THE ROLE OF PRIORITY IN DETERMINING THE RESTRICTED DISTRIBUTION OF TETRAHYMENA THERMOPHILA

Authors: McGee, Amberly, and Zufall, Rebecca A.

Source: The Southwestern Naturalist, 66(1) : 1-4

Published By: Southwestern Association of Naturalists

URL: https://doi.org/10.1894/0038-4909-66.1.1

The Southwestern Naturalist 66(1): 1–4 March 2021

THE ROLE OF PRIORITY IN DETERMINING THE RESTRICTED DISTRIBUTION OF *TETRAHYMENA THERMOPHILA*

Amberly McGee and Rebecca A. Zufall*

Department of Biology and Biochemistry, University of Houston, Houston, TX 77204, USA
**Correspondent: rzufall@uh.edu*

Abstract—Species distributions may be determined by a variety of biotic and abiotic factors. The model ciliate *Tetrahymena thermophila* exhibits an unusual distribution limited to the eastern United States. In this study, we assess the roles of species interactions and habitat requirements in driving this restricted distribution by studying laboratory populations of *T. thermophila* and *Tetrahymena gruchyi.* We find that priority effects and habitat requirements are likely to be important factors driving the distribution of *Tetrahymena* species.

Resumen—Las distribuciones de especies pueden determinarse por una variedad de factores bióticos y abióticos. El modelo ciliar *Tetrahymena thermophila* exhibe una distribución rara limitada al este de los Estados Unidos. En este estudio, evaluamos las funciones de las interacciones de especies y los requisitos de hábitat que determinan esta distribución restringida mediante el estudio de las poblaciones de *T. thermophila* y *T. gruchyi* en el laboratorio. Encontramos que los efectos prioritarios y los requisitos de hábitat probablemente sean factores importantes que determinan la distribución de las especies de *Tetrahymena.*

There are a large number of factors that can affect a species' distribution. One of these factors involves how a species interacts with other closely related species. For example, closely related species may share similar habitat requirements and thus must compete for resources (reviewed in Webb et al., 2002). This could result in a more competitive species displacing another species or in inhibitory priority effects where an already established species excludes establishment of another species (reviewed in Fukami, 2015). Alternatively, closely related species may thrive under different environmental conditions due to divergence in habitat use among species (Webb et al., 2002).

Tetrahymena is a genus of ciliates, microbial eukaryotes, that are broadly distributed and found in freshwater habitats worldwide (Simon et al., 2008; Doerder and Brunk, 2012; Doerder 2019). *Tetrahymena* species are often morphologically indistinguishable, but >80 species have been identified based on genetic distance at the *cox1* barcode locus (Doerder, 2019). Although many *Tetrahymena* species appear frequently in collections worldwide, other species are restricted to particular geographic areas.

Of particular interest, the model ciliate *Tetrahymena thermophila* has a distribution largely restricted to the eastern United States (Simon et al., 2008; Zufall et al., 2013). The reason that *T. thermophila* has this restricted distribution has no clear explanation because there are no obvious biogeographic barriers or habitat requirements that should drive this distribution.

Here, we begin to test the effects of species interactions and local environmental conditions in driving the restricted distribution of *T. thermophila* by comparison with the closely related and widely distributed species *T. gruchyi. Tetrahymena gruchyi* is found worldwide, including in ponds also containing *T. thermophila* (Doerder, 2019). *Tetrahymena thermophila* is not found in southeastern Texas, where this experiment was performed.

In this study, we tested three possible factors that contribute to the absence of *T. thermophila* in southeastern Texas: competition with another *Tetrahymena* species, priority effects, and habitat requirements. To study these factors, we cultured *T. thermophila* and *T. gruchyi* in controlled microcosms under various conditions. We find that priority effects are important in determining which species persists under favorable growth conditions. However, we also find that environmental conditions play an important role in that *T. thermophila* do not survive well in Texas pond water, whereas *T. gruchyi* grows much better in this environment.

