be U-shaped, with the greatest dispersal at the lowest and highest densities to escape Allee effects and overcrowding respectively (Fronhofer et al. 2016). Further, micro-landscapes have shown that landscape structure can alter how strongly density-dependent dispersal is (de Roissart et al. 2015), and confirmed theory (Travis et al. 2009) that range expansion can promote evolution of negative density dependent dispersal at range edges (Fronhofer et al. 2017a).

For organisms that disperse actively, dispersal decisions can be influenced by information about the landscape, and micro-landscapes offer a unique ability to manipulate this information independently of the landscape itself. For example, theory suggests that species range-edges can be stabilized if dispersal is selected against in range-edge populations (Hargreaves and Eckert 2014). Ciliates expanding along a mortality gradient formed a stable range limit when they were allowed to perceive chemical cues of dead conspecifics from high-mortality patches, and this information countered otherwise strong selection for increased dispersal at the expanding edge (Fronhofer et al. 2017b). While micro-landscapes use relatively simple organisms, conditional dispersal can nevertheless be relatively sophisticated. For example, ciliate immigrants provided information about their original patch and the inter-patch matrix to residents of their new patch, influencing the residents' dispersal decisions (Jacob et al. 2015).

Challenges and Limitations

Micro-landscapes excel at testing theoretical predictions but are limited proxies for complex ecosystems and biogeographic processes. They can test for general principles but not what has happened or mattered most in nature, and the challenges in extrapolating from micro/mescocosm experiments grow with differences between the model and the desired scale of prediction. We

advocate for their use not in place of field experiments or macroecology, but as a complimentary approach. Even in this context, micro-landscape approaches may be less suited for certain applications. General criticisms of micro/mesocosm experiments, including overly simple setups and organisms, and fundamental differences between micro- and macro biology (e.g. horizontal gene transfer), have been reviewed extensively elsewhere (see Srivastava et al 2004, Jessup et al. 2005, Cadotte et al. 2005, Benton et al 2007); below we highlight challenges specific to questions about species distributions. Constraints vary among systems, so optimizing micro-landscape experiments requires thoughtful selection of organisms and landscape design.

Large populations. Lab populations can rapidly reach high densities, essentially skipping the small, vulnerable population sizes that shape the evolutionary ecology of newly founded, recently disturbed, or range-edge populations. The 'problem' of large populations is particularly acute for asexual organisms that can colonize habitat without mates (all but the insect systems in Table 2 reproduced asexually during experiments). Asexual reproduction can limit how alleles move through populations, can make studying mating system effects impractical, and blurs the line between phenotype and species (e.g. is increased frequency of one clone akin to evolution or competitive exclusion?). Thus the organisms best suited for experimental evolution by *de novo* mutation (due to rapid generation times and large populations under typical experimental conditions) may be the least suited to testing theory involving small populations. Evolutionary micro-landscape studies on larger organisms generally quantify selection on standing variation (beetles) or change in frequency among clonal phenotypes (nematodes, plants; Table 2).

Scale. The issue of scale in micro/mescocosm experiments has been widely discussed (e.g. Jessup 2005, Srivastava et al 2004), but is particularly relevant to micro-landscapes. Researchers must consider how to vary habitat size, gaps, and quality, relative to other landscape features (e.g. size vs separation of habitat patches) and to the model organisms' body size and dispersal ability (Fig. 3). Scaling also applies temporally (e.g. adaptation to a deteriorating environment over hundreds of generations may not be relevant to the persistence of populations at the warm edge of a species range if they face dramatic climate warming within a generation). If the appropriate scale is unclear (e.g. the frequency of long-distance dispersal in nature is rarely known), testing multiple levels of a variable of interest may be most powerful (e.g. dispersal rate Bell & Gonzalez 2011, antagonist diversity Davis et al 1998). Given the extensive natural variation in habitat configuration, organism size, and dispersal ability, many experimental designs will have an analogue in nature; the challenge is to choose appropriate scaling for the question at hand and to justify such choices explicitly (e.g. Hufbauer et al 2015).

Replenishing resources. A third challenge is resupplying resources that would be naturally replenished by photosynthesis, reproduction, or decay. While it is easy to add new habitat, it is difficult to replenish stable habitat without disturbance, particularly on solid media (we describe replenishment options in Table S1). Replenishment often adds an ecological phase (e.g. growth from low population size, dispersal/colonization) that may or may not mimic dynamics in the system of interest. For example, organisms in liquid media are often transferred to fresh media at low dilutions, adding sequential bottleneck/growth phases that would be less prevalent in stable habitat patches. Long-term dynamics in stable, structured habitat are perhaps the hardest to replicate using micro-landscapes, no doubt explaining why expansions into fresh habitat or

metapopulation dynamics that require continuous colonization of fresh patches have received disproportionate attention in micro-landscape studies (Table 2a). 'Natural' or field-based micro-landscapes (e.g. moss patches) can help solve this problem (Srivastava et al 2004), though trade increased realism for some degree of experimental control.

Future Directions

The questions that have been neglected by micro-landscape studies are often questions that have been poorly studied in general. A prime example is range contraction. Contracting limits are increasingly common given rapid global change (Hampe and Petit 2005), but their eco-evolutionary dynamics have been remarkably understudied in models or nature (reviewed in Hargreaves and Eckert 2014, Hargreaves et al. 2015) and we found only one micro-landscape experiment (Bell and Gonzalez 2011). Contraction can be simulated relatively easily by worsening an environmental gradient (e.g. salinity: Bell and Gonzalez 2011) or directly altering organism fitness (e.g. manually increasing mortality). Micro-landscape studies of contraction would be particularly helpful as contracting limits are hard to pinpoint (Hampe and Petit 2005) and ethically questionable to create in natural systems.

Similarly, while expansions into uniform habitat have been well-studied (Table 2a), expansion into along environmental gradients has not (Table 2b). The first scenario mimics the early phase of invasions driven by colonization alone, whereas the latter is akin to shifts of cold-induced range edges under climate warming. Climate-driven range shifts have been widely documented but have also varied greatly in direction and magnitude (Freeman et al 2018). Better tests of spread along gradients, including how expansion into poor-quality habitat at leading edges, local adaptation throughout the range, and contraction at trailing edges select on range-wide dispersal are needed to untangle this variation.

The role of biotic interactions in promoting or constraining local adaptation and range expansion has not been well studied in models (Case et al. 2005), nature (Hargreaves et al. 2020), or micro-landscapes. We found only one study that directly examined range limits set by antagonistic interactions (Davis et al 1998), and none of expanding populations encountering resident competing species, even though such interactions can strongly affect climate change-driven range shifts (Alexander et al. 2017). Micro-landscapes have also not yet tested when species will be able to adapt their way out of required interactions (e.g. evolution of selfing or host switching) to colonize new habitat, but microcosms used to study niche expansion (e.g. Bono et al 2019) could be extended for this purpose.

A fourth area ripe for exploration is matrix habitat. Micro-landscapes generally linked habitat patches via corridors of habitat (growth and reproduction possible) or neutral space (growth not possible but risk also minimal, e.g. foodless habitat), rather than a matrix of inhospitable space that incurs risk or damage. In many natural systems dispersers must cross inhospitable habitat with increased mortality risk or dispersal cost to reach new patches. Further, dispersal routes were often defined and local (e.g. corridors between adjacent patches). Embedding patches in inhospitable matrix habitat (e.g. Gonzalez et al. 1998) would allow dispersers to move to non-adjacent patches and experimenters to manipulate dispersal cost, allowing more complex dispersal dynamics to emerge within patchy landscapes.

Finally, micro-landscapes will be particularly powerful when paired with other approaches (and vice versa). For example, by directly testing model predictions, micro-landscapes have helped parameterize, refine, and validate or refute paired models (e.g. Giometto et al. 2014; Fronhofer et al. 2017b, Dallas et al. 2019). We did not find any studies that paired field and micro-landscape studies, but this could help refine hypotheses about the processes

underlying specific distributions. For example, researchers could quantify habitat patchiness and quality in the field, create an analogous micro-landscape, and test the level of long-distance dispersal (rarely traceable in nature) needed to produce the observed pattern of patch occupancy. Paired with macroecology, theory, and field studies, micro-landscapes could be an integral part of the toolkit biologists use to refine our understanding of global biogeography.

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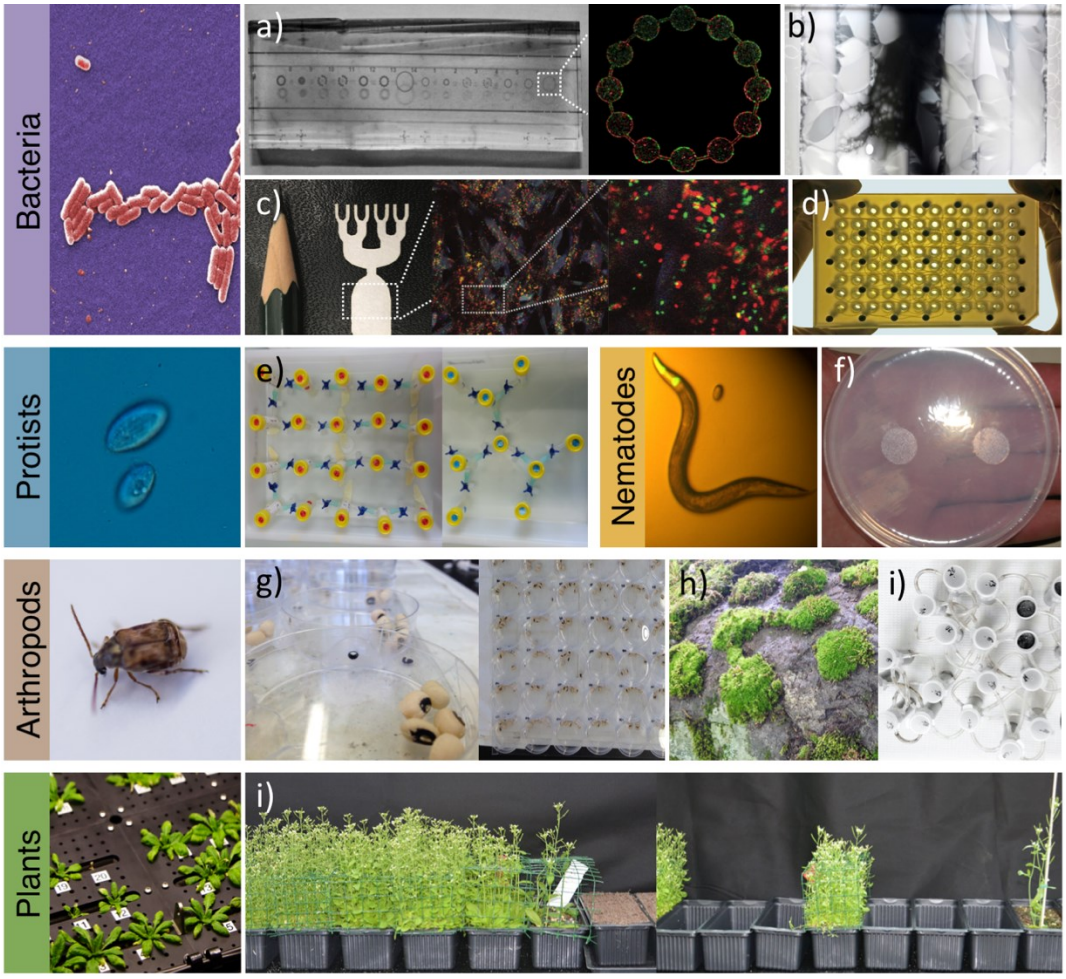

Figure 1: Examples of micro-landscape systems. Bacteria: (a) microfluidic landscapes etched onto glass microscope slide seen in normal light (left) and under fluorescent microscope (right); (b) Microbial Evolution and Growth Arena (MEGA; top-down view) 4 x 2' agar plate; (c) paper branching (dendritic) landscape (shown at increasing magnification from left to right); (d) 96 well plate with some wells connected by corridors. Protists: (e) plastic centrifuge tubes of liquid media linked with tubing. Nematodes: (f) habitat patches of food on neutral agar matrix. Arthropods: (g) Petri dish landscape with adjacent dishes connected by drilled holes (visible in centre of close-up photo on left), (h) patchy moss habitats, (i) modular patch network (vials). Plants: (j) planting trays with soil arranged as continuous (left) or patchy (right) habitat. Photo credits. Organisms: E. coli: Centre for Disease Control; T. pyriformis: F. Altermatt; C. elegans: N. Friedenberg; C. maculatus: J. Fitlow; micro-arthropods: A. Gonzalez & Z. Lindo; A. thaliana: NASA. Micro-landscapes: (a) E. Tekwa; (b) M. Baym; (c) F. Hol; (d) H. Kurkjian; (e) F. Altermatt; (f) N. Friedenberg; (g) B. Ochocki; (h, i) A. Gonzalez; (j) J. Levine.

