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ORIGINAL PAPER

Population consequences of mutational events: effects of antibiotic resistance on the *r*/*K* trade-off

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Abstract What are the effects of a mutational event on population dynamics? This eco-evolutionary question has relevance not only to basic biological theories but also to conservation applications. We evaluated the relationship between maximum population growth rate (r_{max}) and carrying capacity (K) among strains of the bacterium *Pseudomonas fluorescens*. Each of 65 strains differed from their common ancestor by one naturally acquired phenotypic change conferring antibiotic resistance, brought about by a single mutational event, and each was grown in isolation in four environments. We found no evidence of a trade-off between r_{max} and K. Rather, strains with rapid growth rates also had high carrying capacity, with little interaction between strain and environment. We conclude that the extensive variation in overall fitness resulting from single mutational events likely masks whatever population trade-offs may exist.

Keywords *Pseudomonas fluorescens* · Genotype × environment interactions · Fitness · Antibiotic resistance · Population dynamics · Contemporary evolution · Y-model · Fitness components model

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Introduction

Although the feedback between ecology and evolution has been considered for many decades, eco-evolutionary research has only recently gained widespread attention (Carroll et al. 2007). This attention is due in part to improved techniques that allow unprecedented genetic investigation (Hoffmann and Willi 2008), and in part to a growing realization that an eco-evolutionary approach can be applied to modern conservation management (Mace and Purvis 2008). At issue is the connection between genetic variation and key ecological parameters governing the long-term stability of a population such as growth rate and carrying capacity. Here we explore this question by investigating empirically the effect of mutations that arise naturally and independently on population dynamics across a range of environments. Our main goals were to quantify the amount of genetic variation generated by mutation on two key parameters governing population growth, r_{max} (maximum population growth rate) and K (population carrying capacity; Fig. 1), and to investigate the contribution of mutation to generating trade-offs between these parameters.

The notion of a negative association between r_{max} and K was popularized in the 1960's and 70's (MacArthur and Wilson 1967; Pianka 1970) and has been the focus of much research since (reviewed in Reznick et al. 2002; Bell 2008). Fast-growing 'weedy' strategies (*r*-selected strategies) are hypothesized to come at the expense of competitive ability in dense populations (*K*-selected strategies). This hypothesis is appealing for its common sense simplicity, but has been criticized on the grounds that it is difficult to test empirically because r_{max} and K cannot usually be directly measured, and proxy measures used to infer r_{max} and K are often inappropriate (Stearns 1977; Reznick et al. 2002). It is possible to avoid some of these criticisms by turning to microbial populations where r_{max} and K can be estimated from batch culture measures of population density, as resources are initially abundant and steadily depleted as the population grows.

Two mechanistic explanations for a trade-off between r_{max} and K have been suggested. The first sees r_{max} and K as characters that functionally interfere with each other such that a given amount of resources must be allocated to one trait or the other. Provided there is no variance in total resources available to individuals, genetic variation in allocation leads to a

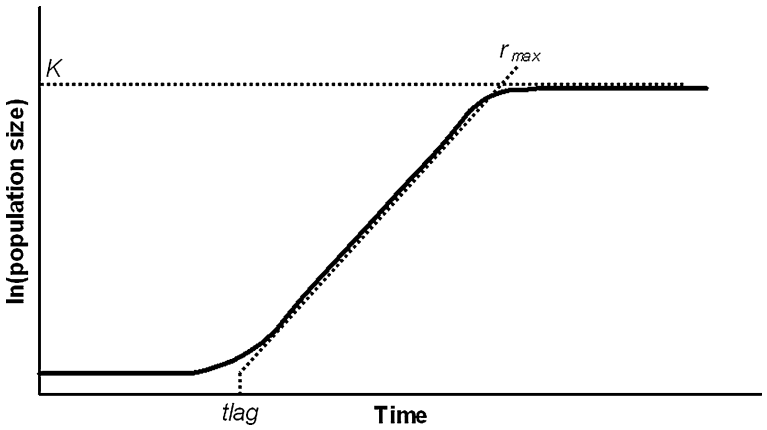

Fig. 1 Schematic population growth curve, illustrating the population carrying capacity (K), maximum population growth rate (r_{max}), and the time lag (*tlag*) before exponential growth phase indicated by the intercept of r_{max} with the initial ln(population size) value

