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RESEARCH ARTICLE

Effects of Phenotypic Robustness on Adaptive Evolutionary Dynamics

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Abstract
Theoretical and experimental studies have provided evidence for a positive role of phenotype resistance to genetic mutation in enhancing long-term adaptation to novel environments. With the aim of contributing to an understanding of the origin and evolution of phenotypic robustness to genetic mutations in organismal systems, we adopted a theoretical approach, elaborating on a classical mathematical formalizations of evolutionary dynamics, the *quasispecies model*. We show that a certain level of phenotypic robustness is not only a favourable condition for adaptation to occur, but also a required condition for short-term adaptation in most real organismal systems. This appears as a threshold effect, i.e. as a minimum level of phenotypic robustness (critical robustness) below which evolutionary adaptation cannot consistently occur or be maintained, even in the case of sizably selection coefficients and in the absence of any drift effect. These results, are in agreement with the observed pervasiveness of robustness at different levels of biological organization, from molecules to whole organisms.

Keywords Quasispecies model · Genetic mutation · Phenotypic evolution · Evolvability

Introduction

Phenotypic robustness has been defined as the property of a biological system to preserve its phenotype in the face of perturbations, such as genetic mutations (Wagner 2011, 2013). This quality is widely considered to be pervasive at different levels of biological organization, from molecules to whole organisms (Kitano 2004; Stelling et al. 2004; Wagner 2005). At the level of the organism, phenotypic robustness to genetic mutations might seem to be a quality of the organism's genotype–phenotype (G–P) map that should hamper the process of adaptation, by making the occurrence of beneficial phenotypic mutations more rare. However, somewhat counter-intuitively, theoretical and computational studies predict a positive role for phenotypic robustness in enhancing long-term adaptation to novel environments (Gibson and Reed 2008; Wagner 2008; Draghi et al. 2010; Hayden et al. 2011). This effect has been demonstrated through simulations (Rodrigues and Wagner 2009; Barve and Wagner 2013) and experimental studies on ribozymes (Hayden et al. 2011). More recently, experimental evolution studies on *E. coli* have shown that phenotypic robustness can promote significantly faster adaptation at the level of a whole organism (Rigato and Fusco 2016; Zheng et al. 2019).

There are two main ways in which phenotypic robustness has been considered to be able to foster adaptation through the accumulation of cryptic genetic variation (CGV) (Wagner 2012). Firstly, in a new environment (Hayden et al. 2011) or in a new genetic background (Hermisson and Wagner 2004), phenotypically unexpressed genetic variation can become expressed, and among the new phenotypes, some variants might result accidentally 'pre-adapted', or 'exapted' to the new environmental conditions. Secondly, and possibly more importantly because less fortuitous, the scattering of genotypes with the same phenotype through the genotype space allows the population to access a greater number of new phenotypes through mutation, increasing the probability of finding phenotypes that happen to have higher fitness (Rodrigues and Wagner 2009). This latter mode has been recently questioned by Mayer and Hansen (2017), who, on the basis of a computational study based on Boolean networks, argued that positive effects of robustness on evolvability can emerge only under strict biological conditions. However, there is possibly a third way, which is the particular focus here and takes into account the fact that robustness can support the spread of already present favourable phenotypic variants. As we will show, this is an

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effect of a damping of the mutation probability owing to a generic property of the G–P map, that in practice increases the evolutionary stability of phenotypic variants. While the first two aforementioned modes are contingent on the structure of variation of the population, including the level of CGV, and on the structure of the neutral networks in the genotype space, the last one is to a large extent independent of these features or the advent of new adaptive challenges (like a new environment, or a modified fitness landscape), and can produce short-term effects.

With the aim of contributing to an understanding of the origin and evolution of phenotypic robustness to genetic mutations in organismal systems, we adopted a theoretical approach, by elaborating on a classical mathematical formalizations of evolutionary dynamics, the *quasispecies model* (Eigen et al. 1989). By appropriate decomposition of a phenotypic version of the quasispecies model, which describes frequency dynamics at phenotypic level, we extracted and analysed a phenotypic-robustness term that is significant in the current debate on the role of robustness in evolution.