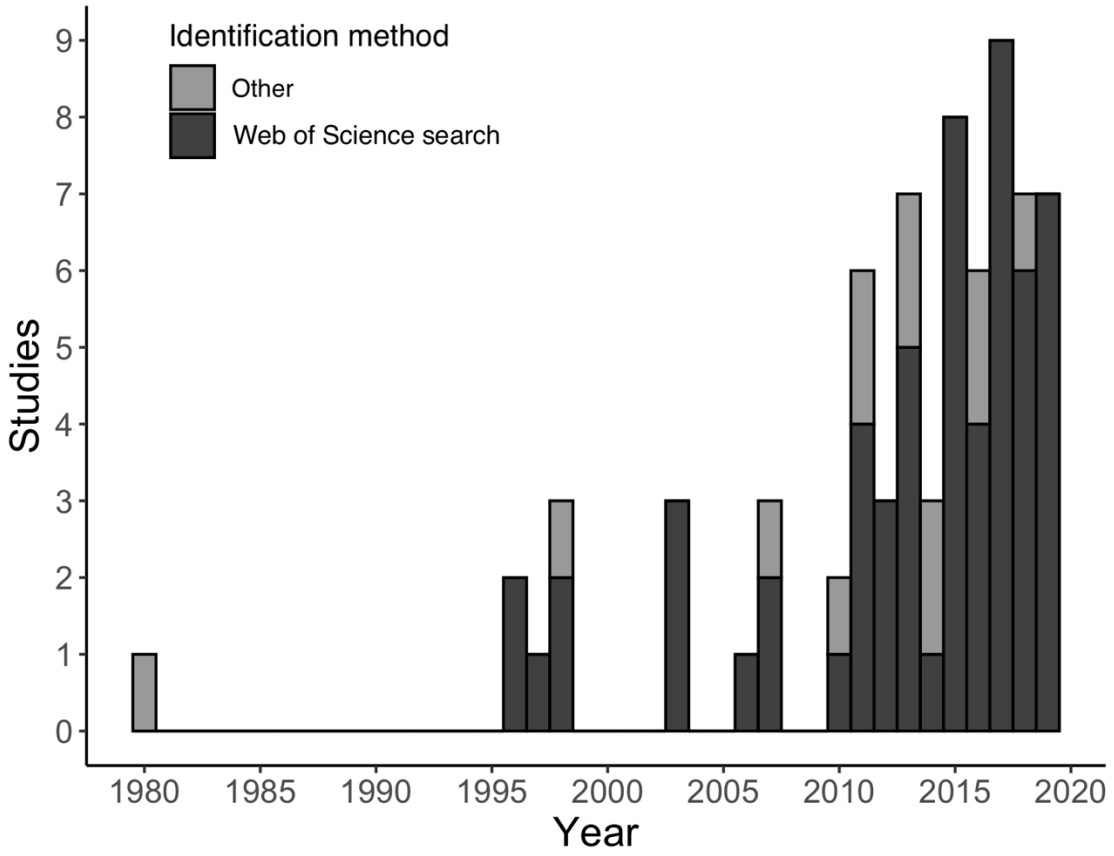

Figure 2: Publishing timeline of micro-landscape experiments used to test the ecological and evolutionary processes that shape species distributions. Figure shows the 72 such studies found using a Web of Science search (term given in text), and from recommendations, citations of or references from relevant studies.

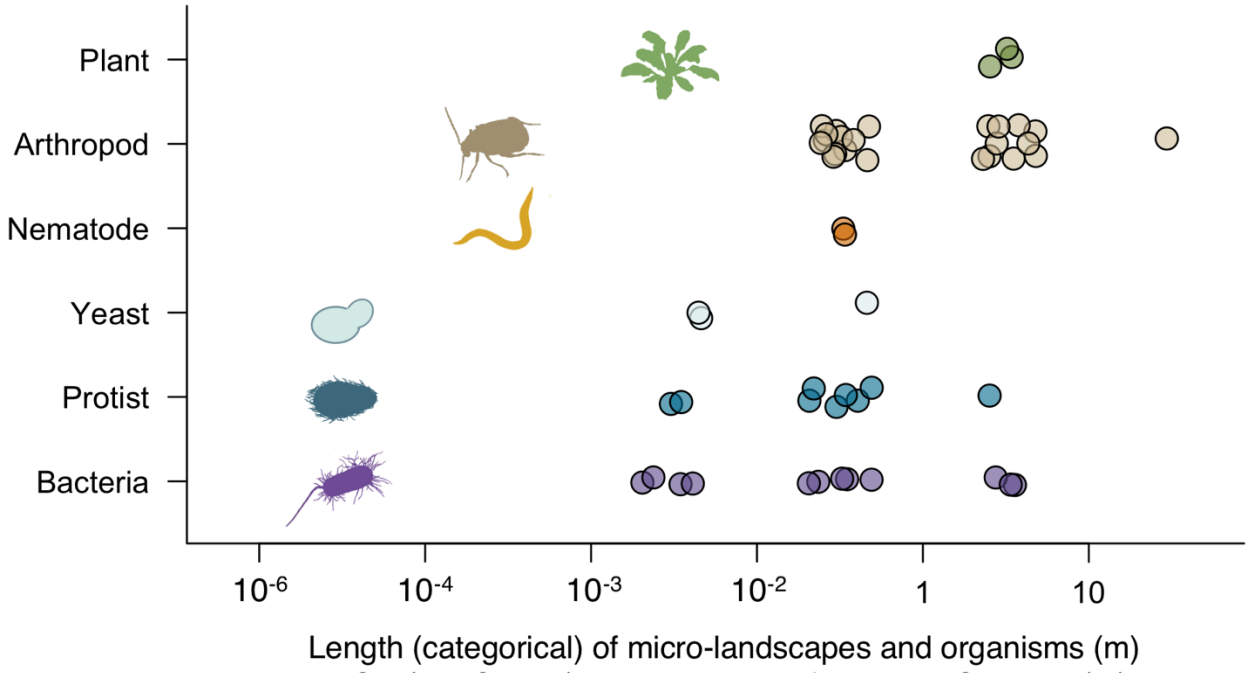

Figure 3: Dimensions of micro-landscapes that allowed organism-mediated dispersal (58 of 72 studies). Points shows the scale of the maximum micro-landscape length per study for the 57 studies for which we could determine both landscape and organism size. Noise added on x and y axis so all points can be seen. Icon position shows the length of the organism; micro-landscapes were 100 to >100 000x longer than the organisms themselves.

Table 1: Examples of experimental micro-landscapes that vary in their habitat connectivity and quality.

Habitat type	
Micro-landscape setup	Example studies
a) Continuous habitat of uniform-quality	
Uniform agar on Petri dish or culture plates	Hallatschek et al. (2007), Taylor & Buckling (2011), Korolev et al. (2012), Van Dyken et al. (2013), Song (2016), Bosshard et al. (2017), Goldschmidt et al. (2017), Ozgen et al. (2018)
Linear liquid tray	Giometto et al. (2014)
Dendritic (branching) paper habitat	Hol et al. (2019)
b) Patchy habitat uniform quality	
Microfluidics chambers/nanohabitat	Hol et al. (2013, 2016), Tekwa et al. (2015, 2017)
96-well plate with wells linked physically or by manual dispersal	Datta et al. (2013), Low-Décarie et al. (2015), Gandhi et al. (2016), Kurkjian (2018a, b)
Liquid vessels linked physically or by manual dispersal	Holyoak & Lawler (1996), Warren (1996), Burkey (1997), Altermatt et al. (2011), Carrara et al. (2012), Seymour & Altermatt (2014), Jacob et al. (2015), Fronhofer & Altermatt (2015), Fronhofer et al. (2015a, 2017a), Altermatt & Fronhofer (2018)
Linked chambers (solid)	Miller & Inouye (2013), Szűcs et al. (2014, 2017), Ochocki & Miller (2017), Weiss-Lehman et al. (2017, 2019), Gilarranz et al. (2017), Wagner et al. (2017)
Single patches of low-quality habitat with migration from source population	liquid: Ching et al. (2013) solid: Hufbauer et al. (2015)

Paper mainland/islands	Hol et al. (2019)
Nutrient patches on agar matrix	Friedenberg (2003a, b), Taylor & Buckling (2010), Dennehy et al. (2007)
Pots of soil	Williams et al. (2016), Williams & Levine (2018)
Moss patches	lab: Staddon et al. (2010), field: Gilbert et al. (1998), Gonzalez et al. (1998)
Leaf islands	De Roissart et al. (2015)
Sponge islands	Henebry & Cairns (1980)
c) Continuous or patchy with gradient/variation in habitat quality (specific gradient)	
Large agar 'MEGA plate' (antibiotics)	Baym et al. (2016)
96-well plate (salt)	Bell & Gonzalez (2011)
Linked liquid vessels (food availability) (mortality)	Donahue et al. (2003) Fronhofer et al. (2017b)
Patches in box (patch moisture) (food quality or availability)	Chisholm et al. (2011), Govindan & Swihart (2012), Govindan et al. (2015)
Linked cages (temperature)	Davis et al. (1998)

Table 2: Main questions related to species distributions addressed to date using micro-landscape experiments, with example studies. Organisms: Bacteria = *Escherichia coli*, or *Pseudomonas* or *Bdellovibrio* species; Beetles = *Callosobruchus maculatus* or *Tribolium castaneum*; Flies = *Bemisia* or *Drosophila* species, Mites = *Tetranychus urtica*; Nematodes = *Caenorhabditis elegans*; Protists were generally *Tetrahymena* species but sometimes *Paramecium*, *Didinium* or *Colpidium* species; Plants = *Arabidopsis thaliana*; Rotifers = *Cephalodella* species; Yeast = *Saccharomyces cerevisiae*.

Topic Broad Question Results	Organism	References
a. Range expansion across unoccupied, uniform habitat		
i) Do range expansions mirror theoretical predictions?		
Mean expansion velocity well approximated by Fisher wave; adding stochasticity improved predictions	Protist	Giometto et al. (2014)
High endogenous (organism-generated) variation in spread rates	Protist	Giometto et al. (2014)
	Beetle	Melbourne & Hastings (2009)
Increased cooperation shifted expansion from pushed to pulled	Yeast	Gandhi et al. (2016)
ii) How do founding population characteristics affect establishment and/or spread?		
More genetic diversity in founding populations increased spread rate	Beetle	Wagner et al. (2017)
More genetic diversity in founding populations increased establishment, particularly in low-quality environments	Fly	Hufbauer et al. (2013)
More genetic diversity interacted with founding population size to determine establishment and subsequent growth	Beetle	Szűcs et al. (2014)

iii) How does dispersal ability evolve during range expansion?		
Evolution of increased dispersal at expanding front	Bacteria	Taylor & Buckling (2011)
	Protist	Fronhofer & Altermatt (2015); Fronhofer et al. (2017a, 2017b)
	Beetle	Szűcs et al. (2017); Ochoki & Miller (2017); Weiss-Lehman at al. (2017)
	Plant	Williams et al. (2016)
iv) How do genetic drift and mutation interact with range expansion?		
Genotypes segregated randomly (drift)	Bacteria	Hallatscheck et al. (2007)
Deleterious mutations accumulated at range front (drift)	Bacteria	Bosshard et al. (2017), Song et al. (2016)
Gene-surfing and loss of genetic diversity at expansion front (drift)	Beetle	Weiss-Lehman et al. (2019)
Selective sweeps took longer in expanding vs. non-expanding populations	Yeast	Korolev et al. (2012)
v) How do intra-specific interactions affect range expansion?		
Interference between strains segregated expanding populations	Bacteria	Ozgen et al. (2018)
Cooperators and defectors segregated during expansion, sometimes accelerating expansion via increased cooperator fitness	Yeast	Van Dyken et al. (2013), Datta et al. (2013)
Commensal strains only expanded after their partner expanded	Bacteria	Goldschmidt et al. (2017)
Female-biased dispersal at expanding front increased expansion speed	Beetle	Miller & Inouye (2013)

b. Spatial gradients in habitat quality		
i) How do environmental gradients affect adaptation and species ranges?		
Dispersal maintained sink populations along climate gradients, expanding species ranges compared to no-dispersal conditions	Flies (3 *spp*)	Davis et al. 1998
Equal dispersal evolution during expansion across uniform vs worsening habitat if dispersers had no prior information	Protist	Fronhofer et al. (2017b)
Information about upcoming low-quality habitat reduced dispersal, preventing range expansion and dispersal evolution	Protist	Fronhofer et al. (2017b), Jacob et al. (2015)
Adaptation at range edge enabled expansion into previously hostile habitat	Bacteria	Baym et al. (2016)
Intermediate migration rates facilitated adaptation to sink habitat	Virus	Ching et al. (2013)
Along a gradient of habitat quality that worsened through time, populations adapted to poor habitat rescued those in newly deteriorating habitat	Yeast Microbe community	Bell & Gonzalez (2011) Low-Décarie et al. (2015)
c. Species interactions		
i) How do inter-specific interactions affect species ranges?		
Inter-specific competition reduced fly ranges along temperature gradient; parasite further altered ranges via preferential parasitism which mediated competition	Flies (3 *spp*) + parasitic wasp	Davis et al. 1998
Antagonists (competitors; predators/prey) less likely to exclude each other in heterogeneous habitat due to spatial refugia	Bacteria	Hol et al. (2013), Hol et al. (2016)