negative relationship between the two traits at the population level. This is the resource acquisition-allocation model, or Y-model (de Jong and van Noordwijk 1992; reviewed in Zera and Harshman 2001; Roff and Fairbairn 2007). The second, the fitness components model, sees r_{max} and K as distinct components of fitness that need not be connected in any functional way. Trade-offs arise through selection as a result of the antagonism among different components of fitness (Bell 2008). Mutation generates substantial genetic variation in both r_{max} and K. Selection eliminates genotypes inferior in both traits and fixes genotypes that are superior in both. At evolutionary equilibrium, only those genotypes that trade-off r_{max} for K (and vice versa) will remain segregating in the population. These two models can be difficult to distinguish in practice, in part because the Y-model can be considered a special case of the fitness components model when the components of fitness are functionally related.

Experimental evolution studies have demonstrated that population growth rates can be density-dependent and can respond to selection (reviewed in Mueller 1997). For example, *Drosophila melanogaster* populations grown at artificially-imposed high densities for several generations evolved higher population growth rates at high than low-densities, while those selected at low densities evolved higher growth rates at low densities (Mueller and Ayala 1981). Our study differs from those focused on *r*- and *K*-selection or density-dependent selection in that ours focuses on indirect effects upon r_{max} and K instead of direct selection upon either trait. The timescale of our study is also novel in that we investigate the effect of single mutational events instead of consistent selective pressure, thus contributing to our understanding of the scaling of trade-offs (Rueffler et al. 2006).

Our study makes use of clonal populations of bacteria that each differ from a common ancestral strain by a single mutational event. There was thus no pre-existing genetic variation for r_{max} or K in our experiment. Any variation among mutant strains for r_{max} and K is the result of mutational events conferring antibiotic resistance. Although it is difficult to make conclusions about the mechanisms underlying trait relationships without detailed physiological and genetic investigation (Zera and Harshman 2001), by combining previous knowledge of the genetics and physiology of antibiotic resistance with the results of our population ecology experiment we can make stronger conclusions about the effect of mutational events on the relationship between traits than we could if we relied only on physiological or ecological information.

The relationship between traits can differ in magnitude and even sign between environments (Sgrò and Hoffmann 2004; Gutteling et al. 2007). In order to draw conclusions about trait relationships and evolutionary trajectories, therefore, more than two environments should be used to test trait relationships (Sgrò and Hoffmann 2004). We tested the relationship between r_{max} and K in four environments, differing in the type of sugar available as a resource. Our goal was to determine the effects of mutational events on the relationship between traits and whether these relationships are environment-dependent. This knowledge will improve our understanding of evolutionary trajectories and the nature of novel mutational events.

Methods

Bacterial strains

The strains used in our experiment come from the collection of nalidixic acid resistant mutants of the soil bacterium *Pseudomonas fluorescens* reported by Kassen and Bataillon

(2006), who provide details on the protocol for mutant isolation. Briefly, a collection of independently-derived strains containing a single phenotypic change conferring resistance to the antibiotic nalidixic acid was obtained by screening over 2,000 populations of the strain SBW25 (Rainey and Bailey 1996) in a conventional fluctuation-assay (Luria and Delbrück 1943). All mutant strains were thus derived from independent mutational events occurring naturally during population expansion without regard to their pleiotropic fitness effects in the assay environment.

