LETTER

Phylogenetic limiting similarity and competitive exclusion

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Abstract

One of the oldest ecological hypotheses, proposed by Darwin, suggests that the struggle for existence is stronger between more closely related species. Despite its long history, the validity of this phylogenetic limiting similarity hypothesis has rarely been examined. Here we provided a formal experimental test of the hypothesis using pairs of bacterivorous protist species in a multigenerational experiment. Consistent with the hypothesis, both the frequency and tempo of competitive exclusion, and the reduction in the abundance of inferior competitors, increased with increasing phylogenetic relatedness of the competing species. These results were linked to protist mouth size, a trait potentially related to resource use, exhibiting a significant phylogenetic signal. The likelihood of coexistence, however, was better predicted by phylogenetic relatedness than trait similarity of the competing species. Our results support phylogenetic relatedness as a useful predictor of the outcomes of competitive interactions in ecological communities.

Keywords

Community phylogenetics, competitive ability, functional trait, interspecific competition, niche conservatism, niche difference, phylogenetic dispersion, phylogenetic relatedness, species coexistence, species extinction.

INTRODUCTION

In *The Origin of Species*, Darwin (1859) proposed one of the earliest hypotheses on competition, stating that the struggle for existence is greater between closely related species than between distantly related species. This idea, which we term the phylogenetic limiting similarity hypothesis, is based on the assumption that close relatives are likely to possess similar niches, an idea synthesised in the recent concept of phylogenetic niche conservatism (Wiens & Graham 2005; Losos 2008; Wiens *et al.* 2010), and that greater niche similarity between species translates into more frequent competitive exclusion. Classic theories in community ecology echo Darwin's thinking on the relationship between niche similarity and competition. For example, the competitive exclusion principle emphasises the impossibility of the coexistence of extremely similar species (Hardin 1960). In proposing the original limiting similarity hypothesis, MacArthur & Levins (1967) showed theoretically that there is a limit to the similarity in the niches of competing species. These influential ideas, coupled with the general perception that species niches are constrained by their evolutionary histories (e.g., Peterson *et al.* 1999), led to the common acceptance of the phylogenetic limiting similarity hypothesis among ecologists, although the hypothesis itself has not been subject to rigorous experimental tests. The hypothesis holds a central place in the field of phylogenetic community ecology, as it suggests that competition exclusion of closely related species would result in the frequent coexistence of distantly related species in ecological communities. It has thus been frequently invoked to explain phylogenetic overdispersion (that is, the phylogenetic relatedness of coexisting species less than expected by chance), a pattern often observed in natural communities (Cavender-Bares *et al.* 2009; Vamosi *et al.* 2009).

Recent evidence, however, suggests that a suite of mechanisms other than competition can also cause communities to be phylogenetically overdispersed (Cavender-Bares *et al.* 2009). Moreover, the opposite pattern of phylogenetic overdispersion, phylogenetic clustering, has also been frequently reported for natural communities (Cavender-Bares *et al.* 2009; Vamosi *et al.* 2009). Therefore, it is difficult to draw definitive conclusions on competition based on patterns of phylogenetic dispersion observed in natural communities, where a variety of factors could potentially confound the relationship between the two. Experiments that directly manipulate the phylogenetic relatedness of competing species provide the best approach to testing whether competition gives rise to phylogenetic limiting similarity, but so far such experiments have been extremely rare. One possible exception is Cahill *et al.*'s (2008) recent meta-analysis of several plant competition experiments, which found no significant relationship between phylogenetic relatedness and the intensity of competition between species. The robustness of this result, however, may have been compromised by heterogeneous growth conditions across different experiments included in the analysis and a lack of tests for phylogenetic conservatism of plant traits relevant for competition.

