Taylor’s Law holds in experimental bacterial populations but competition does not influence the slope

Johan Ramsayer1,†, Simon Fellous1,†, Joel E. Cohen2 and Michael E. Hochberg1,*

1Institute of Evolutionary Sciences, Montpellier (UMR 5554 ISE-M), University of Montpellier 2, Cedex, France
2Laboratory of Populations, Rockefeller and Columbia Universities, 1230 York Avenue, Box 20, New York, NY 10065-6399, USA
*Author for correspondence (mhcobber@uni-m.fr).
†These authors contributed equally to the study.

Keywords: Taylor’s Law; populations; competition; bacteria; Pseudomonas fluorescens; Serratia marcescens

1. INTRODUCTION
Starting half a century ago, Taylor et al. [1–4] and nearly a thousand other papers [5] demonstrated a linear relationship (commonly called ‘Taylor’s Law’) between the log(variance) of the population size and density of a species and the log(mean) of population size or density. This relationship applies to both spatial and temporal population samples [4]. Numerous mechanisms affecting the slope of this linear relationship have been examined theoretically [6,7–10], but few have been investigated experimentally [11].

Kilpatrick & Ives [10] suggested that interspecific competition might reduce the slope of the temporal form of Taylor’s Law, i.e. lower the rate at which log(variance) increases with log(mean) when mean and variance are calculated over time. In a simple dynamic community model, they demonstrated that direct and ‘apparent’ competition could reduce slopes from 2 to a lower value between 1 and 2.

The effect of competition on the spatial form of Taylor’s Law has not been investigated either theoretically or empirically. The spatial form of Taylor’s Law calculates the mean and the variance of populations sampled (possibly simultaneously) at different points in space, when replicate populations are grouped by some external condition such as available nutrients. We examined experimentally the validity of the spatial form of Taylor’s Law and the effect of competition on the slope of Taylor’s Law in simple, well-controlled laboratory experiments. We cultured two species of bacteria, both separately and together, in media of widely varying nutrient richness. The bacterial species interacted competitively when they were together, and the spatial form of Taylor’s Law usefully described the approximately linear increase of log(variance) with increasing log (mean). The slopes were not significantly different from 2. The presence of a competitor had no statistically significant effect on the slope. On the contrary, the slope was slightly (not statistically significantly) higher in the presence of competition.

2. MATERIAL AND METHODS
We used the Gram-negative bacterium Serratia marcescens [12] and a smooth-type stabilized strain of Pseudomonas fluorescens SBW25 [13]. Each of these two species was cultured alone or in competition with the other (i.e. three types of culture). These cultures were grown in 24 well plates each containing 0.5 ml of King’s B (KB) medium (glycerol 10 ml l⁻¹; proteose peptone H3 20 g l⁻¹; dipotassium phosphate K₂HPO₄ 1.5 g l⁻¹; magnesium sulphate MgSO₄ 1.5 g l⁻¹; distilled water qs 1 l). To create a gradient of environmental richness, we used pure KB medium and KB medium diluted 3, 9, 27, 81, 243, 729 and 2187 times (eight levels). Each of the 3 x 8 = 24 treatments was replicated eight times. We inoculated all the cultures containing P. fluorescens or S. marcescens with bacteria harvested from two diversified mass cultures (one per species). The initial density of bacterial cells was proportional to the richness of the medium used. In pure KB medium, we introduced approximately 3 x 10⁷ P. fluorescens cells, or 3 x 10⁵ S. marcescens cells or 3 x 10⁴ cells of each species into 1.5 ml. Initial densities were thus approximately 2 x 10⁵ colony forming units (CFUs) per millilitre for each species whether alone or in combination. Cultures that contained KB medium diluted three times received one-third of these cell numbers, cultures that contained KB medium diluted nine times received one-ninth of these numbers, etc. All populations were grown at 28°C under constant agitation for 24 h, that is, until they reached carrying capacity. Although we cannot rule out that some evolution occurred in the microcosms, we expect that ecological forces dominated dynamics over the 24-h period (over which a maximum of 10 and seven generations would have occurred in S. marcescens and in P. fluorescens, respectively).

Population densities were then estimated by counting the number of CFUs that appeared after plating 15 μl of (serially diluted) cultures on 1 per cent KB agar. After 24- to 36-h growth, colonies of the two bacterial species were readily recognized and counted under a stereomicroscope based on their colour and morphology. Bacterial density in each culture was estimated by averaging counts from two different 15 μl samples. We calculated the mean and the variance of the population density of each bacterial species over the eight replicates in each of the 24 treatments. After log₆ transformation, we obtained 32 points (log(mean), log(variance)): 16 from bacteria grown in monoculture and 16 from bacteria grown with a competitor. Electronic supplementary material, appendix table S1 gives the raw data.

We tested for evidence of competition using ANOVAs, and investigated the effect of competition on the slope of the regression of log(variance) to log(mean) with linear models and ANCOVAs. All statistical computing was performed using R v. 2.9.0 [14].