We chose 66 strains from this collection of 665 for analysis. Eighteen of these strains were non-randomly selected from the few that had been classified by Kassen and Bataillon (2006) as 'beneficial' because they produced higher population densities than their common ancestor after 24 h of growth in permissive Luria Bertrani (LB) medium, the same medium in which antibiotic selection was performed. The remaining 48 strains were chosen at random from the larger collection, and had been classified as 'non-beneficial' because they produced lower population densities than their common ancestor in LB medium (Kassen and Bataillon 2006). Strains were stored at −80°C in solutions of 60% culture: 40% glycerol by volume.

Without sequencing the entire genome of each strain we cannot be certain that each strain diverged by a single mutation. A 500 base pair segment of the quinolone resistance determining region (QRDR) of *gyrA* was sequenced for the 18 beneficial strains (E. Ouellet and R. Kassen, unpublished data). Four different point mutations were found (never more than one per strain) among the 13 strains that had a mutation at this locus, with the remaining five strains potentially having mutations at one of several other loci known to confer resistance to nalidixic acid (Jacoby 2005). Five of the eighteen strains sequenced shared the same point mutation (T for G at position 259), suggesting ∼28% of our strains may share identical resistance-conferring mutations, assuming non-beneficial strains have similar rates of mutation similarity as beneficial strains. It is thus possible for some strains to have independently acquired identical mutations resulting in pseudoreplication. We remain confident in our results due to the strength of the relationships that emerged from our analyses; even if there were pseudoreplication of up to 28% our relationships would remain strong. Some strains may have accumulated more than one fitness-altering mutation (probability estimated by Kassen and Bataillon 2006 to be approximately one in a million), or epigenetic changes may have conferred antibiotic resistance (unlikely since strains' resistance was heritable; see Adam et al. 2008 for an empirical example of antibiotic resistance caused by epigenetic effects). These possibilities are all included along with typical single resistance-conferring mutations in our scale of study: single phenotypic changes on the timescale of single 'mutational events.'

Experimental setup

We first grew all 66 mutant strains at 28°C on 1.2% agar LB plates with nalidixic acid at the same antibiotic concentration (1 mg/l) as used by Kassen and Bataillon (2006). The common ancestor was also grown at 28°C on agar LB plates, but without nalidixic acid. Single colonies were selected from plates (to ensure only one genotype was used) and grown in 30 ml glass vials with 5 ml LB liquid media without antibiotic for 48 h at 28°C. Racks of vials were constantly shaken at $0.35g$ to maintain culture homogeneity and adequate aeration. Two microliters of this culture were transferred into microwells of media to initiate our experiment.

We inoculated all 67 strains (66 mutants + 1 common ancestor) into 24-well microwell plates (Cellstar, Greiner Bio-One) containing 2 ml of one of four media environments that differed only in the source of carbon: glucose, mannitol, mannose, or sorbitol (4.78 mM Na_2HPO_4, 2.20 mM KH_2PO_4, 0.86 mM NaCl, 1.87 mM NH_4Cl, 0.20 mM $MgSO_4{\cdot}7H_2O$, 2.21 mM sugar). Microwell plates were shaken at 0.35*g* at 28°C. Enough plates were used at once to ensure two replicate wells of each strain × environment combination (2 replicates × 67 strains × 4 environments = 536 wells). Each plate included one well with the common ancestor, and one well without any bacteria added (negative control) to detect contamination and measure spectrophotometric optical density (OD) of media without bacteria at each reading point. All negative controls were free of contamination, and all produced similar OD readings regardless of media type or time of measurement (mean ± SD = 0.030 ± 0.001).

Optical density of wells was read at 630 nm on an ELX800 Microplate Reader (Bio-Tek Instruments Inc., Winooski, Vermont, USA) using KC Junior 1.41 software (Bio-Tek Instruments Inc.) every hour following inoculation until the end of exponential growth phase and every 2 h thereafter until 27 h of growth, and once again 19 h later to verify that carrying capacity had been reached. OD is commonly used as an indicator of cell density, with OD and cell number being positively related in a sub-sample of wells tested ($r^2_{87} = 0.564$, $P < 0.001$).

