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Bacterial microcosms obey Taylor's law: effects of abiotic and biotic stress and genetics on mean and variance of population density

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Abstract

Introduction: According to the empirical regularity called Taylor's law, the variance of population density in samples of populations is a power of the mean population density. The exponent is often between 1 and 2. Our experiments investigated how genetics, evolution, and environment shape Taylor's law.

Methods: Genetically different strains (wild type and hypermutator) of the bacterium *Pseudomonas fluorescens* evolved and were assayed under different environmental conditions (with and without antibiotic rifampicin and bacteriophage SBW25ϕ2, separately and in combination).

Results: Experimental treatments altered the exponent b, but not the power law form, of the relation between variance and mean population density. Bacterial populations treated only with rifampicin had a narrow range of mean population densities and exponent $b = 5.43$. Populations exposed to rifampicin plus phage had $b = 1.51$. In ancestral, control, and phage-exposed populations, mean abundance varied widely and b was not significantly different from 2. Evolutionary factors (mutation rate, selection) and ecological factors (abiotic, biotic) jointly influenced b.

Conclusions: Taylor's power law relationship accurately and robustly described variance as a function of mean population density, with overall exponent $b = 1.89$. These and other experiments with different factors acting on bacterial population size support the relevance of models that predict 'universal' patterns of fluctuation scaling.

Keywords: Taylor's law, *Pseudomonas fluorescens*, rifampicin, bacteriophage, genetics

Introduction

In 1961, L. R. Taylor brought to wide attention an approximate empirical power law relationship, variance = a(mean)^b, where $a > 0$ and $b > 0$, between the variance and mean of the size of insect populations (Taylor 1961). Taylor's law describes variation in hundreds of species and has been the subject of approximately a thousand papers (Eisler et al. 2008). Taylor's law has practical importance for designing efficient sampling of agricultural pests and insect vectors of human diseases (Young and Young 1994; Binns et al. 2000; Park and Cho 2004).

Long before Taylor publicized this empirical pattern, Luria and Delbrück (1943) investigated, theoretically and experimentally, mutations of bacteria from virus sensitivity to virus resistance. If virus resistance arose from heritable mutations, then, their theory showed, the distribution of the number of resistant bacteria under given conditions would not be described by the Poisson distribution, which has variance equal to mean, but would be described by an over-dispersed distribution, with variance significantly larger than the mean. They counted the numbers of resistant bacteria and reported the raw counts, means, and variances in series of eight similar cultures in their Table 2. They remarked (Luria and Delbrück 1943, page 504): '... in every experiment the fluctuation of the numbers of resistant bacteria is tremendously higher than could be accounted for by the sampling errors, ... in conflict with the expectations

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from the hypothesis of acquired immunity' and in support of the alternative hypothesis of heritable mutations.

They did not remark that the relation between the variance and the mean in their data is well approximated by a power law. Showing that a power law applies to relationships between the mean and variance of population size suggests orderly underlying processes (Taylor et al. 1983, but see, e.g., Hanski 1982; Anderson et al. 1982). We performed a linear regression of the $\log_{10}$ variance as a function of the $\log_{10}$ mean in the data of Table 2 of Luria and Delbrück (1943) and found that $b = 1.47 \pm 0.57$ (standard error) with $R^2 = 0.53$. This estimate of b differed from 0 significantly (95% level) but did not differ significantly from either 1 or 2.

The present paper uses Taylor's law to extend the research of Luria and Delbrück (1943) beyond genetics into ecology and evolution. We ask here how genetic, ecological, and evolutionary processes interact to affect variance-mean relationships. Are specific conditions necessary to generate Taylor's law, or is Taylor's law robust with respect to other ecological and evolutionary processes?

Models developed in other areas of population biology suggest that Taylor's law may be quite robust. For example, in models of stem cell clones, stochastic 'voter' or 'random walk' models of neutral drift converge to 'universal' patterns which obey Taylor's law with $b = 2$ for 'any mechanism of stem cell self-renewal, however complex' (Klein and Simons 2011, page 3107). If these patterns are universal, then they should apply to bacterial populations. In a Lotka-Volterra model of interspecific competition, under a range of conditions, Taylor's law holds with $1 \le b \le 2$ (Kilpatrick and Ives 2003). These theoretical suggestions need to be tested experimentally.