Here we reported on a protist microcosm experiment testing Darwin's phylogenetic limiting similarity hypothesis under homogenous environmental conditions. To this end, we set up all 45 pairwise interactions of 10 bacterivorous ciliated protist species (Fig. 1) as well as their monocultures in a total of 165 laboratory microcosms that we

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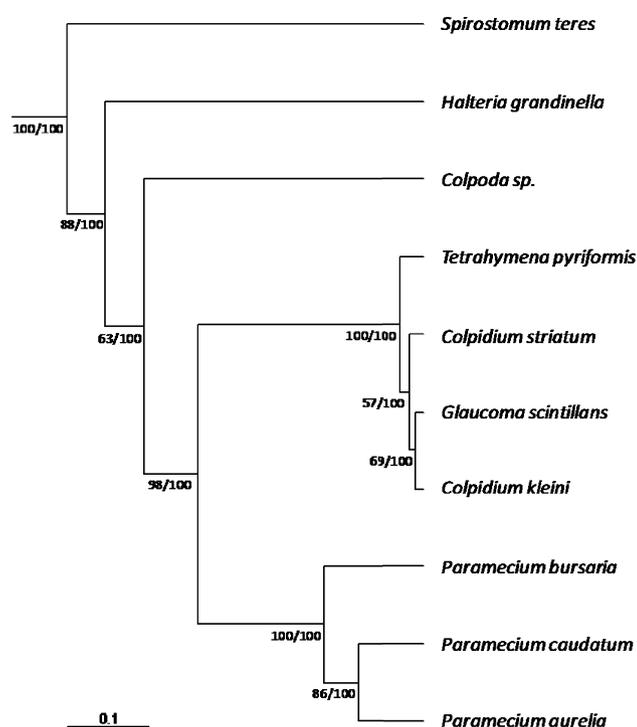
Figure 1 Phylogeny of the 10 ciliate species used in the experiment. This ultrametric tree was derived from a maximum likelihood phylogenetic tree based on small subunit rRNA gene sequences (see the main text for details). Scores on nodes indicate bootstrap support.

tracked over time. This model system of fast-growing protists enables the observation of rapid competitive exclusion and stable coexistence, as shown by the classic work of Gause (1934) that helped establish the competitive exclusion principle. Our study builds on the work of Gause (1934) and others that demonstrated the competitive exclusion of ecologically similar species, but differs from them by examining competition along a continuous phylogenetic relatedness gradient.

MATERIALS AND METHODS

Microcosms

Our experiment used a total of 10 common freshwater bacterivorous ciliated protist species (Fig. 1). Prior to the experiment, the 10 species had been separately cultured in the laboratory on three bacterial species (*Bacillus cereus*, *Bacillus subtilis*, and *Serratia marcescens*). The same three bacterial species, which are widely distributed in nature and known to be edible for various bacterivorous ciliates (Lawler 1993), were also used in our experiment. The use of a multi-species bacterial assemblage allowed protists to potentially exhibit various degrees of niche differentiation when coupled with different competitors, facilitating the test of our hypothesis. Microcosms were 250-mL Pyrex glass bottles each filled with 100 mL of medium. The medium was prepared by dissolving a 0.55 g of protozoan pellet (Carolina Biological Supply Company, NC, USA) in 1 L of deionised water, autoclaving, then inoculating with the bacterial assemblage. This medium supported the growth of bacterial food for the protists, which were food, but not nutrient, limited given the comparatively rich nutrient content of bacteria (Sterner & Elser 2002; Cross *et al.* 2005).

The competition experiment

Each species was introduced to one microcosm with one of the other nine species; *c.* 100 individuals for each protist species were inoculated to start the experiment. This was done for all 45 two-species combinations, each replicated three times. Weekly samples (2 mL) were taken from each microcosm and examined under a stereoscope to record species presence/absence; this was done until week 8, when either competitive exclusion or stable coexistence (i.e., no obvious trend of population decline) was observed. Species were deemed extinct after they were undetected for at least three consecutive weeks (we continued monitoring the experiment until week 10 to ensure that species missing during week 8 remained undetected for another 2 weeks). Control jars with each species grown in monocultures (three replicates per species) were also established and monitored in the same way. At the end of the experiment, we estimated the density of each protist species by sampling ca. 0.40 mL of solution in each microcosm. The sample was distributed as multiple drops onto a Petri dish and the number of individuals of each species was enumerated under a stereoscopic microscope. The exact mass ($\approx$ volume) of the sample was determined using an analytic balance. Finally, we measured the size of the oral opening (μm) of each protist species, a key trait known to affect the selectivity and uptake rate of prey particles (Fenchel 1980a,b,c), with a compound microscope based on 10 randomly selected individuals of the species.