Data analysis

We fitted both logistic and Gompertz equations (Zwietering et al. 1990) to ln-transformed OD data for each well using SAS 9.1 (SAS Institute Inc., Cary, North Carolina, USA) to obtain estimates of growth parameters. Both equations gave comparable results so we report only those obtained from the logistic equation. The growth parameters were maximum linear population growth rate (r_{max}), population carrying capacity (K), and the intersection of the slope r_{max} with the initial ln(OD) value which represents the time lag before the growth phase (*tlag*) (Zwietering et al. 1990) for each replicate of each strain × environment combination (Marquardt iteration method used, other iteration methods produced similar results, data not shown). We excluded from data analyses ten wells that had so little growth that the program could not converge upon parameter estimates. Among these were all eight wells containing the strain 3-1A10. All analyses were thus performed on the remaining 65 mutant strains.

We evaluated the relationship between r_{max} and K by calculating the Pearson correlation coefficient among mutant strains within each environment. Because we were concerned explicitly with the variance contributed by mutation, we excluded the common ancestor from these analyses. Average values of replicates were used. All analyses were conducted using JMP 5.1 or 7.0.2 (SAS Institute Inc.).

We tested for the significance of the strain × environment interaction term in standard two-fixed-factor ANOVAs for each of r_{max} and K to detect variability among strains in growth across environments. We then estimated the variance components of strain, environment, their interaction, and residual error upon r_{max} and K (one model for each of r_{max} and K). We used sum of squares for each effect using the restricted maximum likelihood (REML) method with all effects considered random. The resulting variance components were then used to calculate the fraction of total variance of r_{max} and K attributable to each term in the model. We also calculated the Pearson correlation coefficient for each of r_{max} and K between pairwise environments to further determine whether strains with high r_{max} or K in one environment also had high values in the other environments.

Results

Relationship between r_{max} and K

r_{max} and K were strongly positively correlated among all strains in each environment (glucose $r^2_{64} = 0.466$, $P < 0.0001$; mannitol $r^2_{64} = 0.764$, $P < 0.0001$; mannose $r^2_{63} = 0.773$, $P < 0.0001$; sorbitol $r^2_{64} = 0.763$, $P < 0.0001$; Fig. 2).

Strain × environment interaction

The strain × environment interaction term, along with each term independently, explained significant variation in both r_{max} and K in ANOVA tests (Table 1). The main effects of strain and environment together accounted for more than 85% of the variance in both r_{max} and K, with their interaction accounting for 11.2 and 2.8% of the variance in r_{max} and K, respectively (Table 1). Strains' values for r_{max} and K were positively correlated between environments (adjusted r^2 always >0.447, $P < 0.0001$; Table 2).