We used microbial microcosms under controlled initial conditions and in a controlled environment to investigate the impacts of genetic differences in mutation rate, exposures to an abiotic stressor (antibiotic), and exposures to a biotic stressor (parasitism by a lytic bacteriophage) on a single bacterial species' adherence to Taylor's law over an evolutionary time scale (approximately 50 generations). Ramsayer et al. (2011) used the same species of bacterium to investigate the impacts of competition from another bacterial species and of nutrient concentrations on the adherence to Taylor's law of each competitor, and measured impacts on an ecological time scale (24 h) of, at most, a few generations. The shared purpose of both studies is to identify the ecological and evolutionary factors that contribute to the success or failure of Taylor's law and that influence the values of its parameters.
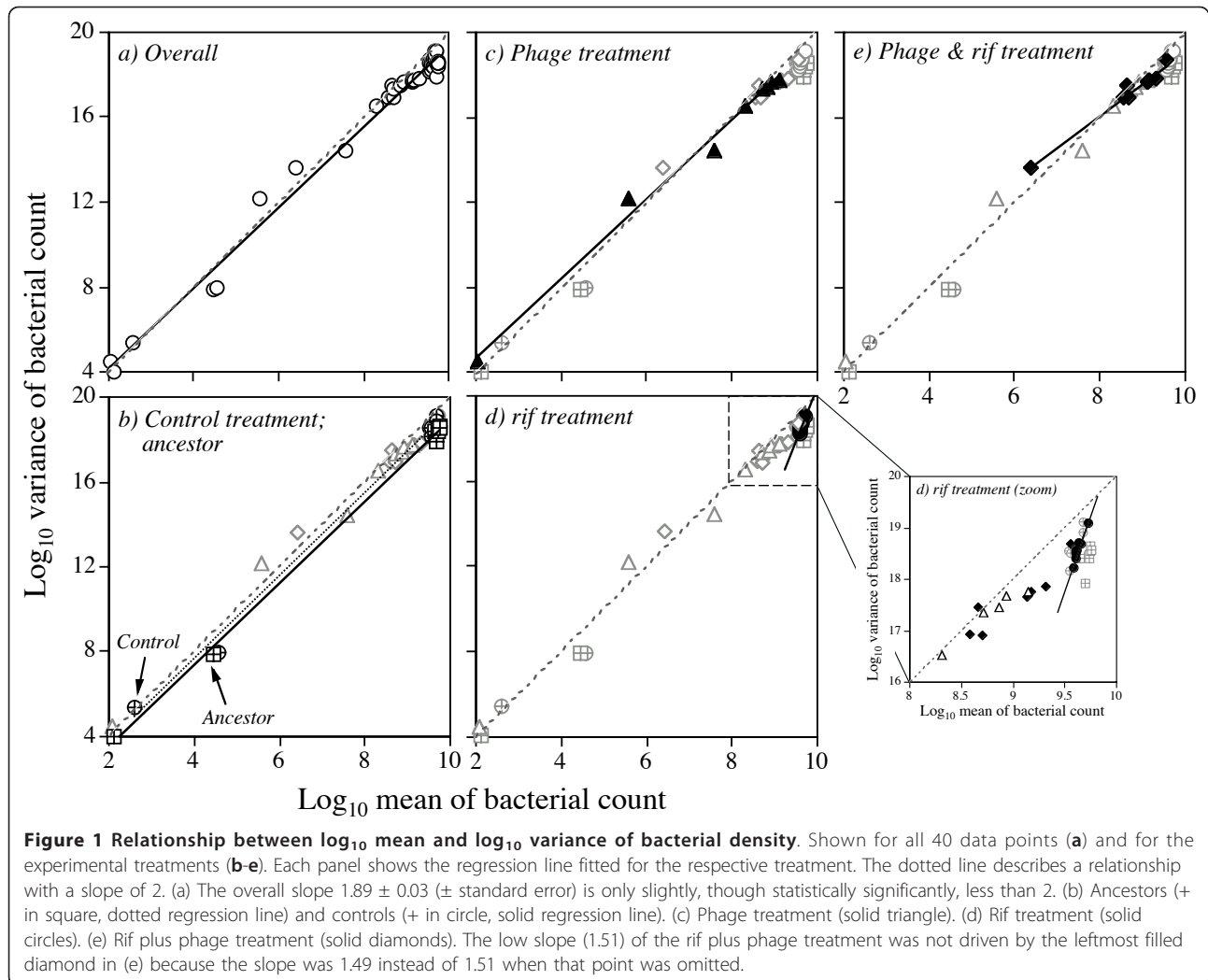
Methods