Phylogeny

To estimate phylogenetic relatedness between the protist species, we obtained the small subunit ribosomal RNA gene sequences of the studied taxa from the GenBank/EMBL databases. For *Colpoda* sp., which we were only able to describe at the genus level, we used the sequences of its congeners as a proxy. Omitting *Colpoda* sp. from the analyses did not alter any of our conclusions. We aligned the gene sequences with ClustalX (Larkin *et al.* 2007) using the default parameters, and manually edited alignments to remove unalignable hypervariable regions. Nucleotides (numbering based on ClustalX alignment) 101–178, 318–665, 810–885, 958–1132, 1188–1465, 1636–1752 and 1825–2055 were retained for phylogenetic analysis.

We then generated an ultrametric phylogenetic tree of the organisms used in this study. Here, we used PAUP (Swofford 2003) to obtain a maximum likelihood tree with the GTR + G + I model, the best model of sequence evolution as determined by Modeltest (Posada & Crandall 1998). *Protoperidinium pellucidum, Gambierdiscus polynesiensis, Prorocentrum gracile, Sarcocystis lacerate* and *Plasmodium falciparum*, which together with ciliates belong to same superphylum of *Alveolata*, were used as outgroup species to root the tree. Because the sequence data violated an assumption of a molecular clock, nonparametric rate smoothing (NPRS) and penalised likelihood were implemented in r8s version 1.71 (Sanderson 2003) to obtain an ultrametric tree that accommodates rate heterogeneity across lineages; both methods yielded similar results, and we only report those based on NPRS here. We then calculated phylogenetic distances as the pairwise branch lengths of the phylogeny using the Phydist function of Phylocom version 3.40 (Webb *et al.* 2008). This analysis was repeated with 100 bootstrap replicates to validate the robustness of our analysis to alternative phylogenetic reconstructions.

Data analysis

We used linear regressions to assess the fraction of competitive exclusion (out of three replicates of each pairwise species interaction treatment) as a function of phylogenetic distance and mouth size difference between the competing species. The same relationships were also assessed using Mantel tests that account for the non-independence in the data, caused by a given species present in multiple pairwise species interaction treatments. Partial mantel tests were used to evaluate whether phylogeny data significantly improved the fit of the model when trait data were already included (and vice versa). We used Spearman's rank correlation to assess the relationship between phylogenetic distance and the tempo of competitive exclusion. For microcosms in which no competitive exclusion occurred, we estimated how interspecific competition affected the inferior competitor using the ratio of its abundance in the bi-species microcosm and its monoculture abundance. We then assessed how this ratio was affected by phylogenetic distance of the competing species, again using Spearman's rank correlation test. We quantified the degree of phylogenetic signal for the size of the oral opening across the 10 protist species, using the K statistic with 9999 randomisations (Blomberg & Garland 2002; Blomberg *et al.* 2003). This was done using the software program Picante (Kembel *et al.* 2010). We also confirmed our phylogenetic signal result using the *pic.funct* function (http://www.utsc.utoronto.ca/~mcadotte/R_scripts.html), which is based on the sum of phylogenetically independent contrast values, with 9999 randomisations (results not shown). All analyses were performed using R 2.7.1 (http://www.R-project.org).

RESULTS

While all species persisted until the end of the experiment in monocultures, competitive exclusion occurred in more than half (72 of 135) of the bi-species cultures. Both regression and Mantel tests revealed that the frequency of competitive exclusion was significantly greater between species that were more closely phylogenetically

related (Fig. 2a, Table 1). In addition, the tempo of competitive exclusion was significantly greater among more closely related species (Fig. 3). Furthermore, in microcosms where both competitors coexisted for the duration of the experiment, interspecific competition more strongly reduced the abundance of the inferior competitor (relative to its abundance in monoculture) as the phylogenetic relatedness between the two competitors increased (Fig. 4).