Intra-specific competition did not result in competitive exclusion from habitat patches	Beetle	Dallas et al. (2019)
Intra-specific competition increased dispersal and colonization of empty patches	Protist	Fronhofer et al. (2015a)
d. Colonization and persistence in patchy habitat		
i) How does landscape structure affect colonization dynamics?		
Distance from mainland lowered island colonization rate and richness; richness did not increase with island size	Protist community	Henebry & Cairns (1980)
Island colonization dominated by priority effects	Bacteria	Hol et al. (2019)
Colonization rate and local richness higher in branched vs. unbranched habitat	Protist & rotifer community	Seymour & Altermatt (2014)
Range expansion slowed by increasing patchiness	Plant	Williams & Levine (2018)
Metapopulation persistence promoted by low connectivity and intermediate patch turnover rates	Beetle	Govindan et al. (2015)
Presence of low-quality patches (sinks) reduced metapopulation viability in structured landscapes	Virus	Dennehy et al. (2007)
Net dispersal was from high- to low-quality patches, supporting source-sink theory	Protist	Donahue et al. (2003)

ii) Does dispersal/connectivity promote persistence in patchy/fragmented habitat?		
Dispersal and connectivity among patches lessened impacts of disturbance and aided recovery post disturbance	Protist community	Warren (1996), Altermatt et al. (2011)
	Micro-arthropod community	Gonzalez et al. (1998), Gilbert et al. (1998)
	Rotifer	Altermatt et al. (2011)
Corridor length and arrangement affected recolonization rate, with different optima for different disturbance levels	Bacteria	Kurkjian (2018a)
Habitat configuration altered effects of fragmentation after controlling for habitat area	Micro-arthropod community	Chisholm et al (2011)
Dispersal among patches hastened patch extinction, potentially by synchronizing extinction	Bacteria-protist food web	Burkey (1997)
Localized connections in metapopulation network isolated effects of disturbance	Micro-arthropod	Gillaranz et al. (2017)
Dispersal from core population lowered extinction in sink habitat via genetic and demographic rescue	Beetle	Hufbauer et al. (2015)
Intermediate dispersal in patchy habitat maximized predator and prey densities	Protist	Holyoak & Lawler (1996)

iii) How do spatiotemporal patch dynamics affect dispersal?		
Increased patch turnover selected for increased dispersal	Nematode	Friedenberg (2003a, 2003b)
Increased patch isolation decreased dispersal (via adaptation or behavior)	Mite	De Roissart et al. (2015)
iv) How do conspecifics affect dispersal?		
Increased relatedness in a patch increased disperser fitness	Bacteria	Taylor & Buckling (2010)
Information about beyond-patch habitat and matrix altered dispersal decisions	Protist	Fronhofer et al. (2017b), Jacob et al. (2015)
Greatest dispersal at low (Allee effects) and high (overcrowding) population densities	Protist	Fronhofer et al. (2015b)

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Table A1: Options for propagating micro-landscapes as study organisms deplete nutrients or occupy the entire landscape.

Replenishment	Description	Example	Benefits	Drawbacks
No replenishment of food or space				
	Entire experiment occurs in same micro-landscape	Baym et al. (2016)	No disturbance Micro-landscape can be fully enclosed	Growth becomes limited by food and space, so larger landscapes generally needed
Food replenished only				
	New food added to existing landscape	De Roissart et al. (2015)	Longer experiments possible	Potential disturbance; avenue for contamination
Food and space replenished				
Treadmill	New patches of landscape added, organisms disperse into new habitat on their own	Fronhofer et al. (2017)	Can simulate a long expansion with minimal space	Dispersal limited to few patches at a time; only suitable for some questions (e.g. range expansions)
Transfer to fresh landscape	Individuals transferred manually to new micro-landscape	Friedenberg (2003a)	Nutrients and space are replenished	Disturbance; must decide whether to maintain population sizes or subsample

Table A2: Examples of stressors used to create variation in environmental quality in micro-landscape experiments. Stressors are of three types: limitation of resources, fitness gradient imposed by researchers, or an actual negative stressor. Plural organism names indicate multiple species were used.

Gradient		
Organism	Stressor details	Example studies
Resource limitation		
Virus	Ratio of good habitat patches (infectable bacteria) vs. sink habitat (bacteria that virus could bind to but not infect)	Dennehy et al. (2007)
Beetle	Resource quality (ratio of wheat to corn flour)	Hufbauer et al. (2015)
Beetle	Resource availability (amount of flour / patch)	Govindan et al (2015)
Researcher-imposed fitness gradient		
Protist	Mortality (removal of individuals)	Fronhofer et al. (2017b)
Beetle	Patch turnover rate (removal of occupied patches and introduction of new patches)	Govindan et al (2015)
Negative stressor		
E. coli	Antibiotic	Baym et al. (2016)
Yeast	Salt	Bell & Gonzalez (2011)
Soil microbes	Herbicide	Low-Décarie et al. (2015)
Fruit flies	Temperature	Davis et al. (1998)

Table A3: Studies depicted in Figure 2. For each study, we estimated the organism's length in cm from the papers themselves (ideally) or internet sources. We then rounded down to the nearest decimal, e.g. an organism of 30 um = 0.003 cm = 0.01 in the table. This helped deal with organisms with variable body size (most) and studies of multiple organisms. We estimated landscape length as the maximum distance an organism could travel along a landscape during the experiment. For landscapes made of sequentially added patches this was patch length x patch number, for the Baym et al. MEGA plate this was half the MEGA plate length as the antibiotic gradient was mirrored (highest in middle of landscape). We then converted length to the units of measurement (scale). This helped deal with studies where the exact landscape length could not be calculated from the information in the paper.

			Length		
Organism			Organism	Landscape	
Authors	Year	Journal	(cm)	(cm)	scale
Arthropod					
Astrom & Bengtsson	2011	Oecologia	0.1	300	m
Chisholm et al.	2011	Ecography	0.1	50	dm
Dallas et al.	2019	J Anim Ecol	0.1	12	dm
Davis et al.	1998	Nature	0.1	–	m
Drake & Griffen	2013	Ecol & Evol	0.1	31.5	dm
Gilarranz et al.	2017	Science	0.1	50	dm
Gilbert et al.	1998	Proc R Soc B	0.1	34	dm
Gonzalez et al.	1998	Science	0.1	50	dm
Govindan & Swihart	2015	Ecology	0.1	–	dm
Govindan & Swihart	2012	PLOS One	0.1	21	dm
Lomnicki	2006	Evol Ecol Res	0.1	20	dm
Miller & Inouye	2013	Ecol Lett	0.1	410	m
Morel-Journel et al.	2019	Ecol Lett	0.1	650	m
Morel-Journel et al.	2018	Ecography	0.1	455	m
Ochocki & Miller	2017	Nat Comm	0.1	2000	dak
Staddon et al.	2010	Ecol Lett	0.1	–	dm
Starzomski & Srivastava	2007	Oikos	0.1	34	dm
Strevens & Bonsall	2011	J Anim Ecol	0.1	36.5	dm
Szucs et al.	2017	PNAS	0.1	–	m
Tung et al.	2018	Oikos	0.1	–	m
Wagner et al.	2017	J Anim Ecol	0.1	–	m
Weiss-Lehman et al.	2017	Nat Comm	0.1	180	m
Weiss-Lehman et al.	2019	Proc R Soc B	0.1	180	m
Bacteria					
Baym et al.	2016	Science	0.0001	400	m
Bosshard et al.	2017	Genetics	0.0001	9	cm

Goldschmidt et al.	2017	ISME	0.0001	10	dm
Hallatscheck et al.	2007	PNAS	0.0001	10	dm
Hol et al.	2013	PLOS One	0.0001	12.7	dm
Hol et al.	2016	PNAS	0.0001	12.7	dm
Hol et al.	2019	Ecol Lett	0.0001	2.6	cm
Kurkjian	2018	Meth Ecol Evo	0.0001	–	cm
Ozgen et al.	2018	Sci Adv	0.0001	9	cm
Song et al.	2016	Env Micro Bio	0.0001	100	m
Taylor & Buckling	2010	Am Nat	0.0001	10	dm
Taylor & Buckling	2011	Evolution	0.0001	135	m
Nematode					
Friedenberg	2003	Ecol Lett	0.1	10	dm
Friedenberg	2003	Am Nat	0.1	10	dm
Plant					
Lustenhouwer et al.	2019	J Ecol	10	–	m
Williams & Levine	2018	Ecology	10	453	m
Williams et al.	2016	Science	10	840	m
Protist					
Altermatt & Fronhofer	2018	Freshwater Bio	0.001	75	dm
Donahue et al.	2003	Am Nat	0.001	24.4	dm
Fronhofer & Altermatt	2015	Nat Comm	0.001	72	dm
Fronhofer et al.	2017	J Evo Biol	0.001	72	dm
Fronhofer, Nitsche, Altermatt	2017	Glob Ecol Biogeo	0.001	84	dm
Giometto et al.	2014	PNAS	0.001	200	m
Henebry & Cairns	1980	Am Midland Nat	0.001	46.5	dm
Holyoak & Lawler	1996	J Anim Ecol	0.001	62.3	dm
Jacob et al.	2015	J Anim Ecol	0.001	2.5	cm
Jacob et al.	2019	Oikos	0.001	2.5	cm
Protists and Animals / Bacteria					
Altermatt et al.	2011	PLOS One	0.001	12.7	dm
Carrara et al.	2012	PNAS	0.001	–	dm
Seymour & Altermatt	2014	Ecol & Evol	0.001	245	m
Burkey	1997	Am Nat	0.001	15	cm
Yeast					
Gralka et al.	2016	Ecol Lett	0.001	10	dm
Korolev et al.	2012	Phys Biol	0.001	10	cm
Van Dyken et al.	2013	Current Biol	0.001	3.5	cm

Literature Cited

Alexander, J. M. et al. 2015. Novel competitors shape species' responses to climate change. - Nature 525: 515–518.

Alleaume-Benharira, M. et al. 2006. Geographical patterns of adaptation within a species' range: interactions between drift and gene flow. - J. Evol. Biol. 19: 203–215.

Altermatt, F. and Fronhofer, E. A. 2018. Dispersal in dendritic networks: Ecological consequences on the spatial distribution of population densities. – Freshw. Biol. 63: 22–32.

Altermatt, F. et al. 2011. Effects of connectivity and recurrent local disturbances on community structure and population density in experimental metacommunities. - PLoS ONE 6: e19525.

Altermatt, F. et al. 2015. Big answers from small worlds: a user's guide for protist microcosms as a model system in ecology and evolution. - Methods Ecol. Evol. 6: 218–231.

Antonovics, J. 1976. The nature of limits to natural selection. - Ann. Mo. Bot. Gard. 63: 224–247.

Bay, L. K. et al. 2008. Meta-population structure in a coral reef fish demonstrated by genetic data on patterns of migration, extinction and re-colonisation. - BMC Evol. Biol. 8: 248.

Baym, M. et al. 2016. Spatiotemporal microbial evolution on antibiotic landscapes. - Science 353: 1147–1151.

Bell, G. and Gonzalez, A. 2011. Adaptation and evolutionary rescue in metapopulations experiencing environmental deterioration. - Science 332: 1327–1330.

Benton, T. G. et al. 2007. Microcosm experiments can inform global ecological problems. - Trends Ecol. Evo. 22: 516–521.

Bono, L. M. et al. 2019. Evolvability costs of niche expansion. - Trends Genet. 36: 14–23.

Bosshard, L. et al. 2017. Accumulation of deleterious mutations during bacterial range expansions. - Genetics 207: 669–684.

Brown, C. D. and Vellend, M. 2014. Non-climatic constraints on upper elevational plant range expansion under climate change. - Proc. Biol. Sci. 281: 1–9.