The gram-negative bacterium *Pseudomonas fluorescens* SBW25 has been employed to study adaptation in heterogeneous environments (Venail et al. 2008; Buckling et al. 2009) and coevolution with a lytic bacteriophage SBW25ϕ2 (Brockhurst et al. 2007; Poullain et al. 2008). We analyzed in a new way the data of Escobar-Páramo et al. (2012) on the population dynamics and evolution of four strains of *P. fluorescens* SBW25 exposed to a biotic and an abiotic stress. The four strains, derived from the wild-type (*WT*) (Rainey and Bailey 1996), included an isogenic hypermutator (*MutS*) knock-out mutant with a per base mutation rate of approximately 10^{-5} per generation (Pal et al. 2007) (compared to approximately 5×10^{-7} per base pair per generation in *WT*), and lines resistant to the antibiotic rifampicin (*Rif+*), one derived from the *WT* and the other from the *MutS* strain. Thus, the experimental replicate populations of *P. fluorescens* were seeded from four different strains of bacterial cells: wild type *WT/Rif-* (Rainey and Bailey 1996), hypermutator *MutS/Rif-* (Pal et al. 2007), rifampicin-resistant wild type *WT/Rif+*, and rifampicin-resistant hypermutator *MutS/Rif+*. Over approximately 50 generations, 12 replicate populations of each strain were submitted (or not submitted, in the control) to antibiotic rifampicin (rif), an abiotic stressor, and to the lytic bacteriophage SBW25ϕ2, a biotic stressor, separately and in combination, for a total of four treatments. At the end of the experiment, densities (number of colony-forming units per milliliter) of evolved populations were assayed on King's B (KB) agar plates in the presence (KB + rif) or absence (KB) of rif to distinguish total count from the antibiotic-resistant subpopulation. Escobar-Páramo et al. (2012) give further details of experimental methods.

We calculated means and variances of final bacterial density across the replicate populations of each combination of bacterial mutation rate, experimental treatment, and assay plate environment. The table in Additional file 1 gives the resulting 40 pairs of means and variances. As means and variances were calculated over distinct replicate populations at a given time, and not for individual replicates observed over time, we examined whether these final means and variances and those of the ancestral lines obeyed the ensemble (or equivalently, spatial) form of Taylor's law, and if so, whether b of Taylor's law depended on the ancestral lines, experimental treatments, or assay plate environment (SAS 2009; Mathworks 2011).

Results

Mean bacterial densities covered nearly 8 orders of magnitude (Figure 1a). On assay plates with rif, mean population density varied from nearly 10^2 to nearly 10^{10}. The high densities arose from *Rif+* (rif-resistant) clones. In the assay plates without rif, mean population density was always $> 10^8$ [see table in Additional file 1].

Figure 1 Relationship between log$_{10}$ mean and log$_{10}$ variance of bacterial density. Shown for all 40 data points (**a**) and for the experimental treatments (**b-e**). Each panel shows the regression line fitted for the respective treatment. The dotted line describes a relationship with a slope of 2. (a) The overall slope 1.89 ± 0.03 (± standard error) is only slightly, though statistically significantly, less than 2. (b) Ancestors (+ in square, dotted regression line) and controls (+ in circle, solid regression line). (c) Phage treatment (solid triangle). (d) Rif treatment (solid circles). (e) Rif plus phage treatment (solid diamonds). The low slope (1.51) of the rif plus phage treatment was not driven by the leftmost filled diamond in (e) because the slope was 1.49 instead of 1.51 when that point was omitted.