Protist mouth size exhibited a significant phylogenetic signal (Blomberg's $K = 1.41$, $P = 0.009$). The frequency of competitive exclusion was significantly greater between species that were more similar in mouth size (Fig. 2b, Table 1). Phylogenetic distance, however, accounted for more variation in the frequency of competitive exclusion than mouth size difference (Table 1). Phylogenetic distance remained a significant predictor of competitive exclusion in a partial Mantel test that controlled for mouth size difference, but mouth size difference was no longer significant in the partial Mantel test that controlled for phylogenetic distance (Table 1).

DISCUSSION

The occurrence of phylogenetic overdispersion in natural communities has been often attributed to competitive exclusion of closely related species as suggested by the phylogenetic limiting similarity hypothesis. However, the fact that phylogenetic overdispersion may also arise from a suite of other mechanisms (Emerson & Gillespie 2008; Cavender-Bares *et al.* 2009), such as facilitative interactions (Valiente-Banuet & Verdu 2007), complicates this interpretation. The difficulty in elucidating the role of competition in driving observed phylogenetic dispersion patterns in nature, coupled with the lack of experimental tests of the phylogenetic limiting similarity hypothesis, seriously compromises our understanding of how phylogenetic relatedness influences competitive interactions. Our experimental results demonstrate that competitive exclusion occurred more frequently and more rapidly between species that are more phylogenetically closely related, providing strong support
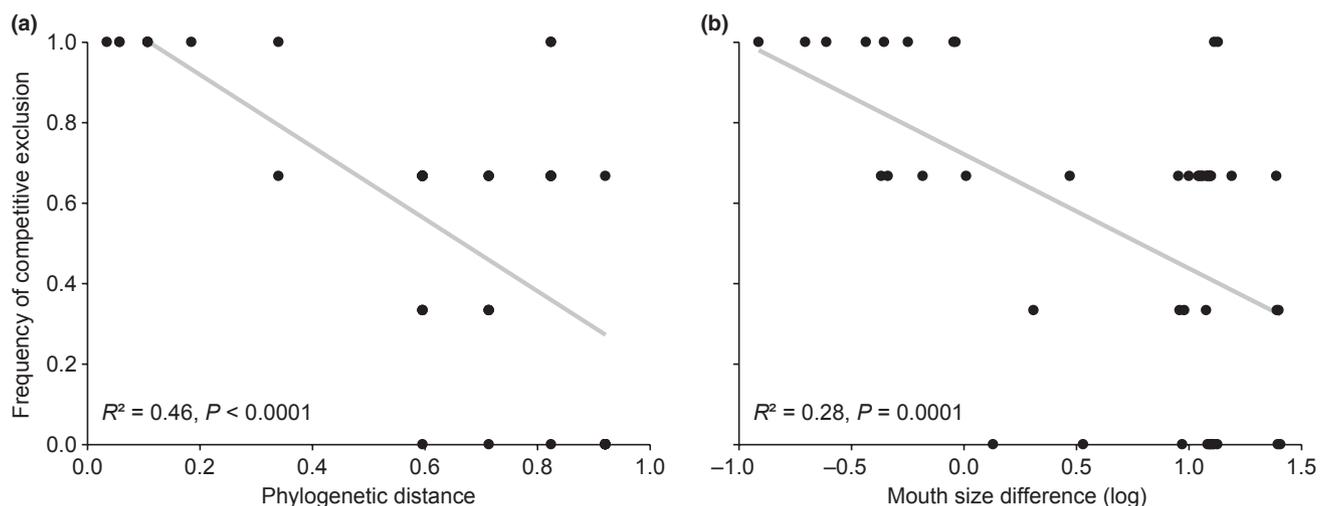
Figure 2 Frequency of competitive exclusion as a function of phylogenetic distance and trait dissimilarity. The frequency of competitive exclusion, calculated as the proportion of replicates in which competitive exclusion occurred in each species pairwise interaction treatment, was plotted against (a) phylogenetic distance and (b) mouth size difference. The grey line is the regression line.