Materials and Methods—*Isolate Collection and Culturing*—K. L. Dimond collected *T. thermophila* strain NH0127-2 in New Hampshire, and identified it as described in Zufall et al. (2013; *cytochrome oxidase 1* (*cox1*) GenBank accession MN481562). NH0127-2 was used in all experiments as the focal strain. This strain is 99.8% identical to previously described *T. thermophila* from New England (JX406164). We collected *T. gruchyi* (previously *T.* sp. nsp7; Doerder, 2019) strain SLAM1 from Sheldon Lake State Park (Harris Co., Texas) and identified it as described in Zufall et al. (2013; *cox1* GenBank accession MN481563). SLAM1 is 99.8% identical at *cox1* to *T. gruchyi* from Doerder (2014; KJ028685) and is amicronucleate.

Briefly, we collected water samples from ponds and enriched

Table 1—Outcomes of experimental populations.

Culture condition	Species addition regime[a]	Final species present (no. of replicates)
2% BP, 12-well plate	C	*Trahymena gruchyi* ($n = 3$)
	T	*T. thermophila* ($n = 3$)
	G	*T. gruchyi* ($n = 3$)
2% BP, petri dish	C	*T. thermophila* ($n = 3$)
	T	*T. thermophila* ($n = 3$)
	G	*T. gruchyi* ($n = 3$)
Pond water, petri dish	C	*T. gruchyi* ($n = 1$)
	T	NA
	G	*T. gruchyi* ($n = 2$)

[a] C = populations in which both species were added at the same time. T = populations in which *T. thermophila* was introduced first. G = populations in which *T. gruchyi* was introduced first; NA = not applicable.

them with a selection medium of proteose peptone and antibiotics (Doerder and Brunk, 2012). We isolated single *Tetrahymena*-like cells microscopically and grew clonal lines for DNA extraction. We polymerase chain reaction–amplified the mitochondrial marker *cox1* using the primers in Zufall et al. (2013) and compared resulting sequences against GenBank.

Experimental Populations—We maintained experimental populations under three conditions: (1) 2.5 mL of 2% bacterized peptone (bacterized with *Klebsiella pneumoniae*; 2% BP; Cassidy-Hanley, 2012) in wells of 12-well plates at 24°C, (2) 25 mL of 2% BP in petri dishes at 24°C, or (3) 25 mL of filtered pond water in petri dishes at 30°C. We collected pond water from a pond at Sheldon Lake State Park where *T. gruchyi* was found. We filtered water using qualitative filter paper (VWR International, Radnor, Pennsylvania; grade 413, ~5-μm pore size). We confirmed by microscopy that filtering removed all ciliates, but not bacteria or small microbial eukaryotes including nanoflagellates. We supplemented filtered pond water with an autoclave-sterilized rice grain when added to petri dishes. We cultured populations grown in 2% BP at 24°C. We cultured pond water populations at 30°C to support bacterial growth. Both *Tetrahymena* species divide rapidly in rich medium at 24 and 30°C, with 3–4 and 6–7 divisions/day, respectively.

To test which species is a better competitor when both species colonize at the same time, we inoculated three replicate populations of each type with an approximately equal number of *T. thermophila* and *T. gruchyi* cells into an experimental well or plate. We added three individual cells of each species from mid-log phase cultures grown in 2%BP (~10^4 cells/mL) to the 12-well plates; we added 1 mL of each culture to the petri dishes.

To test for priority effects, we inoculated replicate populations with 20 μL (12-well plates) or 2 mL (petri dishes) of mid-log-phase cultures of one species only. We allowed these single-species cultures to establish for 1 week. In the 12-well plates, we then moved 250 μL of each culture to a new well and added five cells of the alternate species. In the petri dishes, we added 1 mL of mid-log-phase culture of the alternate species directly to the established cultures.

We maintained single-species cultures for an additional 2–3 weeks as a control for growth of each species under each condition. We also used these populations to validate our sequencing approach for species monitoring (see below). Both *Tetrahymena* species survived and grew under all conditions, except *T. thermophila* in pond water, where only one of three replicate populations survived.

Species Monitoring—We checked the populations where both species were added at the same time for which species were present after 1 week (for the 2% BP cultures) or 2 weeks (pond water cultures). In all but the pond water populations, both species were still present at this time, so we took a second sample 2 weeks after initiating the populations. We first checked the populations where one species was added 1–1.5 weeks after the second species was added. Our assay for species identification is not quantitative (see below), so we ran all experiments until we detected the presence of only a single species; these results are reported in Table 1.