Model Assumptions

The quasispecies model is a mutation-selection dynamical system that describes the evolution of an infinitely large population of haploid, asexually reproducing genotypes on a constant fitness landscape (Nowak 2006). This is a deterministic model and our derivations are based on three assumptions: (i) The view on phenotype is restricted to the *target phenotype*. This is defined as the phenotype that would be expressed by a given genetic makeup of the organism under some given environmental conditions during development, in absence of any perturbations (Nijhout and Davidowitz 2003). This is not to neglect environmental effects on the phenotype, either in the form of phenotypic plasticity or developmental noises (Fusco and Minelli 2010), but rather to concentrate on the contribution of the organism's genotype to its phenotype. Thus phenotypic plasticity, i.e. the fact that individual genotypes can produce different phenotypes when exposed to different environmental conditions, and developmental instability produced by random perturbations of development are not accounted for here. (ii) The genotype includes the whole genome of the organism, as a single allele determining the phenotype (*omnigenic model*; Boyle et al. 2017). This perspective is supported by two complementary arguments. On one side, a phenotypic trait generally depends on the expression of numerous genetic determinants, although with effects of variable magnitude (Fisher's *infinitesimal model*; Turelli 2017). On the other side, virtually each locus can, more or less directly, affect a vast array of phenotypic traits (*ubiquitous pleiotropy*; Visscher and Yang 2016). The omnigenic model is supported by empirical evidence, the most recent coming from genome-wide association studies (GWAS) (review in Boyle et al. 2017), but with reference to our derivations, this choice allows us to avoid specific assumptions on more detailed features of the organism's G–P map, including the level of epistasis, pleiotropy, and neutrality, for which, despite substantial theoretical modelling (e.g., Orr 2000; Wagner 2008; Wagner and Zhang 2011; Pavlicev and Wagner 2012), observational data (e.g., Pavlicev et al. 2008; Wagner et al. 2008; Wang et al. 2010; Tanaka et al. 2015; Shikov et al. 2020) shows high disparity in the structural properties of G–P maps across biological systems. (iii) A key generic feature of the G–P map at the level of the organism (when the phenotype is intended as target phenotype) is that this is a many-to-one relationship. Stated differently, multiple genotypes can map on the same phenotype (Wagner 2011; Ahnert 2017; Mayer and Hansen 2017).

Elaborating on the quasispecies formalization of evolutionary processes, here we show that a certain level of phenotypic robustness is not only a favourable condition for adaptation to occur, but also a required (although not sufficient) condition in most real organismal systems. This appears as a threshold effect, i.e. as a minimum level of phenotypic robustness (critical robustness) below which evolutionary adaptation cannot consistently occur or be maintained, even in the case of sizably selection coefficients and in the absence of any drift effects.

Phenotypic Robustness and Phenotypic Stability

Phenotypic robustness is a property of the genotype–phenotype map. Here, for the derivations to follow, we will adopt a narrow, quantitative definition of *phenotypic robustness* (ρ), that is the probability that mutation of a given genotype g across one generation takes to a genotype g' that exhibits the same phenotype of g.

From this definition of robustness, a definition of *phenotypic stability* (ϕ_{pp}) follows. This is the probability that the replication of a given genotype g with phenotype p takes to a genotype that exhibits the same phenotype p. Indicating with η_g the mutation probability per genome per generation, phenotypic stability results to be the sum of the probabilities of two mutually exclusive events, namely (i) that there is no mutation $(1-\eta_g)$ and (ii) that in case of mutation the mutant genotype maps to the same phenotype ($\rho\eta_g$), that is

$$\phi_{pp} = (1-\eta_g) + \rho\eta_g. \tag{1}$$

Quasispecies Model Analysis

The quasispecies model (Eigen et al. 1989) is a single locus, multiallele, mutation-selection model where each allele differs from the others by at least a single point mutation.