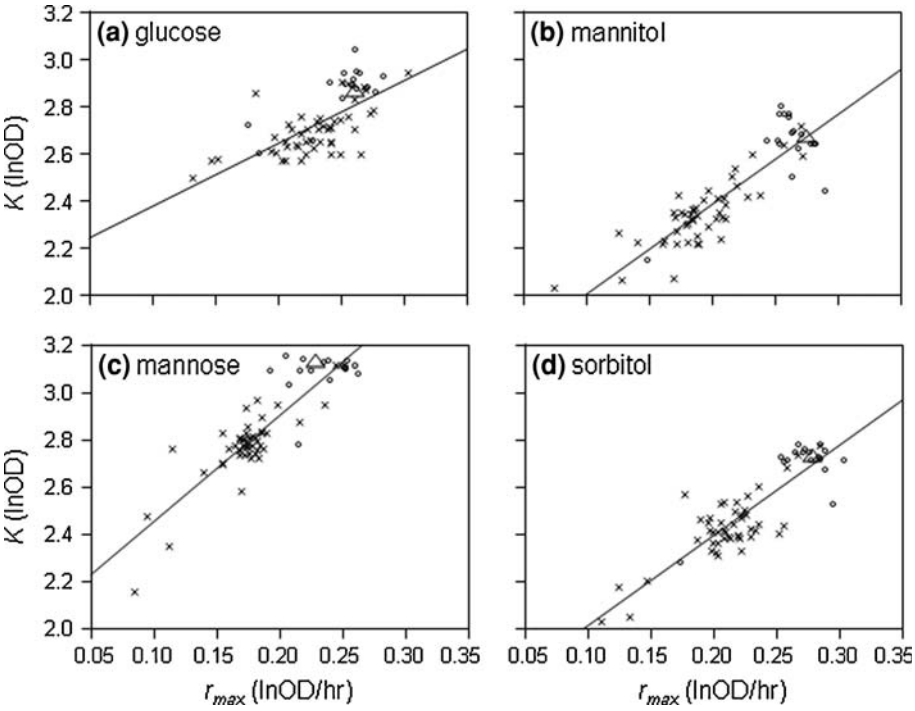

Fig. 2 Relationship between maximum population growth rate (r_{max}) and carrying capacity (K) in ln-transformed optical density (OD) units with regression lines in each of four environments: glucose (**a**), mannitol (**b**), mannose (**c**), and sorbitol (**d**). Circles represent 'beneficial' strains, crosses represent 'non-beneficial' strains (see Methods for definitions), and the triangle represents the average of six replicates of the common ancestor (common ancestor was not included in regression analysis, and is presented for comparison purposes only)

Table 1 Results of two-factor ANOVAs and variance components analyses for r_{max} and K

Term	*df*	r_{max}			K		
		F ratio	*P*	Total variance (%)	*F* ratio	*P*	Total variance (%)
Strain	64	151.50	<0.0001	64.72	106.86	<0.0001	37.42
Environment	3	187.74	<0.0001	20.63	1,111.85	<0.0001	54.46
Strain × environment	192	7.47	<0.0001	11.19	4.67	<0.0001	5.27
Error	258			3.46			2.85
Total	517			100.00			100.00

Table 2 Adjusted Pearson correlation coefficients of strains' r_{max} and K values between environments

	Glucose	Mannitol	Mannose	Sorbitol
Glucose		0.746	0.630	0.687
Mannitol	0.621		0.770	0.834
Mannose	0.447	0.733		0.849
Sorbitol	0.660	0.924	0.817	

Values in the bottom-left of the table are for r_{max}, and those in the top-right are for K. All values are significant ($P < 0.0001$), and degrees of freedom (df) = 64 for all comparisons except those involving mannose for which $df = 63$

Discussion

There was a positive relationship between growth rate and carrying capacity among strains in each environment. Strains that had high r_{max} and K in one environment also had high values in the other environments, and the strain × environment interaction explained little variation in r_{max} and K. This suggests that our results are not environment-specific. Our results are supported by research on *Escherichia coli* bacteria, in which mutational events conferring a fitness advantage in one environment also conferred a fitness advantage in most other environments (Ostrowski et al. 2005). *P. fluorescens* readily specializes on different sugar medium environments following selection over a few hundred generations (Jasmin and Kassen 2007). Any environment-specificity contributed by our mutational events was masked by overall variation in fitness.

The positive relationship between r_{max} and K is consistent with the fitness components model. However, this result may also be consistent with the Y-model provided any functional trade-off between r_{max} and K is masked by mutational variation in total resource availability such that some strains invest lots of resources into both r_{max} and K while others invest little in both (van Noordwijk and de Jong 1986; Reznick et al. 2000). Either way, if our mutant strains were exposed to prolonged selection in a stable environment we might expect the variation contributed by mutation to be reduced as those strains with shallow r_{max} and low K would quickly be eliminated. Any trade-off between r_{max} and K could then be observed, and would in fact be expected. Long-term evolution experiments using *E. coli* support this prediction (Novak et al. 2006). After evolutionary time periods most populations were found to consist of strains that displayed a negative association between r_{max} and K (Novak et al. 2006), consistent with the hypothesis that selection removed the variation in overall fitness contributed by mutation over time, revealing a functional trade-off

between r_{max} and K. The main conclusion we can draw is that mutation is not bound by any inherent functional or physiological constraint that generates a trade-off between r_{max} and K. Rather, such trade-offs are the product of the combination of mutation and selection.

