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Year: 2017

Version:

Experimental Approaches for Testing if Tolerance Curves Are Useful for Predicting Fitness in Fluctuating Environments

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Most experimental studies on adaptation to stressful environments are performed under conditions that are rather constant and rarely ecologically relevant. Fluctuations in natural environmental conditions are ubiquitous and include for example variation in intensity and duration of temperature, droughts, parasite loads, and availability of nutrients, predators and competitors. The frequency and amplitude of many of these fluctuations are expected to increase with climate change. Tolerance curves are often used to describe fitness components across environmental gradients. Such curves can be obtained by assessing performance in a range of constant environmental conditions. In this perspective we briefly list theoretical and experimental evidence why results obtained under constant environmental conditions might be misleading for processes in nature and therefore may not be suitable for predicting fitness and future species distribution and abundance. We further suggest experimental avenues that can provide a better foundation for forecasts of the distribution of biota.

Keywords: tolerance curves, constant and fluctuating environments, climate change, species distributions, environmental stress

Tolerance curves are often used to describe performance or physiological traits across environments (Sarkar, 2004; Angilletta, 2006, 2009; Stinchcombe et al., 2012). Such curves are typically obtained by assessing the performance of genotypes in different constant environments; e.g., at a range of constant temperatures (e.g., Schou et al., 2016). Observations from these experiments allow researchers to get detailed information on performance across environmental gradients and they have been proposed to allow spatial and temporal visualization of fitness components of genotypes, and thereby for example pinpointing species or populations that are specialists or generalists, and for detecting genotype by environment interactions (Dobzhansky and Spassky, 1963). Tolerance curves have multiple purposes in diverse biological disciplines and they seem attractive for predicting performance and distribution of species in more natural fluctuating environments (Kassen, 2002; Chevin et al., 2010; Schou et al., 2016; Sexton et al., 2016).

PROBLEMS WITH TOLERANCE CURVES

Tolerance curves based on observations from a range of constant environments, which is the common experimental approach, can however be heavily criticized, both from a theoretical and

an empirical standpoint, when it comes to predicting performance in fluctuating environments. Recent papers have pointed out the problem that tolerance curves are considered invariant to previous exposures to different environments, speed, and order of fluctuations (see e.g., Sinclair et al., 2016). From physiology we know that the speed, amplitude, and duration of environmental fluctuations strongly affect fitness (Schulte et al., 2011). For example expression of stress proteins such as heat shock proteins are prime examples where the expression patterns differ dramatically in species occupying fluctuating and stable temperature habitats and also within species, the expression patterns, and the adaptive importance of the plastic response differ at constant high and fluctuating high temperatures (Podrabsky and Somero, 2004; Tomanek, 2010).

Interestingly, when we look back at earlier literature on experimental evolution in fluctuating environments, the results indicate that different fluctuations might lead to separate adaptations that are not visible in traditional tolerance curves obtained across constant temperatures. For example a study with ciliates showed no adaptation to fluctuating environments when measured at constant temperatures, but strains adapted to rapidly fluctuating thermal environments had increased expression of the heat shock protein Hsp90, indicating evolution of tolerance to cope with acute stress (Ketola et al., 2004). From studies that have tested the effect of evolution in fluctuating environments on tolerance curves and performance also in fluctuating environments (Bennett and Lenski, 1993; Leroi et al., 1994; Kassen and Bell, 1998; Hughes et al., 2007; Ketola and Saarinen, 2015), only one study shows a positive link between tolerance curve parameters obtained across constant environments and the performance in fluctuating environments (Hughes et al., 2007) and one suggests a strong negative link (Ketola and Saarinen, 2015).

In addition to empirical evidence, theoretical work suggests that tolerance curves are poor predictors of performance in fluctuating environments (Levins, 1968; DeWitt and Langerhans, 2004; Botero et al., 2015). For example, evolutionary theories predict that responses to fluctuating and constant environmental conditions will favor genetically determined broad and narrow tolerance curves, respectively (Slatkin and Lande, 1976; Lynch and Gabriel, 1987; Gomulkiewicz and Kirkpatrick, 1992; Gilchrist, 1995). However, the main message from models that take into account inducible and reversible plastic responses to environmental fluctuations, in addition to genetically determined tolerance curves, suggests that inducible plasticity is superior to genetically fixed strategies (broad or narrow tolerance curves) under nearly all conditions (Levins, 1968; DeWitt and Langerhans, 2004), and especially in frequently fluctuating environments (Padilla and Adolph, 1996). However, if environmental cues for predicting future environmental conditions are unreliable inducible plasticity also becomes a burden (Reed et al., 2010) and risk spreading strategies, such as bet-hedging are expected to evolve (Levins, 1968; DeWitt and Langerhans, 2004). Thus, when populations are exposed to environments with different predictabilities, amplitudes, and frequencies of fluctuations, different adaptation mechanisms are expected to operate (Botero et al., 2015).