Table 1 Results of Mantel and partial Mantel tests relating the frequency of competitive exclusion (out of three replicates of each pairwise species interaction treatment) (CE) to trait and phylogenetic distances

y	x_1	x_2	Pearson's r	P-value
Simple Mantel tests				
CE	P		−0.67	< 0.0001
CE	M		−0.53	0.001
Partial Mantel tests				
CE	M	P	−0.57	0.006
CE	P	M	−0.33	0.08

Partial Mantel tests evaluated whether the second predictor variable (x_2) significantly improves the fit of the model predicting the frequency of competitive exclusion (y) when the first predictor variable (x_1) is already included.
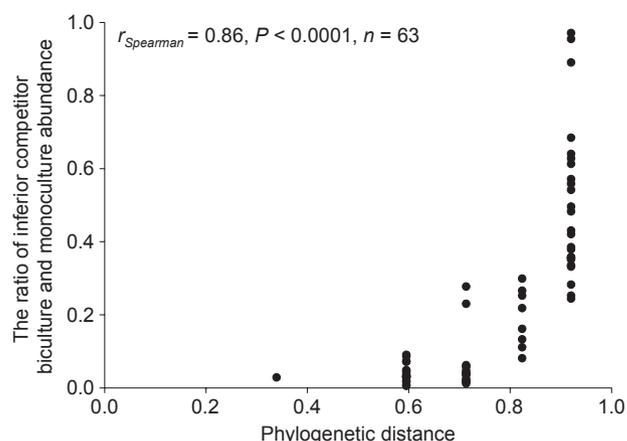P, phylogenetic distances; M, mouth size differences.
Pearson's r in the partial Mantel tests represents partial correlation coefficients.
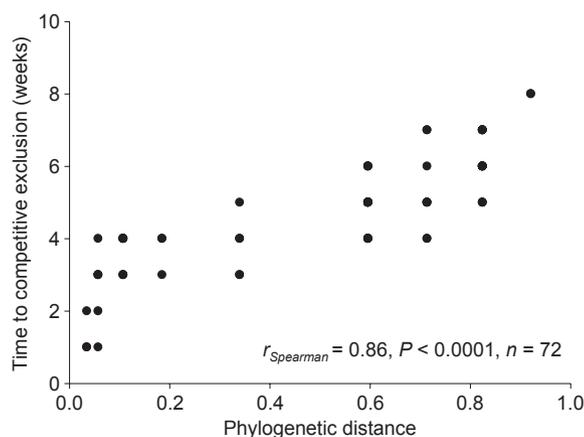
Figure 3 The time to competitive exclusion (in weeks) within each species pairwise interaction treatment as a function of phylogenetic distance between the competing species.

Figure 4 The ratio of the abundance of the inferior competitors in the bi-species microcosms in which they persisted and their monoculture abundance, as a function of phylogenetic distance between the two competing species. Species abundances were measured at the end of the experiment. Larger ratios correspond to smaller effects of competition on species abundance.

for the hypothesis. Competition among more closely related species also more strongly depressed the abundance of the inferior competitors that managed to persist, further strengthening our results. While our study provides arguably the strongest direct experimental evidence for the phylogenetic limiting similarity hypothesis, two recent experiments also offered additional, although more indirect and less quantitative, support for the hypothesis. In a fungal community assembly experiment that started with the same number of species, Maherali & Klironomos (2007) found low realised species richness in communities containing species from a single family and higher realised richness in communities containing species from two or three different families. In a bacterial invasion experiment, Jiang *et al.* (2010) showed that the frequency of successful establishment of an alien bacterial invader decreased as its phylogenetic relatedness with native bacterial communities increased. Strong competition between close relatives presumably resulted in low species richness and failed invasions in the former and latter study, respectively.