An alternative explanation of positive covariance between r_{max} and K is that mutation affected neither resource acquisition nor fitness components, but shifted variation in resource allocation decisions to upper hierarchical levels thus masking trade-offs at subsequent levels (Worley et al. 2003). For example, if an animal's resources are first allocated between reproduction and somatic growth, and reproduction resources are subsequently allocated between offspring size and offspring number, a positive relationship between offspring size and number would result from mutation contributing far greater variation in allocation at the first hierarchical level (reproduction vs. growth) than the second. According to this explanation, our strains with high r_{max} and K have greater resources available for reproduction at the expense of resources for other functions, compared to their common ancestor. Thus strains with low r_{max} and K might have invested vast resources in cellular defences or motility rather than reproduction (for example), and would be expected to be competitively superior in environments favouring these traits. We believe this explanation is unlikely for the majority of our strains, as we expect based in part on our knowledge of the physiology of our mutational system that strains with low r_{max} and K result from mutations affecting overall fitness.

Knowledge of the physiology and genetics of our experimental system allows us to make hypotheses regarding the mechanism underlying the relationship between r_{max} and K at our scale of study that would not be possible if we were to rely only upon ecological data. A striking feature of our results, which confirms that of earlier work (Kassen and Bataillon 2006), is that the pleiotropic costs of resistance to quinolone antibiotics are highly variable both in terms of r_{max} and K. We suspect that the source of this variance in costs of resistance stems from the specific genetic targets conferring resistance. Low costs of resistance are likely to arise from mutations that induce conformational changes in DNA gyrases that prevent quinolones from binding to the enzyme-DNA complex. Such mutations should be energetically inexpensive since they result largely from amino acid substitutions that affect the tertiary folding of proteins (Hawkey 2003). High-cost mutations, on the other hand, are expected to result from mutations in regulatory genes associated with efflux systems, as the constitutive expression of such systems is likely to be energetically expensive to the cell. Preliminary evidence in support of this comes from our observation that the majority (13/18) of mutations amongst our fittest strains stem from one of four mutations within the 500 bp quinolone-resistance-determining region of *gyrA* (Ouellet and Kassen, unpublished data), which alters the target of quinolone rather than efflux systems (Hawkey 2003; Jacoby 2005). Mutants with low-cost mutations would invest plenty of resources in both r_{max} and K, while those with mutations affecting efflux systems would have few resources to invest in either r_{max} or K. Mutations affecting efflux systems might also affect resource acquisition rates if the efflux pump is overly-general and ejects resources from the cell before they can be used.

Evidence suggests the average effect of mutations on fitness is weak and deleterious (Eyre-Walker and Keightley 2007), but it is also important to understand the pleiotropic effects of mutational events to model evolutionary predictions (Johnson and Barton 2005). We have demonstrated that, at least for our study system, experimental environments, and characters evaluated, mutational events likely contribute greater variation to overall fitness than to trade-offs between fitness components.

Human degradation of natural ecosystems is expected to continue at an unprecedented rate (Millennium Ecosystem Assessment 2005), leading to phenotypic changes in wild populations that are generally greater in magnitude than those caused by natural perturbations (Hendry et al. 2008). In this setting the influence of phenotypic changes on population dynamics is not merely an academic concern. Research should advance beyond documenting changes in life-history trait values due to environmental change, and investigate the influence of those trait changes on individual fitness, population dynamics, and community- and ecosystem-scale dynamics. We have investigated the influence of one phenotypic change (antibiotic resistance, resulting from a mutational event) on two population parameters (growth rate and carrying capacity) in a controlled system that allows population-level study but lacks the multiple interactions typical of natural systems. Future research should determine the generality of population effects of mutational events and investigate eco-evolutionary dynamics in multi-species communities (Johnson and Stinchcombe 2007; Lau 2008).

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