Tetrahymena thermophila and *T. gruchyi* are morphologically indistinguishable, so we used a sequencing approach to monitor which species were present in each population. We sampled 1 mL of each population and extracted total DNA using standard phenol–chloroform protocols (Ausubel et al., 1998). We polymerase chain reaction–amplified *cox1* as described above. We purified polymerase chain reaction products by gel isolation (QIAquick gel extraction; Qiagen, Venlo, The Netherlands) and sequenced purified products (LoneStar Labs, Houston, Texas). We analyzed sequence chromatograms with Geneious R11 (https://www.geneious.com).

To confirm that we could distinguish the presence of both species, we sequenced our control populations that contained only one species each, and newly initiated competition populations that contained both species. We could clearly distinguish, by multiple peaks in the chromatogram, when both species were present at equal or near equal abundances and had no false positives when only one species was present. However, this approach does not quantify the relative abundance of each species, nor does it detect with certainty whether a species is absent from a population or merely present in a low relative frequency. Thus, our results indicate when both species are present in moderate frequencies or which one species has come to dominate a population. In addition, we do not distinguish live from dead cells, but expect the vast majority of DNA to be from live cells.

Results—*Priority Effects*—In the 2% BP populations, 100% of populations in which one species was added first resulted in the established species dominating the culture at the end of the experiment (Table 1). This was true for both small and large populations (i.e., petri dish and 12-well plate cultures). *Tetrahymena thermophila* did not grow well enough in pond water to determine the effect of introducing one species first in this environment (see below). This result indicates a strong role for inhibitory priority effects resulting in the inability of the later arriving species to grow to high densities. Our experimental design does not allow us to determine whether the later arriving species is completely excluded or merely kept at very low densities. But in either case, it is clear that which species arrives first has a strong effect on the success of later arriving species.

Competition—We found conflicting results in our competition experiments. In the 2% BP populations in

12-well plates, *T. gruchyi* consistently outcompeted *T. thermophila*. However, in the larger 2% BP petri dish populations, *T. thermophila* always outcompeted *T. gruchyi* (Table 1). There is no obvious explanation for these differing results. It is possible that the two species respond differently to oxygen concentration, which is higher in the petri dishes as a result of a larger surface area. Alternatively, differences in inoculation conditions may contribute to different outcomes, with *T. gruchyi* performing better at lower starting densities. *Tetrahymena gruchyi* also outcompeted *T. thermophila* in the petri dishes with pond water, likely because of *T. gruchyi*'s superior ability to grow in this medium (see below).

Environmental Conditions—In 2%B P, both *T. thermophila* and *T. gruchyi* quickly grow to high densities; however, this is not true when grown in filtered pond water. We collected and filtered pond water from the same location from which we collected *T. gruchyi*. Thus, we know that *T. gruchyi* can survive in this water in nature. We found that *T. gruchyi* can also grow in filtered Sheldon Lake pond water in the laboratory; however, not until after adding rice grains to support bacterial growth were the *T. gruchyi* pond water populations able to reach high densities. *Tetrahymena thermophila,* in contrast, struggled to grow at all in the pond water cultures. *Tetrahymena thermophila* rarely divided and in all but one culture, quickly went extinct. Thus, in the pond water assays, *T. gruchyi* always dominated the population (Table 1).

Discussion—*Tetrahymena* have been extensively sampled from natural populations over many years (Simon et al., 2008; Lynn and Doerder, 2012; Doerder, 2019). One striking observation from these collections is that *T. thermophila* is only ever found in the eastern United States (Simon et al., 2008; Zufall et al., 2013). Thus in this work, we set out to begin to uncover the mechanisms that could drive this restricted distribution. Our results indicate two possible mechanisms that are likely contributing to the restricted distribution of *T. thermophila*. First, *T. thermophila* cannot thrive in the same environments as other species of *Tetrahymena*. In this example, *T. gruchyi* grows well in Texas pond water, but *T. thermophila* does not. Second, *T. thermophila* could be excluded from ponds by the presence of other species of *Tetrahymena*. We have shown that for these two species of *Tetrahymena,* whichever species is first to colonize a laboratory culture with a suitable medium is destined to dominate that population.