Let us imagine a sufficiently large population of n replicating sequences (or, asexually reproducing haploid genotypes). Sufficiently large means that we can neglect the effects of drift. Sequences can replicate at different rates, according to their fitness and can mutate upon replication. Denote by x_i the relative frequency of the ith sequence type, thus we have $\sum_i x_i = 1$. The population structure is given by the vector $\mathbf{x} = (x_1, x_2, \dots x_n)$. Denote by q_{ji} the per-replication probability of a sequence j to mutate into a sequence i and by W_i the absolute fitness (absolute growth rate) of the ith sequence type. The fitness landscape is given by the vector $\mathbf{W} = (W_1, W_2, \dots, W_n)$ and the average population fitness is $\overline{W} = \sum_i x_i W_i$ (see Nowak 2006). In its continuous-time version, the quasispecies equation expresses the time derivative of the frequency of the ith sequence type as

$$\dot{x}_i = \sum_j x_j W_j q_{ji} - x_i \overline{W}. \tag{2}$$

Equation (2) describes the evolution of population of genotypes on an invariant fitness landscape, where the absolute fitness of a genotype does not depend of its own frequency (frequency-independent selection).

Introducing the Genotype–Phenotype Dualism into the Quasispecies Model

Since the principle of the quasispecies dynamics holds for any mutating and reproducing entity, we can use the quasispecies formalism to track frequency changes at phenotypic level, rather than at the level of the genotype. Let us rewrite the quasispecies equation for a focal phenotype p, with frequency x_p, as

$$\dot{x}_p = \sum_j x_j W_j \phi_{jp} - x_p \overline{W}, \tag{3}$$

where W_j is the fitness of the jth phenotype, ϕ_{jp} is the phenotypic mutation probability of j into p and $\overline{W}$ is the population mean fitness ($\overline{W} = \sum_j x_j W_j$). Decomposing the summation in (3) to highlight the two main contributions to the frequency change of p, yields

$$\dot{x}_p = x_p W_p \phi_{pp} + \sum_{j \neq p} x_j W_j \phi_{jp} - x_p \overline{W}. \tag{4}$$

Equation (4) is the phenotypic version of the quasispecies model, assuming different genotypes to map on the same phenotype. The first term of the right-hand side of (4) is the contribution of non-mutant phenotypes p, while the second term is the sum of the contribution of mutations from different phenotypes. ϕ_{pp} is the phenotypic stability term, which in turn contains the robustness term ρ. Derivations similar to Eq. (3) were developed by Reidys et al. (2001) and Takeuchi et al. (2005). However, having a different aim and moving from different assumptions with respect to the present modellization, these two contributions consider the quantification of the G–P map redundancy in a different perspective with respect to the one adopted here, where the precise focus is on the role of phenotypic robustness (ρ) in evolution (see Discussion).

Conditions for Adaptation

Considering Eq. (4), let us define that adaptation occurs when an advantageous phenotype p (i.e. a phenotype with $W_p > \overline{W}$) increases its frequency, that is when $\dot{x}_p > 0$. Then we can write

$$x_p W_p \phi_{pp} + \sum_{j \neq p} x_j W_j \phi_{jp} - x_p \overline{W} > 0, \tag{5}$$

and dividing both sides of the inequality by $\overline{W}$ and rearranging, gives

$$x_p (w_p \phi_{pp} - 1) + \sum_{j \neq p} x_j w_j \phi_{jp} > 0, \tag{6}$$

where w_p and w_j indicate the relative fitness of the phenotypes. Note that this definition of adaptation focuses on the instantaneous ability of the population to adapt, and does not require any equilibrium analysis. At variance with most treatments of the quasispecies equation, the advantageous phenotype does not need to be the most advantageous phenotype and the analysis does not assume a closed system (i.e., a system in which the arrival of mutant phenotypes that are fitter than the focal phenotype can be ignored).