For all 40 data points combined, log variance was highly significantly positively related to log mean of bacterial density ($F_{1, 38}$ = 692, $p < 0.0001$). We refer to the power b in Taylor's law as the slope when b is estimated from log variance = log a + b log mean by linear regression. The point estimate b = 1.89 fell statistically significantly below 2 (Table 1). There was no significant evidence of quadratic nonlinearity (the quadratic term in polynomial regression: $F_{1, 37}$ = 3.58, p = 0.0663).

In the minimal adequate analysis of covariance (ANCOVA) model (Table 2), the only statistically significant effects on log variance were due to log mean, to treatment, and to treatment × log mean interaction, indicating differences in slope among treatments. Bacterial populations treated only with rif showed a very high slope (5.43), whereas populations exposed to rif plus phage showed a relatively low slope (1.51). Both slopes differed significantly from 2 (Table 1).

In contrast, ancestral and control populations and phage-exposed populations had slopes not significantly different from 2, whether assayed in the KB agar plates with or without rif. Any slope within the intersection of the confidence intervals of these three treatments, namely (1.84, 2.01), was compatible with the data from all three treatments. The confidence interval of the pooled data from all treatments, namely (1.83, 1.96), fell almost entirely within the intersection of the confidence intervals of these three treatments (Table 1). The exclusion of slope 2 by the confidence interval of the pooled data evidently derives from the two treatments with rif, with or without phage. Bootstrap analyses [see Additional file 2] confirmed these conclusions.

The rif (without phage) treatment had the slope most different from 2 and was the only one where the confidence interval lay above 2. In this treatment, log$_{10}$ mean ranged from 9.6 to 9.7. This very limited range gives

Table 1 Estimates of intercept and slope (± standard error) from linear regressions of log-variance on log-mean

	Intercept	Slope	95% C.I. [lower; upper]	Adjusted R^2	n
Overall	0.38 ± 0.29	**1.89 ± 0.03**	**[1.83; 1.96]**	0.99	40
Plate					
KB	5.62 ± 1.23	**1.33 ± 0.13**	**[1.06; 1.61]**	0.85	20
KB+rif	0.28 ± 0.36	1.91 ± 0.04	[1.81; 2.00]	0.99	20
Strain					
MutS/Rif-	-0.36 ± 0.83	1.97 ± 0.10	[1.75; 2.19]	0.98	10
MutS/Rif+	3.47 ± 1.78	1.57 ± 0.19	[1.14; 2.00]	0.89	10
WT/Rif-	0.44 ± 0.25	**1.87 ± 0.03**	**[1.80; 1.94]**	0.99	10
WT/Rif+	2.94 ± 0.98	**1.62 ± 0.11**	**[1.37; 1.87]**	0.96	10
Rif- (pooled)	0.22 ± 0.34	**1.90 ± 0.04**	**[1.82; 1.99]**	0.99	20
Rif + (pooled)	2.98 ± 0.75	**1.62 ± 0.08**	**[1.45; 1.79]**	0.96	20
WT (pooled)	0.62 ± 0.31	**1.87 ± 0.04**	**[1.79; 1.94]**	0.99	20
MutS (pooled)	-0.25 ± 0.61	1.96 ± 0.07	[1.82; 2.11]	0.98	20
Treatment					
Ancestor	-0.30 ± 0.31	1.92 ± 0.04	[1.84; 2.01]	0.99	8
Control	-0.21 ± 0.52	1.95 ± 0.06	[1.81; 2.10]	0.99	8
Rifampicin	-33.71 ± 7.12	**5.43 ± 0.74**	**[3.68; 7.18]**	0.90	8
Phage	0.97 ± 0.56	1.86 ± 0.07	[1.69; 2.03]	0.99	8
Rifampicin and phage	3.95 ± 0.81	**1.51 ± 0.09**	**[1.29; 1.73]**	0.97	8