Empirical and theoretical evidence suggests that many tolerance curves presented in the literature are not good predictors of fitness in fluctuating environments. However, very few studies have properly tested the extent of the problem. Lack of this knowledge is problematic in the broad context of understanding evolutionary processes in natural systems and the genetic background for adaptation to environmental fluctuations. One reason why it is of outmost important to obtain a better understanding of tolerance curves is that they are often being used to predict future distributions of species (e.g., Chevin et al., 2010; Huey et al., 2012; Valladares et al., 2014). Further, experimental molecular work that aims at elucidating the genetic architecture of fitness components is typically performed on populations held in constant environments and therefore may not detect adaptive genetic variation of relevance for populations in their natural habitat. Failure to recognize by which means the genotype, population or species is adapted to environmental fluctuations will make it hard to predict how e.g. climate change induced alternations in fluctuations affect biota, and extinction risks (Botero et al., 2015). Here we present avenues for how studies can test the value of tolerance curves and suggest ways that can provide data suitable for predicting performance in fluctuating environments, and distribution and abundance of biota in rapidly increasing stressful and fluctuating environments.

WAYS FORWARD

We argue that it is important to acknowledge the fact that fluctuating and constant environments have different impacts on fitness (Ketola et al., 2012; Sinclair et al., 2016), that responses that are adaptive in fluctuating environments might be maladaptive in constant environments, and *vice versa* (Kristensen et al., 2008; Chevin et al., 2010), and that evolutionary responses to constant and fluctuating environments can be distinct (Botero et al., 2015; Melbinger and Vergassola, 2015; Dey et al., 2016).

Therefore, we propose to investigate the validity of current practices, where data from tolerance curves obtained across a number of constant environments in the laboratory are used to predict fitness across constant and fluctuating environments and future species distributions. Further we suggest that there is a pressing demand for experiments where the fitness impacts of different kinds of environmental fluctuations can be evaluated, and we provide examples and specific recommendations to show how this can be done.

Experimental Evolution

Theories emphasize that populations can adapt differently depending on the type of environmental fluctuations (see above). The experimental evolution setup in which rapidly reproducing species are let to evolve multiple generations in different kinds of environments is capable of resolving whether evolutionary responses to constant and fluctuating environments are similar and whether adaptations are specific to particular kinds of fluctuations. For example if experimental bacterial strains can be identified from each other (Bennett and Lenski, 1993; Ashrafi

et al., 2017), it allows comparing fitness of the strains in competition.

For example one can ask if strains adapted to environmental fluctuations are superior in both constant and in fluctuating environments, in comparison to strains evolved in a constant environment. When this information is projected on tolerance curves obtained at constant environments it unambiguously indicates if fitness in fluctuating environments is reflected in the tolerance curves.

Ketola and Saarinen (2015) did not utilize marker strains and they used fitness surrogates in their experiments. Still, this study provides insights on the value of tolerance curves obtained from constant thermal environments. The idea in Ketola and Saarinen (2015) was to test if fluctuations increased growth or yield of bacterial clones during fluctuations, which could be seen as an adaptation to prevailing conditions. Accordingly, strains evolved in fluctuating regimes had higher growth rate under fluctuations than strains evolved in constant environments. Thus, these experimentally evolved bacteria provided clear evidence for an evolved ability to tolerate fluctuations. However, when thermal tolerance curves were based on several measurements obtained across the range of environments experienced during the process of experimental evolution, no evidence of adaptation to fluctuations could be deduced from the tolerance curve. On the contrary, the strains adapted to fluctuations were outperformed by strains evolved at constant environments. These data clearly demonstrate that evolutionary processes are distinct in fluctuating and constant temperature environments. Recent work on the insect, *Drosophila simulans*, draws similar conclusions (Manenti et al., 2016).

We propose that experimental evolution experiments that test different and ecologically relevant frequencies of variation are highly needed. Such experiments will resolve if fluctuations that are fast or slow, frequent or infrequent, predictable or not, and have high or low amplitude, have their characteristic adaptations—as theories predict (see above). Such studies will elucidate if tolerance curves from constant environments fail to capture adaptations in certain kinds of fluctuations, as we propose. Based on the reasoning put forward in papers by Schulte et al. (2011) and Sinclair et al. (2016) it is likely that adaptations to fast fluctuations are especially hard to observe from tolerance curves obtained across constant environments. Thus, more work should be done with different kinds of environmental fluctuations (Ketola et al., 2004; Buckling et al., 2007; Manenti et al., 2014), to disentangle critical frequencies, amplitudes, or predictabilities, where traditional tolerance curves may start to fail. Experimental evolution studies have a lot to provide in this context.