The left-hand side of inequality (6) presents a decomposition of the condition for adaptation in two additive terms. The first term ($x_p(w_p \phi_{pp} - 1)$) represents the contribution of non-mutant phenotypes and critically depends on phenotypic stability. This, in turn, derives from a very generic property of the G–P map, the many-to-one relationship between genotype and phenotype spaces (quantified by the robustness term ρ in Eq. (1)), and in this form does not depend on any specific structuring of the G–P map. The second term ($\sum_{j \neq p} x_j w_j \phi_{jp}$) represents the mutational contribution from different phenotypes to the frequency of the focal phenotype. This is analogous to the probability of back mutations in the standard application of the quasispecies equation, a term often neglected to simplify further analytical elaborations (e.g., Nilsson and Snoad 2002; Sasaki and Nowak 2003; Gorodetsky and Tannenbaum 2008; Draghi et al. 2011). Here, in the context of a phenotypic quasispecies model, we note that this term (always ≥ 0) is contingent on the specific phenotype, the current distribution of genotypes in the genotype space and other local detailed features of the G–P map. These features are expected to vary extensively across levels of biological organization, organisms and characters within the same organism (Hansen

2006; Wagner and Zhang 2011; Pavlicev and Wagner 2012; Szamecz et al. 2014). To investigate the effects of robustness on adaptation, it is thus useful to evaluate the contribution of the first term in the absence of any contribution of the second term. This is not to neglect the effects of the G–P map structure on adaptation, but rather to focus on a more generic property of the G–P map, which applies (although with variable effects, see below) to a wide set of adaptive contexts and organisms. Thus, by setting the second term of inequality (6) to zero we get

$$x_p(w_p\phi_{pp} - 1) > 0, \tag{7}$$

that simplifies to

$$w_p\phi_{pp} > 1. \tag{8}$$

Inequality (8) is the necessary condition to be satisfy for adaptation to occur without relying on factors contingent on the detailed structure of the G–P map. Since the phenotypic stability term ϕ_{pp} contains the robustness term, by substituting (1) into (8), the required minimum level of robustness to satisfy (8) results to be

$$w_p(1 - \eta_g + \rho\eta_g) > 1. \tag{9}$$

Rewriting the relative fitness term as $w_p = (1 + s_p)$, were s_p is the selection coefficient of the advantageous phenotype p, we get

$$(1 + s_p)(1 - \eta_g + \rho\eta_g) > 1, \tag{10}$$

and by isolating the ρ term, we finally obtain

$$\rho > \rho_c = \frac{(1 + s_p)\eta_g - s_p}{(1 + s_p)\eta_g}. \tag{11}$$

The right-hand side of inequality (11) is the minimum level of phenotypic robustness required for adaptation to consistently occur or to be maintained under the quasispecies model, that we indicate as the *critical robustness* (ρ_c). This depends exclusively on the genome mutation probability η_g and on the selection coefficient s_p. As the mutation probability increases, higher levels of phenotypic robustness are required for adaptation to occur, whereas for increasing values of the selection coefficients, lower levels of phenotypic robustness are required (Fig. 1). ρ_c can vary from $-\infty$ to 1. When $\rho_c \leq 0$, no robustness is required for adaptation. This happens for low mutation rates and for high selection coefficients, but for whole-genome genotypes this is not a common combination (see Discussion).