Least-squares regressions of $\log_{10}$ variance of bacterial density on $\log_{10}$ mean density (in colony-forming units per milliliter) were fitted across all data (*overall*) or fitted separately for the different assay environments (*plate*), bacterial strains, and long-term treatments. For each regression, the 95% confidence interval (C.I.) for the slope is given. Bold type indicates slopes significantly different from +2 (upper and lower boundaries calculated as mean slope ± $t_{n-1, 95\%}$ × standard error). False discovery rate method was used to correct for multiple testing (García 2004). Also given are the adjusted R^2 and the number of data points (n).

little information about how log variance would vary over multiple orders of magnitude of changes in log mean.

Rif plus phage was the other treatment with slope significantly different from 2. In this treatment, $b < 2$ and log mean ranged from 6.4 to 9.6. This range of log mean is more informative than the very narrow range of the log mean in the rif treatment, but is notably less than that of the other three treatments (ancestor, control, phage only) where log mean varied over at least 6 orders of magnitude (from < 3 to > 9). The range of mean population densities and the slope of the rif plus phage treatment resembled those of the phage (alone) treatment far more than those of the rif (alone)

Table 2 Analysis of covariance after sequential removal of nonsignificant ($p > 0.1$) factors from the full model

Source	df	Mean squares	F	P
Log mean	1	1.555	14.30	0.0008
Treatment	4	0.707	6.49	0.0008
(Rif+, Rif-) strain	1	0.148	1.36	0.2533
Treatment × log mean	4	1.390	3.19	0.0280
(Rif+, Rif-) strain × log mean	1	0.408	3.75	0.0630
Residual	28	0.109		

Analysis of covariance of the $\log_{10}$ variance of bacterial density, as a function of the $\log_{10}$ mean density, treatment (rifampicin, phage, rifampicin plus phage, control, ancestor), and bacterial resistance strain (*Rif+*, *Rif-*), in a minimal model.

treatment. Further, in the rif plus phage treatment, initially rif-resistant lines had the lowest means, whereas in the rif (alone) treatment, initially rif-resistant lines tended to have the highest means. The difference is evidently due, in part at least, to the action of the phage on the bacteria.

Discussion

A linear relation between log variance and log mean (Taylor's law) described accurately and robustly how variability in population size changed with mean population size in *P. fluorescens* bacteria. Though strains with different mutator genotypes and antibiotic-resistant phenotypes evolved and were assayed under different environmental conditions, no factors in our experiments significantly altered the linearity of the relationship.

In a combined analysis of covariance when all cofactors were considered simultaneously, the experimental treatments were the only factors that affected the slope b of Taylor's law (Table 2). In this integrated analysis, neither the bacterial strain nor the assay plate significantly affected b. Among the treatments, the slope was indistinguishable for ancestors, controls, and populations treated with phage only.

The exceptionally high slope of the rif treatment and the exceptionally low slope of the treatment with rif plus phage require further confirmation by experiments that are designed to produce a wider range of variation

in log mean. Such variation in log mean density might be achieved by varying the concentrations of rif, phage, and KB nutrients. Ramsayer et al. (2011) showed that variation in KB concentration produced wide variation in mean bacterial densities and that Taylor's law applied. Our study extends and generalizes these findings on Taylor's law to the effects of abiotic and biotic environments, and evolutionary potential in the form of mutation rates.

Mean population sizes ranged much more widely for some genetic strains and experimental treatments than for others, but whether these differences in mean affected the variance within sets of replicates, that is, the mean-variance relationship, was not clear without further investigation.