One major assumption of the phylogenetic limiting similarity hypothesis is the greater niche similarity among more closely related species, which was borne out in our analysis of the size of the oral opening of the studied species. Protist mouth size is known to affect

the selectivity and uptake rate of prey particles (Fenchel 1980a,b,c). Thus, one possible explanation for the low frequency of coexistence between closely related species is that their similarity in mouth size translates into the exploitation of similar bacterial resources, resulting in more competitive exclusion. Consistent with this, we found that the frequency of competitive exclusion depended on the difference in mouth size of the competing species. Mouth size difference alone, however, accounted for less variation in competitive exclusion than phylogenetic distance, suggesting that that other traits [e.g., protist mouth structure (Fenchel 1980b)] involved in resource uptake may also be important for competition. It also suggests that phylogenetic relatedness was a reasonable proxy of the similarity of these relevant traits, although they were not measured in this study. Given the non-trivial task of the *a priori* identification of key functional traits associated with competition (Navas & Violle 2009) and the common scenario of phylogenetic niche conservatism (Peterson *et al.* 1999; Prinzing *et al.* 2001; Webb *et al.* 2002; Donoghue 2008), phylogenetic relatedness may be expected to hold its value as a useful surrogate of ecological similarity among species. Indeed, a similar logic has prompted ecologists to investigate the relationship between phylogenetic diversity and ecosystem functioning, with several studies showing that plant phylogenetic diversity tends to be a better predictor of primary productivity than plant functional trait diversity (Cadotte *et al.* 2008, 2009).

The results of our experiment are strikingly consistent with Darwin's phylogenetic limiting similarity hypothesis, lending support to the idea that competition serves as an important candidate mechanism behind the phylogenetic overdispersion pattern exhibited by natural communities. Several points regarding the generality of our result, however, are worth noting here. First, while our study considers classic theory of species coexistence that emphasises the role of niche differences, recent coexistence theory suggests that competitive outcomes may be formulated as the combined effects of both species niche differences and differences in their competitive ability (Chesson 2000; Adler *et al.* 2007; Mayfield & Levine 2010). An important corollary of this theory is that phylogenetic limiting similarity is only one of several possible outcomes of competition, which may depend upon the strength of species niche and

competitive ability differences as well as the strength of their phylogenetic signal (Mayfield & Levine 2010). Although our study examined protist mouth size as a trait potentially related to resource use, we did not quantify species niches and competitive abilities that may permit an assessment of the above prediction. There is, however, an urgent need for empirical tests of the prediction, which, if true, would complicate the interpretation of phylogenetic dispersion patterns in natural communities. Second, our experiment was conducted under fixed, uniform conditions. However, environmental conditions tend to vary over space and time in nature; different environmental conditions are known to favour different species, potentially leading to different competitive outcomes (e.g., Fox 2002; Jiang & Morin 2004). Nevertheless, we expect more closely related species in our experiment to still share more similar niches under a different environment, which may lead to qualitatively similar results as found in this study. Third, although microscopic protists were used as model organisms in our experiment, our results may actually be more relevant for understanding phylogenetic community structure of macro-organisms. This is because unlike our experimental microcosms that supported finite protist population sizes and were closed to dispersal, natural populations of protists and other micro-organisms may not experience competitive exclusion in their suitable habitats as frequently, due to frequent among-population dispersal associated with their typically large population sizes (Finlay 2002; Finlay & Fenchel 2004). Macro-organisms, on the other hand, generally have much reduced population sizes and tend to exhibit dispersal limitation (Finlay 2002; Finlay & Fenchel 2004) similar to our protist microcosm populations, and are thus likely to face competitive exclusion. In apparent congruence with this idea, analyses of microbial communities rarely revealed phylogenetic overdispersion (Horner-Devine & Bohannan 2006; Bryant *et al.* 2008; Edwards & Zak 2010), contrasting with frequent cases of phylogenetic overdispersion reported for macrobial communities (Cavender-Bares *et al.* 2009). Finally, we note that experimental studies that link phylogeny with species interactions, which may hold the key to elucidating mechanisms underlying phylogenetic community structure patterns in natural communities, are extremely lacking (but see Maherali & Klironomos 2007; Jiang *et al.* 2010). It is our hope that our experiment, by validating the phylogenetic limiting similarity hypothesis at the root of phylogenetic community ecology, will encourage more experimental work in this emerging field.

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