Burkey, T. V. 1997. Metapopulation extinction in fragmented landscapes: Using bacteria and protozoa communities as model ecosystems. - Am. Nat. 150: 568–591.

Cadotte, M. W. et al. 2005. Constructing nature: Laboratory models as necessary tools for investigating complex ecological communities. - In: Advances in Ecological Research. Population Dynamics and Laboratory Ecology. Academic Press, pp. 333–353.

Carrara, F. et al. 2012. Dendritic connectivity controls biodiversity patterns in experimental metacommunities. - PNAS 109: 5761–5766.

Case, T. J. et al. 2005. The community context of species' borders: ecological and evolutionary perspectives. - Oikos 108: 28–46.

Cheptou, P.-O. et al. 2017. Adaptation to fragmentation: evolutionary dynamics driven by human influences. - Philos. Trans. R. Soc. Lond. B. Biol. Sci. 372: 20160037.

Ching, J. et al. 2013. Migration enhances adaptation in bacteriophage populations evolving in ecological sinks: Migration and adaptation among bacteriophage populations. - Evolution 67: 10–17.

Chisholm, C. et al. 2011. Metacommunity diversity depends on connectivity and patch arrangement in heterogeneous habitat networks. - Ecography 34: 415–424.

Chuang, A. and Peterson, C. R. 2016. Expanding population edges: theories, traits, and trade-offs. - Global Change Biol. 22: 494–512.

Clausen, J. et al. 1940. Experimental studies on the nature of species. III. Environmental responses of climatic races of *Achillea*. - Carnegie Insitution of Washington, Washington DC.

Dallas, T. et al. 2019. When can competition and dispersal lead to checkerboard distributions? - J. Anim. Ecol. 88: 269–276.

Darwin, C. 1859. On the Origin of Species by Natural Selection. J Murray, London.

Darling, E. et al. 2008. Increased seed dispersal potential towards geographic range limits in a Pacific coast dune plant. - New Phytol. 178: 424–435.

Datta, M. S. et al. 2013. Range expansion promotes cooperation in an experimental microbial metapopulation. - PNAS: 201217517.

Davis, A. J. et al. 1998. Making mistakes when predicting shifts in species range in response to global warming. - Nature 391: 783–786.

De Roissart, A. D. et al. 2015. Spatial and spatiotemporal variation in metapopulation structure affects population dynamics in a passively dispersing arthropod. - J. Anim. Ecol. 84: 1565–1574.

Dennehy, J. J. et al. 2007. Virus population extinction via ecological traps. - Ecol. Lett. 10: 230–240.

Donahue, M. J. et al. 2003. Patterns of dispersal and dynamics among habitat patches varying in quality. - Am. Nat. 162: 302–317.

Eckert, C. G. et al. 2008. Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. - Mol. Ecol. 17: 1170–1188.

Fahrig, L. and Merriam, G. 1994. Conservation of fragmented populations. - Conserv. Biol. 8: 50–59.

Freeman, B. G. et al. 2018. Expanding, shifting and shrinking: The impact of global warming on species' elevational distributions. - Global Ecol. Biogeogr. 27: 1268–1276.

Friedenberg, N. A. 2003a. Experimental evolution of dispersal in spatiotemporally variable microcosms. - Ecol. Lett. 6: 953–959.

Friedenberg, N. A. 2003b. Determinism in a transient assemblage: The roles of dispersal and local competition. - Am. Nat .162: 586–596.

Fronhofer, E. A. and Altermatt, F. 2015. Eco-evolutionary feedbacks during experimental range expansions. - Nat. Comm. 6: 6844.

Fronhofer, E. A. et al. 2015a. Condition-dependent movement and dispersal in experimental metacommunities. - Ecol. Lett. 18: 954–963.

Fronhofer, E. A. et al. 2015b. Density-dependent movement and the consequences of the Allee effect in the model organism *Tetrahymena*. - J. Anim. Ecol. 712–722.

Fronhofer, E. A. et al. 2017a. Evolution of density-dependent movement during experimental range expansions. - J. Evol. Biol. 30: 2165–2176.

Fronhofer, E. A. et al. 2017b. Information use shapes the dynamics of range expansions into environmental gradients. - Global Ecol. Biogeogr. 26: 400–411.

Gandhi, S. R. et al. 2016. Range expansions transition from pulled to pushed waves as growth becomes more cooperative in an experimental microbial population. - PNAS 113: 6922–6927.

Gilbert, F. et al. 1998. Corridors maintain species richness in the fragmented landscapes of a microecosystem. - Proc. R. Soc. Lond. B. Biol. Sci. 265: 577–582.

Gilbert, K. J. et al. 2017. Local adaptation interacts with expansion load during range expansion: Maladaptation reduces expansion load. - Am. Nat. 189: 368–380.

Gilarranz, L. J. et al. 2017. Effects of network modularity on the spread of perturbation impact in experimental metapopulations. - Science 357: 199–201.

Giometto, A. et al. 2014. Emerging predictable features of replicated biological invasion fronts. - PNAS 111: 297–301.

Goldschmidt, F. et al. 2017. Successive range expansion promotes diversity and accelerates evolution in spatially structured microbial populations. - The ISME Journal 11: 2112–2123.

Gomulkiewicz, R. et al. 1999. The effects of density dependence and immigration on local adaptation and niche evolution in a black-hole sink environment. - Theor. Popul. Biol. 55: 283–296.

Gonzalez, A. et al. 1998. Metapopulation dynamics, abundance, and distribution in a microecosystem. - Science 281: 2045–2047.

Govindan, B. N. and Swihart, R. K. 2012. Experimental beetle metapopulations respond positively to dynamic landscapes and reduced connectivity. - PLOS ONE 7: e34518.

Govindan, B. N. et al. 2015. Intermediate disturbance in experimental landscapes improves persistence of beetle metapopulations. - Ecology 96: 728–736.

Halbritter, A. H. et al. 2013. How comparable are species distributions along elevational and latitudinal climate gradients? - Global Ecol. Biogeog. 22: 1228–1237.

Hallatschek, O. et al. 2007. Genetic drift at expanding frontiers promotes gene segregation. - PNAS 104: 19926–19930.

Hampe, A. and Petit, R. J. 2005. Conserving biodiversity under climate change: the rear edge matters. - Ecol. Lett. 8: 461–467.

Hargreaves, A. L. and Eckert, C. G. 2014. Evolution of dispersal and mating systems along geographic gradients: implications for shifting ranges. - Funct. Ecol. 28: 5–21.

Hargreaves, A. L. and Eckert, C. G. 2019. Local adaptation primes cold-edge populations for range expansion but not warming-induced range shifts. - Ecol. Lett. 22: 78–88.

Hargreaves, A. L. et al. 2014. Are species' range limits simply niche limits writ large? A review of transplant experiments beyond the range. - Am. Nat. 183: 157–173.

Hargreaves, A. L. et al. 2015. Fitness declines towards range limits and local adaptation to climate affect dispersal evolution during climate-induced range shifts. - J. Evol. Biol. 28: 1489–1501.

Hargreaves, A. L. et al. 2020. Local adaptation to biotic interactions: a meta-analysis across latitudes. - Am Nat. (in press).

Henebry, M. S. and Cairns, J. 1980. The effect of island size, distance and epicenter maturity on colonization in freshwater protozoan communities. - Am. Midl. Nat. 104: 80–92.

Hol, F. J. H. et al. 2013. Spatial structure facilitates cooperation in a social dilemma: Empirical evidence from a bacterial community. - PLoS ONE 8: e77042.

Hol, F. et al. 2016. Bacterial predator–prey dynamics in microscale patchy landscapes. - Proc. R. Soc. Lond. B. Biol. Sci. 283: 20152154.

Hol, F. J. H. et al. 2019. Bacteria-in-paper, a versatile platform to study bacterial ecology. - Ecol. Lett. 22: 1316-1323.

Holt, R. D. and Keitt, T. H. 2000. Alternative causes for range limits: a metapopulation perspective. - Ecol. Lett. 3: 41–47.

Holt, R. D. et al. 2005. Theoretical models of species' borders: single species approaches. - Oikos 108: 18–27.

Holyoak, M. and Lawler, S. P. 1996. The role of dispersal in predator--prey metapopulation dynamics. - J. Anim. Ecol. 65: 640–652.

Howell, P. E. et al. 2018. Increasing connectivity between metapopulation ecology and landscape ecology. - Ecology 99: 1119–1128.

Hufbauer, R. A. et al. 2013. Role of propagule pressure in colonization success: disentangling the relative importance of demographic, genetic and habitat effects. - J. Evol. Bio. 26: 1691–1699.

Hufbauer, R. A. et al. 2015. Three types of rescue can avert extinction in a changing environment. - PNAS 112: 10557–10562.

Jacob, S. et al. 2015. Social information from immigrants: multiple immigrant-based sources of information for dispersal decisions in a ciliate. - J. Anim. Ecol. 84: 1373–1383.

Janzen, D. H. 1967. Why mountain passes are higher in the tropics. - Am. Nat. 101: 233–249.

Jessup, C. M. et al. 2005. Microbial experimental systems in ecology. - In: Advances in Ecological Research. Population Dynamics and Laboratory Ecology. Academic Press, pp. 273–307.

Jessup, C. M. et al. 2004. Big questions, small worlds: microbial model systems in ecology. - Trends. Ecol. Evol. 19: 189-197.

Klopfstein, S. et al. 2006. The fate of mutations surfing on the wave of a range expansion. - Mol. Biol. Ecol. 23: 482–490.

Körner, C. and Paulsen, J. 2004. A world-wide study of high altitude treeline temperatures. - J. Biogeogr. 31: 713–732.

Kirkpatrick, M. and Barton, N. H. 1997. Evolution of a species' range. - Am. Nat. 150: 1–23.

Korolev, K. S. et al. 2012. Selective sweeps in growing microbial colonies. - Phys. Biol. 9: 026008.

Kurkjian, H. 2018a. Metapopulations in miniature: connectivity, subpopulation extinction, and recovery in microbial microcosms (Doctoral dissertation, UC Berkeley). - Retrieved from https://escholarship.org/uc/item/0d11302q

Kurkjian, H. M. 2018b. The metapopulation microcosm plate: A modified 96-well plate for use in microbial metapopulation experiments. - Methods Ecol. Evol. 10: 162-168

Low-Décarie, E. et al. 2015. Community rescue in experimental metacommunities. - PNAS 112: 14307–14312.

MacArthur, R. H. 1972. Species' distributions -In: Geographical Ecology: Patterns in the Distribution of Species. - Harper & Row pp. 127 - 198.

Massot, M. et al. 2008. Climate warming, dispersal inhibition and extinction risk. - Global Change Biol. 14: 461–469.

Melbourne, B. A. and Hastings, A. 2009. Highly variable spread rates in replicated biological invasions: Fundamental limits to predictability. - Science 325: 1536–1539.

Merriam, C. H. 1894. Laws of temperature control of the geographic distribution of terrestrial animals and plants. - Harvard University

Miller, T. E. X. and Inouye, B. D. 2013. Sex and stochasticity affect range expansion of experimental invasions. - Ecol. Lett. 16: 354–361.

Ochocki, B. M. and Miller, T. E. X. 2017. Rapid evolution of dispersal ability makes biological invasions faster and more variable. - Nat. Comm. 8: 14315.

O'Malley, M. A. et al. 2015. How do microbial populations and communities function as model systems? - Q. Rev. Biol. 90: 269–293.

Ozgen, V. C. et al. 2018. Spatial interference scale as a determinant of microbial range expansion. - Sci. Adv. 4: eaau0695.

Pachepsky, E. and Levine, J. M. 2011. Density dependence slows invader spread in fagmented landscapes. - Am. Nat. 177: 18–28.

Pateman, R. M. et al. 2012. Temperature-dependent alterations in host use drive rapid range expansion in a butterfly. - Science 336: 1028–1030.

Phillips, B. L. et al. 2006. Invasion and the evolution of speed in toads. - Nature 439: 803.

Sexton, J. P. et al. 2009. Evolution and ecology of species range limits. - Annu. Rev. Ecol. Syst. 40: 415–436.

Seymour, M. and Altermatt, F. 2014. Active colonization dynamics and diversity patterns are influenced by dendritic network connectivity and species interactions. - Ecol. Evol. 4: 1243–1254.

Shine, R. et al. 2011. An evolutionary process that assembles phenotypes through space rather than through time. - PNAS 108: 5708–5711.

Sjögren, P. 1991. Extinction and isolation gradients in metapopulations: the case of the pool frog (*Rana lessonae*). - Biol. J. Linn. Soc. 42: 135–147.

Song, C. et al. 2016. Living on the edge: emergence of spontaneous gac mutations in *Pseudomonas protegens* during swarming motility. - Environ. Microbiol. 18: 3453–3465.