When different strains and treatments were analyzed separately (Table 1), rather than as parts of a single experiment, estimates of slope differed by strain and treatment, though no strain or treatments rejected the power law form of Taylor's law. In particular (strain section in Table 1), *WT* strains had slopes significantly less than 2, while *MutS* strains had slopes not significantly different from 2. Hence, for a given increase in mean population size, the variances of *WT* populations increased by a smaller amount than the variances of *MutS* populations. In this instance, the lower genetic diversity of *WT* compared to *MutS* is associated with a lower rate of increase in the variance of population size. This possible association of genetic strain with slope suggested that the mutation rate of the strain (as a source of genetic variation) played a role in determining the slope of the mean-variance relationship.

The speculative inferences of the previous paragraph should be viewed as hypotheses for verification in future experiments rather than as statistically well-grounded conclusions, for two reasons. First, the confidence intervals of the slopes of *WT* strains overlapped greatly with the confidence intervals of the slopes of the corresponding *MutS* strains (Table 1). Second, the difference between strains attained statistical significance here because this analysis pooled all the different treatments which *WT* strains experienced and pooled all the different treatments which *MutS* strains experienced, thereby increasing the sample size of each to attain apparent statistical significance. The earlier analysis of the experiment as a whole took account of the factorial design of the experiment and distinguished the populations of each strain that received each treatment; that analysis revealed no significant effect of strain on slope.

In addition to the possible genetic association with slope, *MutS/Rif-* populations had higher slope than *MutS/Rif+* populations and, likewise, *WT/Rif-* populations had higher slope than *WT/Rif+* populations (again, with overlapping confidence intervals in each comparison). Together, these results suggested (weakly) that a combination of mutation and selection determined the values of *b*: hypothetically, *MutS* may have increased *b* over the course of the experiment by increasing the variance in growth rates as a result of increased rates of mutation, whereas the lower slope of the *Rif+* populations reflects the action of selection in the past. This suggestion merits further experimental and field testing. Do some natural populations have low slope because of the action of selection on them?

If the rif treatment affects the slope in future experiments where log mean density varies more widely, then we could interpret the effect as follows: Culturing bacteria with rif selected for genes that conferred rif resistance, driving a divergence in population growth rates among populations in which different resistant alleles (demonstrated at the molecular level by Escobar-Páramo et al. 2012, their Table 2) arose by different mutations. Populations cultured with rif always gave rise to one or more resistant alleles and did not go extinct. The growth rate of populations derived from different mutants that arose among the bacteria cultured with rif was significantly different, according to a one-way analysis of variance (not shown here). This putative variance-increasing effect of rif-driven selection did not seem to operate in the simultaneous presence of phage, because in the rif plus phage treatment, the slope was *lower*, rather than *higher*, than 2. The molecular sequence data of Escobar-Páramo et al. (2012, their Table 2) may explain why the slope was significantly below 2 in the rif plus phage treatment: in this treatment, all *MutS* strains converged to a single allele, while in *WT*, the phage went extinct in half of the populations and the others reverted to the rif-resistant phenotype and the resulting strains were all biofilm producers. These observations suggest an interaction between abiotic (rif) and biotic (phage) selective pressures on the bacterial populations (see further discussion in Escobar-Páramo et al. 2012). Indeed, coevolving phage has been shown to affect adaptive radiation of *P. fluorescens* under variable environmental conditions (Benmayor et al. 2008).

An alternative explanation (suggested by a reviewer) of why all slopes were significantly greater than 1 (which is the slope given by Poisson distributions) could be that sampling error increased with mean abundance. For example, if some cells clumped at high density, variance among high-density cultures could increase spuriously. Unfortunately, we cannot test this idea in our data, because we had no replicate counts per plate for a given population on a given plate type. However, a clear advantage of using microcosms to study Taylor's law is that sampling error can be rejected empirically in future experiments, for example, by plotting variance as a

function of sampling effort for different values of mean abundance.

Conclusions

The near approximation to $b = 2$ (Figure 1a) and the simplicity of the ANCOVA results (Table 2), plus the excellent fit to Taylor's law with $b = 2$ in the experiments of Ramsayer et al. (2011), despite completely different sets of factors acting on population size in those experiments and these, support the relevance to these experiments of the 'universal' patterns of scaling in models and data of stem cell populations (Klein and Simons 2011). Examining microbial microcosms in the light of Taylor's law is a potentially powerful tool for investigating mechanisms and consequences of ecological and evolutionary interactions. Future theoretical and experimental studies should test the generality of our findings for the same and other taxa amenable to experimental evolution.