Quantitative Genetic Experiments

There are a large number of quantitative genetic studies exploring the amount of genetic variation in e.g. heat shock survival or in tolerance curve parameters (Scheiner, 1993; Kellermann et al., 2009; Ketola et al., 2014; Kristensen et al., 2015). However, as stated here the adaptive benefit of heritable variation in some proxy of fitness in a constant environment might be minor in fluctuating environments if it is not genetically correlated with fitness in fluctuating environments (Ketola et al., 2014; Manenti et al., 2016). What is crucially missing in most quantitative genetic experiments performed so far is correlating the abovementioned proxies to fitness in fluctuating environments. For example, in a massive experiment we reared half-sib families of *D. melanogaster* under four different thermal environments, two constant and two fluctuating temperatures, and quantified egg-to-adult viability under these four thermal conditions. Two constant temperatures were used to draw simple linear tolerance curves and to estimate its parameters. Next we resolved if these parameters (elevation and the slope) were genetically correlated with egg-to-adult viability in fluctuating environments. We found that the elevation was under positive selection in fluctuating environments but not the slope. However, interestingly the elevation and the slope together explained a rather small proportion of the variance in egg-to-adult viability in fluctuating environments, supporting the idea that the traditional tolerance curves obtained in constant environments might not be enough for describing performance when environments fluctuate (Ketola et al., 2014).

We advocate that quantitative genetic studies aiming at generating ecologically relevant tolerance curves should use similar designs but investigate more temperatures, or vary other environmental components, and a range of different frequencies and intensities of fluctuations. These should also involve investigating some proxies of tolerance to fast environmental changes, for example including measuring physiological responses to more acute stress like inducible heat shock proteins, metabolic rate, knock down temperatures, or chill coma recovery time. Crucially, these proxies, along with tolerance curve parameters, should also be genetically correlated with lifetime fitness or survival in different kinds of fluctuating "test environments" (Ketola et al., 2014) to resolve which of the proxies are more reliable in predicting tolerances to fluctuations (Rezende et al., 2010). In addition to classic quantitative genetic setups (e.g., full-sib half-sib breeding designs, isofemale lines, or the use of pedigreed populations), using clonal species should also be considered, as this could be an affordable approach to conduct these very demanding experiments.

In addition to experiments, data could also be retrieved from natural populations. Quantitative genetic studies of pedigreed wild populations have revealed selection on tolerance curve characteristics (Nussey, 2005; Charmantier et al., 2008; Debes et al., 2017). Depending if there is between year variation in the amount of environmental fluctuations, field studies on pedigreed populations could allow estimating reaction norms for fitness on the continuum of environmental fluctuations. This approach serves as an important source of information by answering the question whether the same genotypes do well in constant and fluctuating natural environments.

Next Generation Genetics

Omics technologies have a lot to offer in relation to pinpointing the physiological and genetic architecture of complex traits in constant and fluctuating environments. For example sequencing of strains/populations from experimental evolution studies evolved in constant and fluctuating environments, or testing

performance of gene knock down/knock in strains in both types of environments will provide valuable insight into whether the same or different genes contribute to the tolerance in fluctuating and constant environments (Sørensen et al., 2016; Deatherage et al., 2017). Recent work by Sørensen et al. (2016) is very illuminating in this respect. Exploring gene expression of *D. melanogaster* flies exposed to different kinds of environments revealed that: "transcriptional responses to mean temperature, acute exposure to heat and fluctuations act through largely independent mechanisms." This quote from the paper matches the take home message that we wish to convey namely that adaptations to constant and fluctuation environments are partly under distinct genetic control.

Also the ability to use genomic and other omics approaches on non-model species is developing rapidly (Shafer et al., 2015). This provides a range of opportunities to pinpoint genes and mechanism responsible for fitness and plastic responses in the field on a wide range of species. This does not leave laboratory experiments out-of-date but do provide additional opportunities that should be exploited.

CONCLUSIONS

The ability to predict effects of increasing environmental fluctuations on fitness traits and the fate of species and populations in given environments is an important topic within the fields of ecology, evolutionary biology, and genetics. It is our impression that there is a misperception among many researchers that tolerance curves are universal descriptors of what happens if environments fluctuate. This problem has been well-described in a few recent papers (Schulte et al., 2011; Sinclair et al., 2016). We are not suggesting that tolerance curves are useless as a predictive tool for many purposes, but argue that we need to perform more ecologically relevant experiments and test when tolerance curves are predictive of what happens if environments fluctuate. From a theoretical point of view

fluctuations are acknowledged to impact strongly the directions and mechanisms of adaptation and empirical work has by and large neglected this complication. Wythers et al. (2005) illustrated the importance of adding reality into experimental work on thermal adaptation. In their study they showed that by allowing acclimation and other plastic responses to occur in ecosystem models, they dramatically altered predictions for the productivity and respiration rates of plants. Others have also shown that predicted species distributions are sensitive to whether these complications are taken into account (Duputié et al., 2015). Such results highlight that experimental work on the ability of tolerance curves from constant environments to describe selection pressures in fluctuating environments is highly needed. The forecast that environmental fluctuations are expected to increase with current climate change make this effort even more pressing (Christensen et al., 2013).

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