Srivastava, D. S. et al. 2004. Are natural microcosms useful model systems for ecology? - Trends Ecol. Evol. 19: 379–384.

Staddon, P. et al. 2010. Connectivity, non-random extinction and ecosystem function in experimental metacommunities. - Ecol. Lett. 13: 543–552.

Szűcs, M. et al. 2014. The roles of demography and genetics in the early stages of colonization. - Proc. R. Soc. B. 281: 20141073.

Szűcs, M. et al. 2017. Rapid adaptive evolution in novel environments acts as an architect of population range expansion. - Proc. Natl. Acad. Sci. USA 114: 13501–13506.

Taylor, T. B. and Buckling, A. 2010. Competition and dispersal in *Pseudomonas aeruginosa*. - Am. Nat. 176: 83–89.

Taylor, T. B. and Buckling, A. 2011. Selection experiments reveal trade-offs between swimming and twitching motilities in *Pseudomonas aeruginosa*: Trade-offs between bacterial motilities. - Evolution 65: 3060–3069.

Tekwa, E. W. et al. 2015. Patchiness in a microhabitat chip affects evolutionary dynamics of bacterial cooperation. - Lab on a Chip 15: 3723–3729.

Tekwa, E. W. et al. 2017. Defector clustering is linked to cooperation in a pathogenic bacterium. - Proc. R. Soc. Lond. B Biol. Sci. 284: 20172001

Travis, J. M. J. and Dytham, C. 1999. Habitat persistence, habitat availability and the evolution of dispersal. - Proc. R. Soc. Lond. B Biol. Sci. 266: 723–728.

Travis, J. M. J. et al. 2009. Accelerating invasion rates result from the evolution of density-dependent dispersal. - J. Theor. Biol. 259: 151–158.

Van Dyken, J. D. et al. 2013. Spatial population expansion promotes the evolution of cooperation in an experimental prisoner's dilemma. - Curr. Biol. 23: 919–923.

Wagner, N. K. et al. 2017. Genetic mixture of multiple source populations accelerates invasive range expansion. - Methods Ecol. Evol. 21–34.

Wang, B. C. and Smith, T. B. 2002. Closing the seed dispersal loop. - Trends Ecol. Evol. 17: 379–386.

Warren, P. H. 1996. Dispersal and destruction in a multiple habitat system: An experimental approach using protist communities. - Oikos 77: 317–325.

Weiss-Lehman, C. et al. 2017. Rapid trait evolution drives increased speed and variance in experimental range expansions. - Nat. Comm. 8: 14303.

Weiss-Lehman, C. et al. 2019. Stochastic processes drive rapid genomic divergence during experimental range expansions. - Proc. R. Soc. Lond. B Biol. Sci. 286: 20190231.

Williams, J. L. and Levine, J. M. 2018. Experimental evidence that density dependence strongly influences plant invasions through fragmented landscapes. - Ecology 99: 876–884.

Williams, J. L. et al. 2016. Rapid evolution accelerates plant population spread in fragmented experimental landscapes. - Science 353: 482–485.

Linking Text: Exploring novel ways to make micro-landscapes

Exploring novel landscape configurations is a logical way to further micro-landscape studies. Many micro-landscapes creatively repurposed standard equipment to great effect (Fronhofer and Altermatt 2015, Ochocki and Miller 2017, Williams and Levine 2018), but custom-made designs can greatly expand key landscape features such as resource patchiness and gradients, and habitat connectivity and turnover. Recent studies demonstrate the value and flexibility of custom-made landscapes. Specifically, these studies have leveraged much larger dimensions (MEGA plate, Baym et al. 2016) and fine-scale control over connectivity (metapopulation microcosm plate, Kurkjian 2018) to ask increasingly complex questions about species distributions. However, complete ground-up customization can be difficult from a design and production perspective; this could partially explain why these types of designs are relatively scarce in the literature.

An increasingly accessible source of highly customizable equipment is 3D printing; it is a flexible manufacturing process which can create both modular pieces and entire micro-landscapes. 3D printing eases prototyping and production by allowing rapid on-site printing, making design iteration more straightforward and often more affordable than ordering custom equipment from a third party. This accessibility should make it easier to experiment with novel micro-landscapes, and ultimately gives researchers extreme control over the entire production process. Print designs can also be easily shared between researchers, encouraging collaboration and reproducibility, and making it easier to build upon promising protocols and designs. Another benefit is that 3D printing is scalable based on need. There are a diverse range of printers and plastics which span the cost and performance spectrum. Basic printers and plastic filaments are generally very affordable, with higher-cost, high-performance printers (e.g. high resolution, larger print volume) and plastics (e.g. autoclavable, medical-grade) readily available for more

complex or demanding tasks. Thus, 3D printing has a relatively low barrier to entry with a high potential ceiling. As such, 3D printing can easily slot into a variety of research applications, micro-landscape and beyond.

3D printing could also make general-purpose research equipment (e.g. petri dishes), potentially filling a broader niche for customizable and reusable plastic items. Finding alternatives to single-use plastics is a timely issue because bioresearch discards a vast amount of plastic annually (Urbina et al. 2015); adopting new reusable alternatives is one way to reduce this waste and increase research sustainability. Of course, reusable items are not new to research (e.g. glassware), but these items traditionally lacked 3D printing's ease of customization and production. The diversity of printable plastics means that researchers could easily find a material suitable for their needs (e.g. high impact resistance, autoclavable, biocompatible). Researchers could then produce equipment on-site, increasing accessibility while further reducing waste by cutting down on packaging and shipping. Despite these attractive features, 3D printed plastics are underexplored in bioresearch.

A key question facing 3D printed items is whether they can be repeatedly sterilized. Sterility is a broad requirement for biological work and will influence 3D printing's attractiveness for items ranging from simple petri dishes to complex micro-landscapes. Importantly, plastic properties (e.g. thermal tolerance) can limit which sterilization methods are feasible. For example, some plastics can be autoclaved whereas others must be cleaned closer to room temperature to avoid warping or melting. There are very successful room-temp sterilization methods such as ethylene oxide gas and gamma radiation, but these methods are probably not convenient, or even accessible, for most labs. As such, labs looking to use non-autoclavable plastics will likely need to use more simple and accessible methods (e.g. ethanol). Studies testing

a variety of simple sterilization methods find room temperature sterilization feasible but not always straightforward, with some of the most accessible methods (e.g. 70% ethanol) taking a long time to work with sometimes inconsistent results (Gualandi et al. 2012, Neches et al. 2016, Guerra et al. 2018). These studies only tested one round of initial sterilization; we have not found any studies to date which have examined whether items may be repeatedly sterilized. Repeatable, accessible sterilization is imperative for items to be truly reusable across a wide range of disciplines.

Chapter 2: Sterility and cost limit 3D-printed plastics as replacements for single-use items

Cole Larsen & Anna Hargreaves

Abstract

Bioresearch produces an estimated 5.5 million tons of plastic waste annually. One way to reduce this waste is to replace some single-use plastic items, such as petri dishes, with reusable items. A potential source of these replacements is 3D printing, a process which can produce highly customizable plastic items. However, 3D printing is underexplored as a source of plastic bioresearch equipment. A key question is whether printed items can be repeatedly and quickly sterilized using common laboratory methods such as ethanol. To address this, we tested whether petri dishes made of three plastics varying in cost and thermal tolerance could be repeatedly sterilized via soaking in 70% ethanol and/or autoclaving. We found that 70% ethanol could not sterilize dishes for any of the plastics (7 trials of 2 min soaks, 5 plates/plastic), and that contamination appeared to increase over the course of the study. As such, non-autoclavable plastics are questionable replacements for single-use items because quick, easy sterilization is likely unfeasible. Autoclaving could likely sterilize the lone autoclavable plastic we tested (7 cycles, 5 plates), but external contamination confounded these results. Autoclavable plastics may be repeatedly sterilized but are expensive and more challenging to print, which limits their broad appeal. Additionally, several of the autoclavable plates began to warp after as few as three sterilizations, potentially signaling another limitation. Our results show that sterilization challenges and cost currently make 3D printing an unlikely replacement for single-use items where sterility is required, leaving it better suited for prototyping, small-scale projects or those used in non-sterile applications.

Introduction

Bioresearch uses and discards a vast amount of plastic. One estimate places plastic waste from bioresearch facilities at 5.5 million tons per year (Urbina et al. 2015), which is roughly 2% of global plastic waste (based on 2010 estimate; Ritchie and Roser 2018). A major component of research-generated plastic waste are single-use items, which began replacing glassware and other reusables in the mid 1900's, increasing convenience at the expense of waste (Hopewell et al. 2009). Finding ways to reintegrate reusable items into research could improve sustainability. To this end, it is worth exploring new ways to produce reusable items as new technologies offer opportunities not readily available with pre-plastic equipment.

One bioresearch field that is particularly dependent on single-use plastics is microbiology. For example, during long-term evolution studies laboratories can churn through petri dishes and culture plates, potentially using over 1200 dishes during the course of an experiment (Lenski et al. 1991, Lenski and Travisano 1994). Single-use items enable this speed and volume because of their convenience; they do not need to be sterilized before use and can be discarded when finished. However, many studies are conducted on smaller scales, and the historical use of glass petri dishes and other reusable items proves that, while convenient, single-use plastics are not the only viable equipment options.

While reusable items clearly reduce plastic waste, they come with their own drawbacks. Reusable items may be more expensive per unit, but this can be offset with sufficient use. Reusable items must be cleaned between uses, which means completely sterilized for many applications. This cleaning can come with its own environmental costs as some methods (e.g. bleach, gas) use toxic chemicals. Additionally, lengthy or complex cleaning procedures can pose logistical constraints on labs that need large amounts of readily available items. These limitations

mean reusable items must be sufficiently cost-effective and easily sterilized if they are to replace items such as petri dishes or other labware.

A potential source of reusable plastic items is 3D printing. In addition to creating standard items such as petri dishes, 3D printing gives researchers control over the entire design and production process. This makes customization straightforward, allowing researchers to produce items otherwise unavailable from general suppliers, from standard items with unusual dimensions to completely novel equipment. 3D printing can use a wide variety of plastic polymers (though most printers can only print a limited suite effectively). Plastics vary in thermal tolerance, impact resistance, color, and transparency, allowing researchers to select a plastic which best suits their needs. Despite its flexibility and increased accessibility, 3D-printing has not been widely tested as a source of plastic labware (but see Neches et al. 2016 & Kadilak et al. 2017).

One critical requirement for integrating reusable 3D printed items into microbiology studies is sterilization. 3D printed items would be most practical if they could be sterilized both easily and repeatedly, reducing the efficiency and cost advantage of disposable plastic items. Pioneering studies have tested biocompatibility of specific plastics for specific organisms (e.g. zebrafish, *Daphnia magna*; Zhu et al. 2015, Macdonald et al. 2016), but did not test equipment sterility per se. Other early experiments focused on sterilization have shown that printed items can be printed sterile, and that some sterilization methods are effective (Guerra et al. 2018, Neches et al. 2016). For example, 3D printed polycaprolactone was successfully sterilized via 8 h ethanol soaks and 2 h UV light exposure (Guerra et al. 2018). However, these studies only tested at most one sterilization run per item, so it is unclear whether items can be sterilized repeatedly, and some of the successful methods (e.g. UV light) can deteriorate plastic. Further,

simply being able to sterilize an item is likely insufficient to allow widespread uptake of 3D printed items to replace single-use plastics; the sterilization process must be quick enough that it does not intolerably slow research and repeatable (without damage) enough that the per-use cost of printed items approaches that of disposables.

Here, we test whether petri dishes made of three printed plastics could be repeatedly sterilized using relatively quick and common laboratory methods. We tested two accessible and low-cost plastics (ABS, PLA), which are the two most widely printed polymers and the ones used by entry-level 3D printers, and a third, more expensive medical grade plastic (SG resin) that is known to be autoclavable. We tested two common sterilization methods: 70% ethanol soaks (all three plastics) and autoclaving (SG resin only), and compared contaminant growth on treated printed plastics to that on standard Petri dishes, which are pre-sterilized polystyrene dishes purchased from a lab equipment supplier (Fisher Scientific, Table 1). Printed dishes went through repeated sterilization trials to test whether successful sterilization, if achieved, was reliably replicated across time. Our results suggest that 70% ethanol is not effective over short time scales (2 to 30 min soaks), and that autoclaving is likely effective but can lead to warping.