It is unknown what conditions are necessary for *T. thermophila* to grow. We know that it grows well under a variety of laboratory conditions, including bacterized peptone and Cerophyll bacterized with *Klebsiella* (Cassidy-Hanley, 2012). We also found that *T. thermophila* can grow, though not well, in autoclaved pond water supplemented with a rice grain and *Klebsiella*. Together these results suggest that there is something about Sheldon Lake pond water, possibly the species of bacteria or other microbial eukaryotes that are present, the abiotic conditions, or the lack of autocrine activators of cell growth (Schousboe et al., 1998) that prevents *T. thermophila* from successfully growing and competing in this environment. In contrast, *T. gruchyi* and other *Tetrahymena* species (unpubl. data) seem to grow successfully in this environment. For this study, we were unable to gain access to water from ponds where *T. thermophila* is naturally found. It would be informative to repeat these experiments using water from ponds where both species are found to assess the effects of competition and priority effects under more natural conditions and better elucidate the role of the environment in these interactions.

In many of the sites that have been sampled over the years, multiple *Tetrahymena* species are found to co-exist, with some containing more than 5 species (Doerder, 2019), indicating that at the level of a pond, species can successfully co-exist. However, in other ponds, there is clearly a dominant species of *Tetrahymena*. In several such ponds, the dominant species is *T. gruchyi,* but in other ponds it is *T. thermophila,* with the former being found frequently in the southeastern United States and the latter in the northeastern United States (Doerder, 2019). Thus, it remains to be determined precisely which factors contribute to the coexistence of multiple *Tetrahymena* species or the success of one species over others. Studies of the abiotic conditions of ponds where *T. thermophila* persist versus those where they are not found will begin to help elucidate this problem. In addition, further experiments controlling the microbial species present in populations will help determine whether food source (e.g., which bacterial species are present), or competition from other heterotrophs, such as nanoflagellates that also feed on bacteria, are driving the results seen the pond water populations. Finally, if priority effects are indeed an important factor in determining the species distributions, we need to further investigate how individuals migrate and colonize new sites.

We are grateful to K. Dimond for providing the *T. thermophila* strain and experimental support and to P. Doerder for thoughtful discussion. We thank K. Plum for translation help. We are also grateful for the helpful suggestions from multiple reviewers. This work was supported in part by NIH NIGMS (award no. R01GM101352) and NSF DEB (1911449).

Literature Cited

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. 1993. Current protocols in molecular biology. Wiley-Liss, New York.

Cassidy-Hanley, D. M. 2012. *Tetrahymena* in the laboratory: strain resources, methods for culture, maintenance, and storage. Pages 237–276 in Methods in cell biology (K. Collins, editor). Academic Press, Amsterdam.

Doerder, F. P. 2014. Abandoning sex: multiple origins of asexuality in the ciliate *Tetrahymena*. BMC Evolutionary Biology 14:112.

Doerder, F. P. 2019. Barcodes reveal 48 new species of *Tetrahymena, Dexiostoma,* and *Glaucoma*: phylogeny, ecology, and biogeography of new and established species. Journal of Eukaryotic Microbiology 66:182–208.

Doerder, F. P., and C. Brunk. 2012. Natural populations and inbred strains of *Tetrahymena*. Pages 277–300 in Methods in cell biology (K. Collins, editor). Academic Press, Amsterdam.

Fukami, T. 2015. Historical contingency in community assembly: integrating niches, species pools, and priority effects. Annual Review of Ecology, Evolution, and Systematics 46:1–23.

Lynn, D. H., and F. P. Doerder. 2012. The life and times of *Tetrahymena*. Chapter 2. Pages 9–27 in Methods in cell biology (K. Collins, editor). Academic Press, Amsterdam.

Schousboe, P., D. N. Wheatley, and L. Rasmussen. 1998. Autocrine/paracrine activator of cell proliferation: purification of a 4-6 kD compound with growth-factor-like effects in *Tetrahymena thermophila*. Cellular Physiology and Biochemistry 8:130–137.

Simon, E., D. Nanney, and F. P. Doerder. 2008. The "*Tetrahymena pyriformis*" complex of cryptic species. Biodiversity and Conservation 17:365–380.

Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and community ecology. Annual Review of Ecology, Evolution, and Systematics 33:475–505.

Zufall, R. A., K. L. Dimond, and F. P. Doerder. 2013. Restricted distribution and limited gene flow in the model ciliate *Tetrahymena thermophila*. Molecular Ecology 22:1081–1091.

Submitted 9 January 2020. Accepted 12 October 2021.
Associate Editor was Frederic Robert Govedich.