Additional material

Additional file 1: Table of means and variances. This table gives $\log_{10}$ mean and $\log_{10}$ variance by assay plate, bacterial origin, resistance status, treatment, and number of replicates.

Additional file 2: Data analysis, including bootstrapping and results. This Additional file gives details of data analysis, including bootstrapping, and the results of the bootstrap analyses.

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Authors' contributions
OK conducted the statistical analysis, prepared the figure, and helped write the paper. PEP helped design, and conducted, the laboratory experiments, contributed to interpreting the results, and helped write the paper. MEH helped design the laboratory experiments, suggested their relevance to Taylor's law, and helped write the paper. JEC carried out the preliminary statistical analyses, performed the bootstrap analyses, and was responsible for integrating and revising the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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Additional file 1

Table. Log_{10} mean and $\log_{10}$ variance of bacterial population density (cfu/ml) by assay plate, bacterial origin, resistance status, treatment, and number of replicates.

assay plate	bacterial origin	resistant (r) or susceptible (s)	treatment	number of replicates	log10 mean	log10 variance
rif	WT	s	+phage	12	2.051153	4.484462
rif	mutS	s	+phage	12	5.572678	12.1733
rif	WT	r	+phage	12	7.581703	14.42449
KB	WT	r	+phage	12	8.309453	16.5388
KB	mutS	r	+phage	12	8.713561	17.35055
rif	mutS	r	+phage	12	8.863075	17.45153
KB	mutS	s	+phage	12	8.938478	17.67967
KB	WT	s	+phage	12	9.141711	17.7544
rif	WT	r	+phage+rif	12	6.403264	13.63981
KB	mutS	r	+phage+rif	12	8.583104	16.92544
KB	WT	r	+phage+rif	12	8.658488	17.46579
rif	mutS	r	+phage+rif	12	8.701856	16.90598
KB	mutS	s	+phage+rif	12	9.138303	17.65896
rif	WT	s	+phage+rif	12	9.174338	17.75274
rif	mutS	s	+phage+rif	12	9.320406	17.85293

1

KB	WT	s	+phage+rif	12	9.558809	18.68858
KB	mutS	s	+rif	12	9.591065	18.21748
KB	WT	s	+rif	12	9.606381	18.54311
KB	WT	r	+rif	12	9.607008	18.5088
rif	WT	s	+rif	12	9.612077	18.40239
rif	mutS	s	+rif	12	9.62454	18.56719
rif	WT	r	+rif	12	9.644685	18.70169
rif	mutS	r	+rif	12	9.659995	18.69102
KB	mutS	r	+rif	12	9.728692	19.08134
rif	WT	s	ancestor	12	2.11115	4.027874
rif	mutS	s	ancestor	12	4.463022	7.891704
KB	WT	r	ancestor	12	9.64836	18.39932
KB	WT	s	ancestor	12	9.70164	17.91617
KB	mutS	r	ancestor	12	9.740001	18.40477
rif	mutS	r	ancestor	12	9.74864	18.54862
KB	mutS	s	ancestor	12	9.749929	18.64708
rif	WT	r	ancestor	12	9.763678	18.57285
rif	WT	s	control	12	2.588272	5.398039
rif	mutS	s	control	12	4.568691	7.947714

rif	WT	r	control	12	9.535716	18.55252
KB	mutS	r	control	12	9.548185	18.158
KB	WT	r	control	12	9.560305	18.51625
rif	mutS	r	control	12	9.680487	19.1073
KB	mutS	s	control	12	9.681241	18.9068
KB	WT	s	control	12	9.726999	18.50375

Additional file 2

Contents

DATA ANALYSIS, INCLUDING BOOTSTRAPPING

For each combination of assay plate (KB, KB+rif), bacterial mutator strain (*WT* vs. *MutS*), resistance phenotype (*Rif-* vs. *Rif+*) and treatment (control, rif, phage, rif plus phage), we calculated the mean and variance of bacterial density over the 12 evolved replicate populations. These data gave us $2 \times 2 \times 2 \times 4 = 32$ pairs of values (mean, variance). In the same way, we calculated means and variances for the 12 replicate samples of the four ancestral populations *WT/Rif-; WT/Rif+; MutS/Rif-; MutS/Rif+* using each of the assay plates (KB, KB+rif). These data gave us an additional $4 \times 2 = 8$ (mean, variance) pairs.