Methods

Plastic selection

We chose three plastics that differed in cost, thermal tolerance, and print method: polylactic acid (PLA), acrylonitrile butadiene styrene (ABS), and surgical guide (SG) resin. PLA (from PolyLite) and ABS (from Ultimaker) are two cheap, simple to print, and commonly printed plastics (Table 1). Practically all 3D printers which use filaments can easily print one of these two plastics, so they should be accessible to almost anyone with access to a printer (Ngo et al. 2018). PLA is a biodegradable plastic made from plant material (e.g. corn starch) familiar to

bioresearch as it can be used for medical equipment such as short-term implants (McKeen 2014). ABS is a recyclable petroleum-based plastic with higher thermal tolerances than PLA and good toughness and impact resistance (Kim and Kang 1995, Olivera et al. 2016). Despite ABS' higher thermal tolerance neither it nor PLA is autoclavable. SG resin (from Formlabs) is a more expensive, medical-grade plastic (i.e. biocompatible; Table 1), with a high enough thermal tolerance that it can be autoclaved.

These three plastics also differ in print method. ABS and PLA are printed by fused deposition (FD) modeling, whereas SG resin is printed via stereolithography. Both methods can largely print the same designs but use different plastics and can influence features of the final product such as surface texturing or print resolution. Plastics printed via FD modelling (e.g. PLA, ABS) start as solid filaments, which are heated and extruded by the printer (Ngo et al. 2018). These plastics generally become viscous at relatively low temperatures, making them straightforward to print but unsuitable for use at high temperatures (e.g. autoclaving). In contrast, stereolithography uses a UV laser to solidify liquid resin into shape (Ngo et al. 2018). Stereolithography can print plastics with high thermal tolerances without needing to generate high heat, but products must generally be postprocessed to remove potentially toxic print residues and achieve optimal performance qualities such as autoclavability (Leonhardt et al. 2016, Macdonald et al. 2016). Stereolithography printers and high-performance resins are also generally more expensive than FD modelling printers and basic plastic filaments.

Table 1: Plastics used in the experiment. FD (fused deposition) modeling printers heat and extrude plastic. Stereolithography printers solidify liquid plastic resin using a UV laser. Estimated costs are if the plate is made in-house and do not include the cost of the printer (i.e. are for plastic only).

Plastic	Print method	Estimated cost per plate (CAD)	Autoclavable
Polystyrene (standard dish)	Purchased	$0.15	No
Polylactic Acid (PLA)	FD modelling	$0.61	No
Acrylonitrile butadiene styrene (ABS)	FD modelling	$0.93	No
SG Resin	Stereolithography	$8 to $11	Yes

Plate design

Our design was based on standard 100 mm-diameter petri dishes, making plastic the key difference between printed and supplier-bought dishes. However, we made some structural changes to improve the stability of the printed dishes. We externally thickened the base (2.0 mm) and added an internal ring where the base joins the wall for support (designs made with Autodesk Fusion 360; Figure 1). These features did not perceptibly change lid fit, and the ring did not extend above the agar pour line and so did not affect the overall agar surface area (~22 mL agar/plate). The surface texture of the dishes varied between plastic types due to differences between print methods and printers; PLA and ABS dishes had more apparent surface texturing than SG resin dishes. We printed 5 dishes out of each plastic; we printed the PLA dishes ourselves and outsourced the ABS and SG resin ones. We printed PLA dishes using a Lulzbot Taz 6 printer in the McGill Biology department. ABS dishes were printed using an Ultimaker 2 Extended by The Cube in the McGill Engineering department, and SG resin dishes were printed using a Form2 by FabLab. To ensure that lid fit was not influencing our results, we only printed dish bottoms, and paired them with lids from fresh, pre-sterilized petri dishes.

Sterilization experiments

We tested whether two common sterilization methods, 70% ethanol soaks and autoclaving, could repeatedly sterilize printed dishes. For each method we compared printed dishes to supplier-bought, pre-sterilized dishes (100 mm, from Fisher Scientific). 70% ethanol is a common laboratory cleaning agent used to sterilize surfaces and equipment in a wide range of disciplines and work environments. While ethanol-resistant microorganisms do exist, 70% ethanol is considered a broadly effective method for laboratory cleaning (Andersen 2005), and is safe for plastics with low thermal tolerances. Autoclaving uses heat and high pressure to kill

contaminants and is generally considered the "gold standard" for relatively quick and thorough sterilization. While more broadly effective than 70% ethanol, autoclaving is not an option for most low-cost printed plastics as it will cause them to warp or melt.

Ethanol: We soaked printed dishes and their paired lids (taken from supplier bought Petri dishes) in 70% ethanol for two min (7 trials with 5 printed plates per plastic, and 5 controls). Dishes and lids were submerged two at a time for two 1-min intervals (hereafter '2 min soaks') with a quick shake-off between to remove any small bits of residual agar or dust; we used fresh control dishes each trial and they were not processed in any way before plating. We let the washed items dry in a biosafety cabinet until the 70% ethanol had evaporated. Once items were dry, we poured ~22 mL of LB agar (Appendix 1) into each printed and control dish. Starting with the third trial we randomized pour order and lid-printed dish pairings. After the agar solidified, we incubated the dishes at 25˚C for 24 h then checked for visible bacterial or fungal growth (i.e. contamination) on top of or within the agar. For all trials after the initial one we washed each dish and reused lid with soap and water prior to the ethanol soak to remove residual agar or dust. For the first and second trial we only had 4 ABS dishes because the wall of one cracked slightly before we began work. The broken plate was replaced with a new one for later trials.

Early results indicated that 2 min ethanol soaks of dishes and lids were not effective (Fig. 2); as such, we performed three one-off tests to explore where the ethanol sterilization failed. Unless otherwise specified, for all follow-up tests we used 3 supplier-bought dishes as controls and incubated all plates at 25˚C for 24 h before checking for visible contamination.

Follow-up 1: Much of the observed contamination was on the surface of the agar, pointing to the reused lids a potential source of contamination. To test this, we compared the sterility of printed

plates given fresh vs. reused lids to controls. We cleaned 4 plates per plastic using the standard 2 min ethanol soaks, with 2 plates per plastic receiving completely fresh lids and the other two receiving reused lids (2 plates per plastic per lid treatment). The results suggested that fresh lids did not improve sterility, so the subsequent follow-ups all used fresh lids to isolate the effects of different approaches on printed plastics.

Follow-up 2: Prior studies suggest that longer contact time between plastic items and cleaning agents improves sterilization (e.g. Guerra et al. 2018). As such, we compared 2 x 3 min (hereafter '6 min soaks') to 2 min soaks and control plates. We divided the printed plates as per Follow-up 1 (2 printed plates per plastic per soak condition).

Follow-up 3: We then tested whether even longer contact times improved sterilization. To do this, we compared 30-minute soaks to our standard 2 min soaks. The 30-minute soaks were uninterrupted to maximize contact time. We divided the printed plates as per Follow-up 1 (2 printed plates per plastic per soak condition).

Follow-up 4: Finally, we tested whether the ethanol soak itself introduced contamination to the printed dishes. We soaked 4 pre-sterilized, supplier-bought dishes in 70% as per our standard procedures (2 min soaks) and compared their sterility to 4 untreated control dishes. All plates were incubated at 25˚C and checked every 24 h for visible growth up to 48 h.

Autoclave: We tested whether autoclaving could repeatedly sterilize the SG resin dishes (7 trials with 5 printed plates and 5 controls). SG resin dishes were autoclaved for 25 min at 121˚C, then allowed to cool in the biosafety cabinet with fresh lids on. We then poured ~22 mL LB agar into the cooled SG resin dishes and the untreated control dishes. We used fresh control plates and lids

for the SG resin dishes each round. For all trials after the initial one we washed each printed plate with soap and water prior to autoclaving to remove residual agar.

We conducted two rounds of trials. For the first 3 trials (winter 2019, pre COVID-19 shutdowns) dishes were incubated at 25˚C for 48 h, then checked for visible bacteria or fungal contamination. There was light bacterial contamination on some of the control dishes from these trials, meaning some step in our workflow was introducing contamination. After several weeks of trial and error and equipment cleaning, we determined that the contamination was coming from the biosafety cabinet (BSC), which we eventually managed to decontaminate. However, before we could redo the experiment, the university suspended research due to the COVID-19 pandemic. For the last four trials (July 2020, after labs reopened) dishes were incubated for 72 h and checked every 24 h for contamination. In the first of these July trials all plates were heavily contaminated with mold. We thoroughly cleaned the incubator after this trial and no further mold contamination occurred; data from the mold-contaminated trial are not included in results. However, light contamination from the BSC continued. There was not enough time to decontaminate it before the thesis submission deadline, so results from the autoclaving experiments are unfortunately not definitive.

Data summarization

We recorded whether each dish had any signs of bacteria or fungal growth and the extent and character of growth. We did not perform any statistical analyses; for our purposes it does not matter how contaminated plates are, nor whether larger or smaller fractions of plates are contaminated among treatments or plastic types. One contaminant may be sufficient to derail an entire project, so we treated all contamination events with equal severity. As long as control

plates were clean, any contamination on printed plates indicated that the sterilization method was not effective enough for experimental use.

Results

Ethanol

The 70% ethanol soaks did not consistently sterilize the printed dishes. Printed dishes were frequently contaminated, sometimes severely so (Figure 2). Contamination ranged from one or two bacterial colonies along the plate periphery to hundreds of colonies covering most of the agar. In some cases, colonies grew within or under the agar along the dish bottom. Contamination became more prevalent over time, but this coincided with reduced agar drying as we resolved some incubator difficulties (Figure 3). As such, it is possible that contamination was equally present in early trials but undetected as drier agar is not as conducive to growth. However, since even initial sterilization was not consistently effective, whether contamination increased across consecutive trials in unimportant. There was never visible growth on the control plates (standard pre-sterilized petri dishes) after the 24 h incubation period (Figure 3).

Our follow-up trials showed that fresh lids and longer ethanol soaks did not prevent contamination, and that the ethanol soaks were unlikely culprits. Printed plates with fresh lids were all contaminated after 24 h, as were the printed dishes with reused lids. This strongly suggests contamination was from the dishes; these results to do not rule out reused lids as a source of contamination but demonstrate that some contamination is present on the plates themselves. Longer soaks were equally ineffective; all printed dishes were visibly contaminated 24 h after 6 or 30 min soaks, as well as the plates they were compared to (2 min soaks). The 4 standard petri dishes soaked in 70% ethanol were visibly clean after five days, suggesting the ethanol baths themselves were not the source of contamination. All control plates were visibly

clean for all follow-up trials except for one from the 6 min trial, the only time this happened across all ethanol trials.

Autoclave

Autoclave trials were promising but confounded by contamination in the biosafety cabinet, with the same low levels of contamination on both the control and SG resin plates (Figure 4). When present, there were generally 1 to 4 small colonies per contaminated plate after 48 h on both control and SG resin dishes. The 72 h tests showed similar results, with similar contamination present on both plate types (Figure 4). Tests where we left open control (manufacturer-sterilized) dishes filled with agar in the biosafety cabinet strongly indicated that the cabinet was introducing contamination. None of the contamination events mirrored the rampant growth seen on the ethanol-soaked dishes, and all contamination in the autoclave trials occurred on the agar surface only (not under the agar as occurred after ethanol cleaning). This corroborates our deduction that contamination in the autoclave trials was coming from the biosafety cabinet rather than the plates themselves.

Repeated autoclaving did cause slight warping of the SG resin plate walls. The walls of two SG resin dishes warped slightly during the initial three autoclave trials, and all five plates showed signs of warping after the second round of four trials. Warping did not prevent lids from fitting properly, but it is possible that continued autoclaving would eventually make the dishes unusable. Warping seemed restricted to the walls, which were the thinnest parts of the plate (0.7mm thick); the thicker bases were not visibly altered. This suggests that warping is a product of design rather than an inherent limitation of the plastic.

Discussion

We tested whether 3D printed petri-style dishes could be repeatedly and conveniently sterilized, a requirement if printed items are to integrate into many bioresearch areas. Our results are discouraging. Short soaks (2 up to 30 min) in 70% ethanol were ineffective (Figures 2, 3). Autoclaving was likely effective, though results were confounded by contamination from the biosafety cabinet. Autoclaving is *the* standard for sterilization in both bioresearch and medical applications, so it is highly unlikely that some property of SG resin prevented its success. However, even if successful at sterilization, autoclaving limits plastic choices and production methods to more expensive options, and limits designs to pieces where all parts are thick enough to repeatedly withstand heat and pressure without warping. As such, 3D printed items are not currently mainstream replacements for bulk single-use plastic items.