3. RESULTS
(a) Evidence for competition
If P. fluorescens and S. marcescens competed for resources, then the presence of each species in a microcosm should reduce the mean population density of the other species.
Experimentially, the density of each species was usually lower in the presence of the other species (figure 1). We defined log(mean)competition − log(mean)monoculture as ‘competitive intensity’ and interpreted more negative values of competitive intensity as indicating greater competition. Most values of competitive intensity were negative (figure 1a,b). ANOVAs of competitive intensity rejected the null hypothesis of no competitive effects of S. marcescens and P. fluorescens on each other with high statistical significance (p < 0.001; table 1). In most cases, competitor presence lowered the log10(bacterial concentration) by more than 0.5 units (figure 1), which represents an almost threefold reduction in the absolute number of bacteria. Competition had thus a very strong effect on population density.

We further tested with ANCOVAs whether competitive intensity was strongest when culture medium richness was lowest. We found no statistically significant support for this hypothesis with S. marcescens (F1,6 = 4.86, p = 0.07) or P. fluorescens (p > 0.1; figure 1). Competition was generally less intense at an intermediate KB dilution (electronic supplementary material, figures S1 and S2), and at some intermediate KB dilutions mean abundance of one bacterial species in the presence of the other exceeded mean abundance in the absence of the other. This U-shaped pattern was more pronounced for P. fluorescens than for S. marcescens. The U-shaped pattern probably occurs because P. fluorescens grows better in slightly diluted KB medium.

(b) Effect of competition on the relation between log(variance) and log(mean)

Mean bacterial densities varied by approximately 2.2 orders of magnitude for P. fluorescens (2.5 × 1010 – 3.9 × 109 CFU ml−1) and 3.0 orders of magnitude for S. marcescens (1.6 × 107 – 1.5 × 1010 CFU ml−1) across all treatments when pooling all data. The variance of bacterial population densities varied among treatments by approximately 4.5 orders of magnitude for P. fluorescens (5.4 × 1013 – 1.6 × 1028) and 5.1 orders of magnitude for S. marcescens (4.6 × 1013 – 5.8 × 1018). Hence, if the relation between log(variance) and log(mean) were approximately linear, then the slope of log(variance) as a linear function of log(mean) would be expected to be approximately 4.5/2.2 = 2.0 for P. fluorescens and 5.1/3.0 = 1.7 for S. marcescens.

To investigate the effect of competition on the relation between log(variance) and log(mean), we performed linear regressions, for each species separately, of the dependent variable log(variance) on the independent variable log(mean), for the treatments with and without competition. Then, we used ANCOVAs to test for a slope difference. ANCOVAs did not indicate any statistically significant differences in slopes (interactions...
between log(mean) and the competition term; \( p > 0.1 \).

For \( P. \) fluorescens, the slopes without and with the competitor were 1.784 ± 0.33 (95% confidence interval) and 2.104 ± 0.426, respectively (figure 2a).

For \( S. \) marcescens, the slopes without and with the competitor were 1.677 ± 0.346 and 1.734 ± 0.304, respectively (figure 2b). Though the slopes are not significantly different from one another, for both species the slope in the presence of the competitor was higher than the slope without the competitor.

This finding for the spatial relationship between log(variance) and log(mean) is contrary to the prediction of Kilpatrick & Ives [10] for the temporal version of Taylor's Law.

When we fitted \( P. \) fluorescens and \( S. \) marcescens data together in a single model that contained factors describing whether observations originated from competitive microcosms and the identity of the bacterial species, the interaction between competition and log(mean) was not significant. As in the previous analysis, this analysis provided no evidence that slopes for the spatial form of Taylor's Law were lower in the presence of a competitor (table 2). The terms describing the bacterial species and its interaction with log(mean) were not significant either (\( p > 0.1 \)).

4. DISCUSSION

Theory has identified numerous processes that could influence relationships between log(variance) and log(mean) [4,6,9,10] in population data, but experimental demonstrations of their role have been elusive. We found that the spatial version of Taylor's Law described well the linear increase in log(variance) with increasing log(mean) for each of two bacterial species grown with or without the other. A reduction in mean population size in the presence of the other species demonstrated competition between the bacterial species when they were grown together. The slope of Taylor's Law was not reduced by interspecific competition in our experiments.

These findings suggest that the spatial version of Taylor's Law does not always satisfy the prediction by Kilpatrick & Ives [10] that interspecific competition lowers slopes in the temporal form of Taylor's Law. The confirmation of the spatial form of Taylor's Law and the failure to detect a reduction in slope from values near 2 as a result of competition in these experiments means that an ecological understanding of at least the spatial version of Taylor's Law remains an open challenge. In light of the widespread usefulness of Taylor's Law in areas such as population ecology [15,16], epidemiology [17], and the spatial and temporal dynamics of agricultural pests [18–20], it is a challenge of significance.

We thank Anthony Ives and two referees for helpful comments on an earlier version of this study. J.R., S.F. and M.E.H. were supported by grants from the Agence National de la Recherche 'EvolStress' (ANR-09-BLAN-099-01) and 'EvolRange' (ANR-09-PEXT-011). J.E.C.'s
participation in this research was supported by grants DMS-0443803 and EF-1038337 from the US National Science Foundation, a grant from the Region of Languedoc-Roussillon through the University of Montpellier 2, the assistance of Priscilla K. Rogerson and the hospitality of Michael Hochberg and family during this work.

1 Taylor, L. R. 1961 Aggregation, variance and the mean. Nature 189, 732–735. (doi:10.1038/189732a0)