The 40 pairs of means and variances of density (listed in Additional file 1) were base-10 log-transformed. In an Analysis of Covariance (ANCOVA), $\log_{10}$ variance was taken as response variable, $\log_{10}$ mean as covariate and assay plate environment, mutator strain, resistance phenotype and treatment as cofactors. Ancestral strain was analysed as a treatment in addition to the four long-term treatments. Table 1 lists all the cofactors.

A model with all two-way interactions between cofactors was statistically fitted to the data. By fitting two-way interactions between cofactors and log mean, we tested for differences in the slope of the log variance - log mean relationship. Backward model simplification was employed, starting with the removal of non-significant interactions with cofactors, to obtain a minimal adequate model (Table 2). Inverting roles by taking log mean as the response variable and log variance as covariate in the model produced qualitatively very similar results (not shown).

We also performed simple linear regressions of log variance on log mean to estimate intercept and slope separately for each combination of assay plate (presence or absence of rif), bacterial strain and treatment (Table 1).

In 7 populations (all *WT*) from the rif plus phage treatment, no phage was detected at the end of the experiment. However, neither mean nor variance of bacterial density differed from populations still harbouring phage ($p > 0.1$), and excluding these 7 populations from the main analysis produced nearly identical results (not shown). We therefore presented the results based on the complete data set, including these populations.

The validity of ANCOVA rests on assumptions that the residuals from each fitted function have a distribution that is independent of the abscissa and is normally distributed. To test the robustness of conclusions based on these assumptions, we used the bootstrap procedure to fit linear and quadratic functions to the 40 pooled data points. Because the minimal model (Table 2) identified only the interaction between log mean density and treatment as statistically significant, we also bootstrapped linear and quadratic functions separately to each of the 5 sets of 8 data points for each treatment. We performed 10,000 bootstrap samples for each analysis, and again independently 1,000 bootstrap samples, to see whether results were sensitive to bootstrap sample size in this range. As the results (not shown) differed little between 1,000 and 10,000 bootstrap samples, we reported only the results from 10,000 samples.

The bootstrap procedure samples the data points with replacement. All the data points sampled could be identical, producing a singular design matrix. In bootstrap samples of the 40 data points, this possibility (which has very low probability) never arose, but it did arise for several individual treatments, which had only 8 data points each. Such bootstrap samples were uninformative and were discarded.

ANCOVA analyses were performed using JMP v8.0 (SAS 2009). Bootstrap analyses were performed using MATLAB R2011b (Mathworks 2011).

RESULTS OF THE BOOTSTRAP ANALYSES

The bootstrap analyses confirmed the results from the above regression analyses, and hence are not tabulated here. Fitting quadratic functions of log mean gave no 95% confidence intervals for the coefficients of the quadratic terms that differed significantly from 0, for all 40 data points or all 5 treatments analysed separately. Hence log variance was approximately linear as a function of log mean. The median slopes (of log variance as a linear function of log mean) from the bootstrap samples did not differ to the nearest 0.01 or differed very slightly from the point estimates of the ordinary linear regressions in Table 1. The exception was the rif treatment, where the median bootstrap slope was 5.46, compared to the linear regression estimate of 5.43.

The bootstrapped confidence intervals of the slopes were always wider, and sometimes substantially wider, than the confidence intervals of the ordinary linear regressions. However, the confidence interval (3.68, 8.74) of the bootstrapped slope in the rifampicin treatment remained significantly above 2, like the confidence interval of the slope in the rifampicin treatment in Table 1.