Why did ethanol sterilization fail when ethanol is a commonly used lab-cleaning product? We believe the primary answer is the surface texturing of 3D printed items. Unlike standard petri dishes and other mass-produced plastic items which are shaped by smooth molds, printed items have many small surface imperfections left by the printing process (pores and crevices, detectable visually or by feel) which can provide refugia for contaminants (Muro-Fraguas et al. 2020). These imperfections may be sealed, for example by atmospheric plasma polymerization; however, this process involves specialized equipment likely unavailable to the average laboratory and reduces but does not eliminate growth (Muro-Fraguas et al. 2020). In our case with unsealed items, this surface texturing could partially explain why contamination appeared to increase over time: the more a dish is used the more contaminants can build up in these refugia (Figure 2). Even if sterilization is sometimes effective, repeated use of unsealed items would almost inevitably lead to contamination at some point.

Longer ethanol soaks may be more effective but are less attractive from a logistical standpoint. Far longer soaks than what we tried (e.g. 8 h) increase contact time between items and ethanol, and were effective for initial sterilization of polycaprolactone items printed by a 3D Tubular Printer (Guerra et al. 2018). However, these longer soaks have not been tested for repeated sterilization of the same item. Even if long soak times prove effective for repeat sterilization, they will be too restrictive for many labs from a time, space, and material (i.e. volume of ethanol) perspective. As such, items printed with non-autoclavable plastics are probably poor bulk alternatives to single-use items if lengthy sterilization demands considerable additional space and resources or slows the pace of research.

In our experiments we did see contamination on the autoclaved dishes (Figure 4), but we believe this was from contaminants in the biosafety cabinet and not an issue with autoclaving per se. Autoclavable plastics are attractive from a sterilization standpoint but are generally expensive and complex to print as high temperatures are required to sufficiently soften the printable filament. The most accessible way to print autoclavable items is using stereolithography, but these machines and autoclavable resins are generally much more expensive than FD modeling printers and non-autoclavable filaments (Table 1). Most FD modeling printers cannot generate enough heat to print autoclavable plastics, and those which can are large industrial-grade machines not practical for most individual labs to own. Prints can be outsourced to specialized printing companies, but this can quickly become expensive for large orders. Restriction to stereolithography also raises downstream issues as resins can leave behind toxic residues, meaning items should be post-processed (i.e. washed, cured, etc.) before use with living organisms (Zhu et al. 2015, Leonhardt et al. 2016). Because of these limitations, autoclavable

plastics are also unlikely to broadly replace single-use plastic items in the near future, but are still promising for experiments which need only a few units or require custom items.

Autoclaving does have the drawback of potentially warping printed items, but the degree of warping is likely to vary between plastics, item designs, and autoclave settings. By the end of the autoclave trials the walls of all 5 SG resin plates had visibly warped. While this did not affect lid fit in our trials it could introduce issues for equipment which requires precise dimensions. However, we only tested one autoclavable plastic on one autoclave setting. It is likely that different autoclavable plastics will respond differently to repeated use, and that different autoclave setting may reduce or increase warping. Further stress tests using more autoclavable plastics, a wider range of item thickness, and different autoclave settings could better clarify when warping would be an issue.

In conclusion, 3D printing is unlikely to solve the research waste generated by microbiology studies, and bioresearch in general, as the most accessible plastics are not easily sterilizable. Nevertheless, 3D printing still shows promise for more specialized applications where customization is more important than bulk output. An example is the rapidly emerging use of laboratory “micro-landscapes” to study how landscape patterns shape species distributions (e.g. Baym et al. 2016, Ochocki and Miller 2017, Kurkjian 2018), where the ability to create customized and repeatable configurations of ‘habitat patches’ would be immediately useful. 3D printing technology is also rapidly advancing, and it is possible that autoclavable plastics will be cheaper and easier to print in the near future. Until then, labs wishing to reduce their plastic waste could always re-consider glassware, as common items like petri dishes are still readily available in glass and custom items can be produced with the help of specialists.

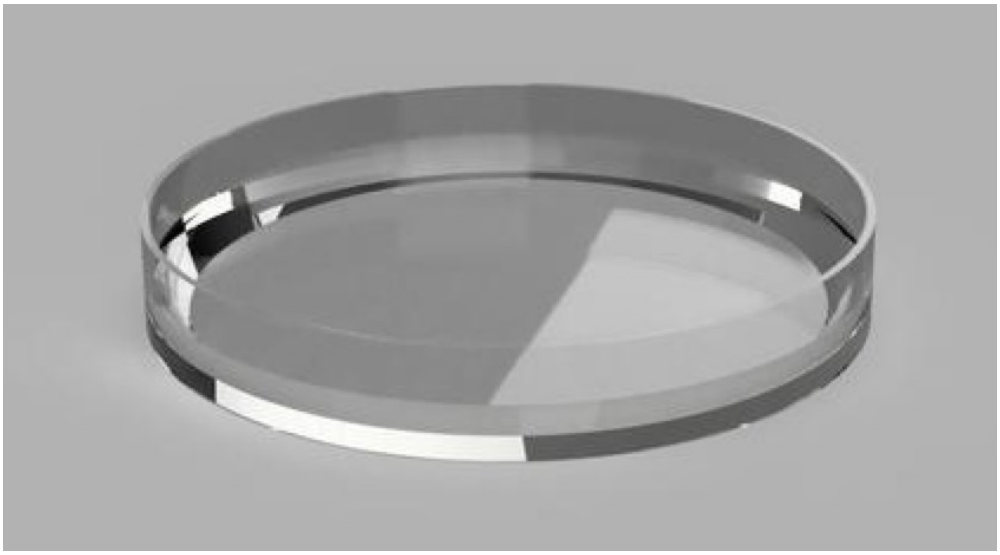

Figure 1: Rendered image of printed dish. Internal diameter (87 mm), internal wall height (12 mm), and wall thickness (0.7 mm) were based on dimensions given by suppliers of standard 100 mm petri dishes. Actual plates differed in color depending on plastic type (Figure 2). This rendering was made using Autodesk Fusion 360.

Figure 2*:* Examples of contamination on 3D-printed dishes following 70% ethanol cleaning (standard 2 x 1 min soak). From left: PLA, ABS, SG resin. Contamination severity was highly variable, ranging from small isolated colony(ies) (left) to colonies covering half the plate (middle).

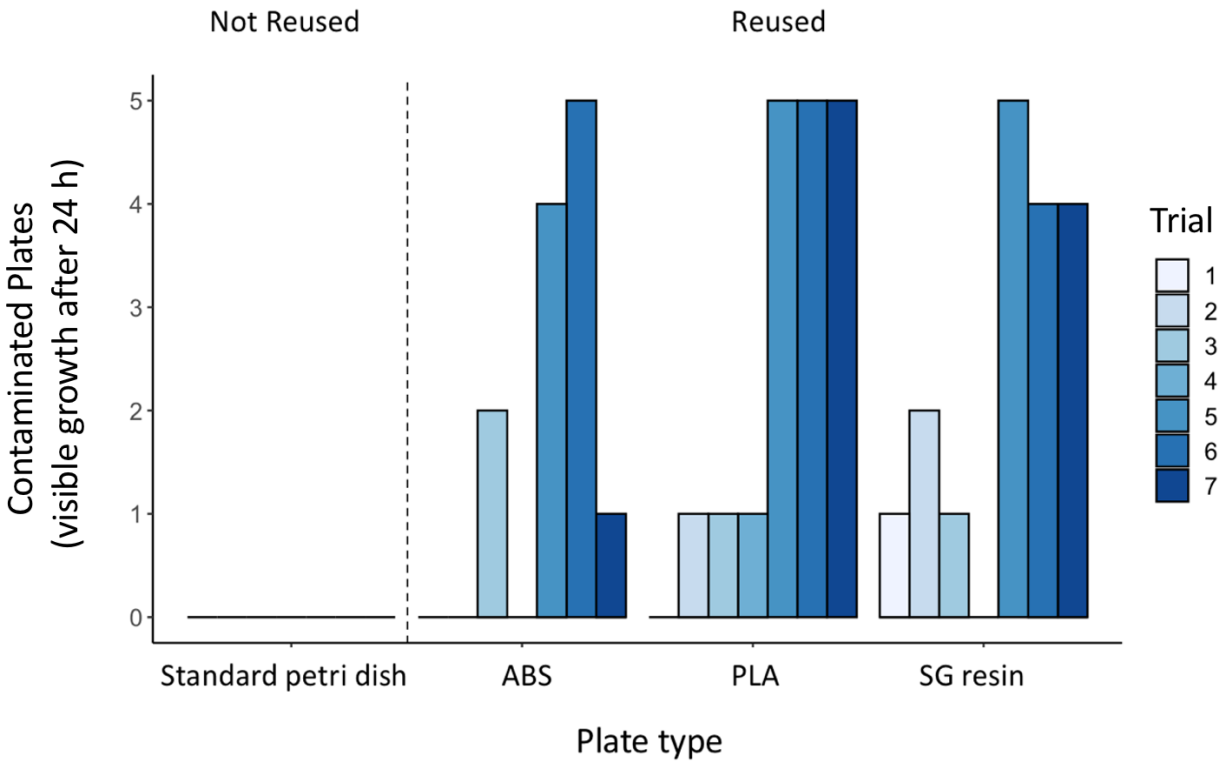

Figure 3: Results from the 2 min 70% ethanol trials showing the number of contaminated dishes per trial (n = 5 dishes per plastic). New standard petri dishes were used each trial, printed dishes and their paired lids were reused (i.e. trial 2 is the 2nd time dishes were cleaned, trial 3 is the 3rd use, etc.). For trials 1 and 2 there were only 4 ABS dishes.

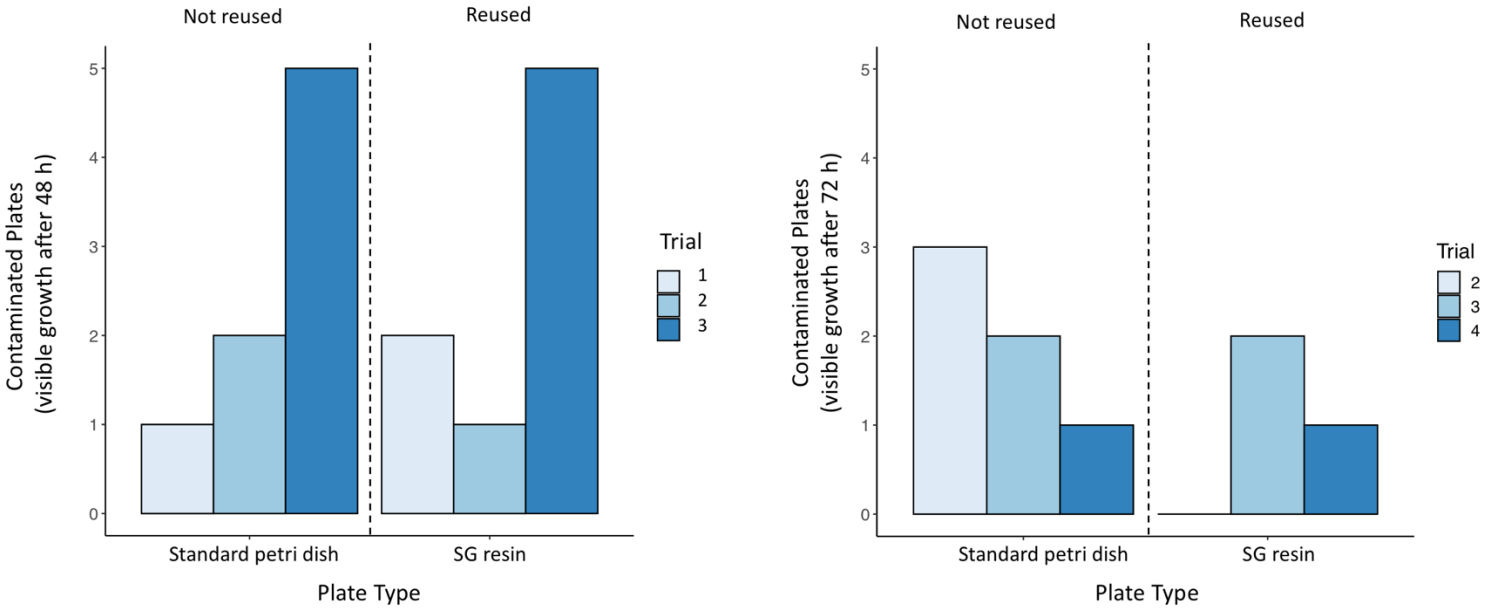

Figure 4: Results from the two (48, 72 h incubation) autoclave tests showing the number of contaminated plates per trial (n = 5 plates per plastic). New standard petri dishes were used each trial, SG resin plates were reused (i.e. trial 2 is the 2nd time dishes were cleaned, trial 3 is the 3rd use, etc.). We only show trials 2-4 from the 72 h tests because the first trial was completely contaminated by mold due to an incubator issue.

Literature Cited

Andersen, R. A. 2005. Algal Culturing Techniques. - Elsevier.

Baym, M. et al. 2016. Spatiotemporal microbial evolution on antibiotic landscapes. - Science 353: 1147–1151.

Guerra, A. J. et al. 2018. Effects of different sterilization processes on the properties of a novel 3D-printed polycaprolactone stent. - Polym. Adv. Technol. 29: 2327–2335.

Hopewell, J. et al. 2009. Plastics recycling: challenges and opportunities. - Phil. Trans. R. Soc. B 364: 2115–2126.

Kadilak, A. L. et al. 2017. A 3D-printed microbial cell culture platform with *in situ* PEGDA hydrogel barriers for differential substrate delivery. - Biomicrofluidics 11: 054109.

Kim, J. K. and Kang, C. K. 1995. Basic studies on recycling of ABS resin. - Polym. Plast. Technol. Eng. 34: 875–890.

Kurkjian, H. M. 2018. The metapopulation microcosm plate: A modified 96-well plate for use in microbial metapopulation experiments. - Methods Ecol. Evol. 10: 162–168.

Lenski, R. E. and Travisano, M. 1994. Dynamics of adaptation and diversification: a 10,000-generation experiment with bacterial populations. - PNAS 91: 6808–6814.

Lenski, R. E. et al. 1991. Long-term experimental evolution in *Escherichia coli.* I. Adaptation and divergence during 2,000 generations. - Am. Nat. 138: 1315–1341.

Leonhardt, S. et al. 2016. Biocompatibility of photopolymers for additive manufacturing. - Current Directions in Biomedical Engineering 2: 113–116.

Macdonald, N. P. et al. 2016. Assessment of biocompatibility of 3D printed photopolymers using zebrafish embryo toxicity assays. - Lab on a Chip 16: 291–297.

McKeen, L. W. 2014. 3 - Plastics used in medical devices. - In: Modjarrad, K. and Ebnesajjad, S. (eds), Handbook of Polymer Applications in Medicine and Medical Devices. Plastics Design Library. William Andrew Publishing, pp. 21–53.

Muro-Fraguas, I. et al. 2020. Atmospheric pressure cold plasma anti-biofilm coatings for 3D printed food tools. - Innov. Food Sci. Emerg. Tech. 64: 102404.

Neches, R. Y. et al. 2016. On the intrinsic sterility of 3D printing. - PeerJ 4: e2661.

Ngo, T. D. et al. 2018. Additive manufacturing (3D printing): A review of materials, methods, applications and challenges. - Compos. Part B-Eng. 143: 172–196.

Ochocki, B. M. and Miller, T. E. X. 2017. Rapid evolution of dispersal ability makes biological invasions faster and more variable. - Nat. Commun. 8: 14315.

Olivera, S. et al. 2016. Plating on acrylonitrile–butadiene–styrene (ABS) plastic: a review. - J Mater. Sci. 51: 3657–3674.

Ritchie, H. and Roser, M. 2018. Plastic pollution. - Our World in Data, retrieved from https://ourworldindata.org/plastic-pollution.

Urbina, M. A. et al. 2015. Environment: Labs should cut plastic waste too. - Nature 528: 479.

Zhu, F. et al. 2015. Assessment of the biocompatibility of three-dimensional-printed polymers using multispecies toxicity tests. - Biomicrofluidics 9: 061103.

Conclusion

Perhaps unsurprisingly, the vast majority of micro-landscape studies we reviewed were led by laboratory researchers, with field ecologists mostly absent. Hopefully more ecologists will look to the laboratory to supplement their field work, thus bringing their unique insights to micro-landscape experiments. However, ecologists planning to use micro-landscapes would do well to find a collaborator, as adopting a new system can pose significant and unforeseen challenges. Those new to model organisms would be unlikely to fully leverage their potential without informed advice or laborious trial and error. Partnerships between ecologists and laboratory scientists could thus be highly fruitful as the latter's experience with the system would help neophytes gauge the system's potential and its limitations for testing specific ecological theories, all while avoiding pitfalls.

The strengths and weaknesses of a chosen system should be deeply explored during the design process. All micro-landscapes and organisms come with inherent costs and benefits, meaning a project's success can hinge on which setup and study species one selects. Broad things to consider are how organisms will move around the landscape (manual transfer, corridors, free movement), if/how nutrients will be replenished, and how the chosen species reproduces (a/sexually, generation time, etc.). Furthermore, experiments can use similar landscape designs but yield different insights. Take as an example range expansions into linear habitat, which have been studied using protists and beetles. Both systems are superficially similar from a design perspective but are suited to ask different questions. Protists are useful for studying de novo mutation because they reproduce rapidly and asexually (e.g. Fronhofer and Altermatt 2015), whereas beetles are useful for studying standing genetic variation and drift in sexually reproducing populations (e.g. Weiss-Lehman et al. 2019). Incorporating expert advice

will help ensure that studies successfully match micro-landscape design and model organism selection to the theory being tested.

In addition to exploring new collaborations and model organisms, increasing design flexibility will help expand micro-landscape capabilities. 3D printing may be a good solution for modest-sized micro-landscapes, although the process is unlikely to replace bulk single-use items like petri dishes anytime soon. While sterility concerns limits 3D printing's broad appeal, many lab organisms do not require fully sterile environments (e.g. beetles); these systems could benefit from customization and cheap plastics without worrying about contamination. The more expensive autoclavable plastics may be situationally useful when only a few landscapes are required as the cost of printing only a few items would be much less prohibitive. Despite sterility and cost issues there is clearly a niche for 3D printing in bioresearch, though just how big this niche is remains uncertain. Future studies could explore 3D printing for non-sterile purposes, test other non-autoclave sterilization methods, or experiment with a wider range of autoclavable plastics. Additionally, other custom manufacturing processes (e.g. glass blowing) could be useful in expanding the scope of micro-landscapes.

Micro-landscapes provide a unique platform for collaborative research which could benefit both field and laboratory scientists. The systems provide empirical tests for theories currently impossible to test at the landscape level and create novel setups which could be useful for a wide range of other laboratory applications beyond testing ecological theory. There are near limitless, and currently largely untapped, potential collaborations where ecologists and lab scientists combine their theoretical and experimental expertise to tackle complex questions. Micro-landscapes thus present unique and exciting opportunities to conduct truly interdisciplinary research.

Literature Cited – sources used outside manuscripts

Altermatt, F. et al. 2015. Big answers from small worlds: a user's guide for protist microcosms as a model system in ecology and evolution. - Methods Ecol. Evol. 6: 218–231.

Barrett, R. D. H. et al. 2005. Experimental Evolution of Pseudomonas fluorescens in Simple and Complex Environments. - Am. Nat. 166: 470–480.

Baym, M. et al. 2016. Spatiotemporal microbial evolution on antibiotic landscapes. - Science 353: 1147–1151.

Brown, J. H. 1995. Macroecology. - University of Chicago Press.

Clausen, J. et al. 1940. Experimental studies on the nature of species. III. Environmental responses of climatic races of *Achillea*. - Carnegie Insitution of Washington, Washington DC.

Freeman, B. G. et al. 2018. Expanding, shifting and shrinking: The impact of global warming on species' elevational distributions. - Global Ecol. Biogeorg. 27: 1268–1276.

Friedenberg, N. A. 2003a. Experimental evolution of dispersal in spatiotemporally variable microcosms. - Ecol. Lett. 6: 953–959.

Friedenberg, N. A. 2003b. Determinism in a transient assemblage: The roles of dispersal and local competition. - Am. Nat. 162: 586–596.

Fronhofer, E. A. and Altermatt, F. 2015. Eco-evolutionary feedbacks during experimental range expansions. - Nat. Commun.. 6: 6844.

Gray, J. M. et al. 2005. A circuit for navigation in *Caenorhabditis elegans*. - PNAS 102: 3184–3191.

Gualandi, C. et al. 2012. Ethanol disinfection affects physical properties and cell response of electrospun poly(l-lactic acid) scaffolds. - European Polymer Journal 48: 2008–2018.

Guerra, A. J. et al. 2018. Effects of different sterilization processes on the properties of a novel 3D-printed polycaprolactone stent. - Polym. Adv. Technol. 29: 2327–2335.

Hargreaves, A. L. et al. 2014. Are species' range limits simply niche limits writ large? A review of transplant experiments beyond the range. - Am. Nat. 183: 157–173.

Holyoak, M. and Lawler, S. P. 1996. The role of dispersal in predator--prey metapopulation dynamics. - J. Anim. Ecol. 65: 640–652.

Holyoak, M. and Lawler, S. P. 2005. The Contribution of laboratory experiments on protists to understanding population and metapopulation dynamics. - In: Advances in Ecological Research. Population Dynamics and Laboratory Ecology. Academic Press, pp. 245–271.

Jessup, C. M. et al. 2004. Big questions, small worlds: microbial model systems in ecology. - Trends Ecol. Evol. 19: 189–197.

Jessup, C. M. et al. 2005. Microbial experimental systems in ecology. - In: Advances in Ecological Research. Population Dynamics and Laboratory Ecology. Academic Press, pp. 273–307.

Kadilak, A. L. et al. 2017. A 3D-printed microbial cell culture platform with *in situ* PEGDA hydrogel barriers for differential substrate delivery. - Biomicrofluidics. 11: 054109.

Kassen, R. and Bell, G. 1998. Experimental evolution in *Chlamydomonas*. IV. Selection in environments that vary through time at different scales. - Heredity: 10.

Kurkjian, H. M. 2018. The metapopulation microcosm plate: A modified 96-well plate for use in microbial metapopulation experiments. - Methods Ecol. Evol. 10: 162–168

Larsen, C. D. and Hargreaves, A. L. 2020. Miniaturizing landscapes to understand species distributions. - Ecography in press.

Lemoine, N. P. et al. 2016. Underappreciated problems of low replication in ecological field studies. - Ecology 97: 2554–2561.

MacArthur, R. H. 1984. Geographical ecology: Patterns in the distribution of species. - Princeton Univ. Press.

Massot, M. et al. 2008. Climate warming, dispersal inhibition and extinction risk. - Glob. Chang. Biol. 14: 461–469.

McCarty, J. P. 2001. Ecological consequences of recent climate change. - Conservation Biology. 15: 320–331.

Merriam, C. H. 1894. Laws of temperature control of the geographic distribution of terrestrial animals and plants. - Harvard Uni.

Neches, R. Y. et al. 2016. On the intrinsic sterility of 3D printing. - PeerJ 4: e2661.

Ngo, T. D. et al. 2018. Additive manufacturing (3D printing): A review of materials, methods, applications and challenges. - Compos. Part B-Eng 143: 172–196.

Ochocki, B. M. and Miller, T. E. X. 2017. Rapid evolution of dispersal ability makes biological invasions faster and more variable. - Nat. Commun. 8: 14315.

O'Malley, M. A. et al. 2015. How do microbial populations and communities function as model systems? - Q. Rev. Biol. 90: 269–293.

Pironon, S. et al. 2017. Geographic variation in genetic and demographic performance: new insights from an old biogeographical paradigm. - Biol. Rev. 92: 1877–1909.

Pradhan, S. et al. 2019. Environmental programming of adult foraging behavior in *C. elegans*. - Current Biology 29: 2867-2879.e4.

Rainey, P. B. and Travisano, M. 1998. Adaptive radiation in a heterogeneous environment. - Nature. 394: 4.

Sexton, J. P. et al. 2009. Evolution and ecology of species range limits. - Annu. Rev. Ecol. Sys. 40: 415–436.

Tan, J. et al. 2017. Spatial storage effect promotes biodiversity during adaptive radiation. - Proc Biol Sci. 284: 1858.

Tekwa, E. W. et al. 2015. Patchiness in a microhabitat chip affects evolutionary dynamics of bacterial cooperation. - Lab on a Chip 15: 3723–3729.

Urbina, M. A. et al. 2015. Environment: Labs should cut plastic waste too. - Nature 528: 479.

Warren, P. H. 1996. Dispersal and destruction in a multiple habitat system: An experimental approach using protist communities. - Oikos 77: 317–325.

Weiss-Lehman, C. et al. 2019. Stochastic processes drive rapid genomic divergence during experimental range expansions. - Proc. R. Soc. B286: 20190231.

Williams, J. L. and Levine, J. M. 2018. Experimental evidence that density dependence strongly influences plant invasions through fragmented landscapes. - Ecology 99: 876–884.

Appendix

Table A1: LB Agar recipe used in Chapter 2.

Ingredient	Amount (g/L)
Glycerol	10
Proteose Peptone 3	20
K_2HPO_4	1.5
$MgSO_4*7H_2O$	1.5
Agar	15