Studying the condition for $\rho_c > 0$, from (11) we get

$$s_p < \frac{\eta_g}{1 - \eta_g}. \tag{12}$$

Since $\frac{\eta_g}{1-\eta_g}$ increases nearly exponentially with η_g (actually, super-exponentially after 0.9), inequality (12) is often easily satisfied, and some level of robustness is required for adaptation to occur irrespective of the selection coefficient value. Moreover, as for large genomes and/or large per-base mutation rates η_g tends rapidly to 1, the condition for adaptation to occur can be approximated to

$$\rho > \rho_c = \frac{1}{1 + s_p}. \tag{13}$$

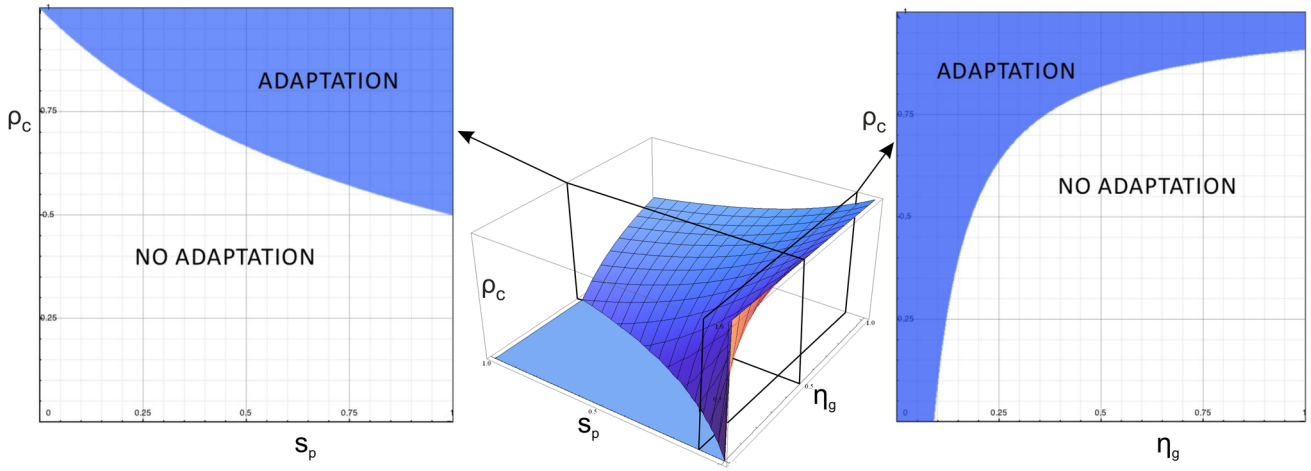

Fig. 1 Center: three-dimensional representation of the critical robustness ρ_c for different combinations of s_p and η_g; Left: critical robustness (boundary between empty and shaded areas) under different selection coefficients, with fixed $\eta_g = 0.5$. The shaded area represents the parameter space where adaptation can occur, while the empty area shows where adaptation cannot occur. Right: critical robustness (boundary between empty and shaded areas) under different genome mutation probability with fixed $s_p = 0.1$

This means that the phenotypic robustness needed for a particular advantageous phenotype to spread throughout the population is inversely related to its selective advantage (s_p) in that particular moment.

Discussion

The mapping from genotype to phenotype plays an important role in evolution, and robustness is a key feature of this map (Hansen 2006; Félix and Barkoulas 2015). Several studies have remarked on the role of phenotypic robustness in enhancing evolutionary adaptation through the effect of cryptic genetic variation (e.g., Hayden et al. 2011; Rigato and Fusco 2016), in particular as long-term effects on evolvability (Payne and Wagner 2019). However, on a short-term scale, i.e. on a time scale of a few generations (see Walsh and Lynch 2018), phenotypic robustness is thought to oppose the process of adaptation by buffering the effects on favourable mutations. Here we have shown that, counterintuitively, not only phenotypic robustness can boost the adaptation process, but that it can also be required for adaptation to occur or to be maintained even in the short term. There is a critical level of phenotypic robustness below which evolutionary adaptation cannot regularly occur, even in the case of sizably selection coefficients and in virtually infinite-size populations, as this threshold does not depend on genetic random drift. The limits to adaptation exposed by the critical robustness are analogous to those posed by the so called *error threshold* of the ordinary quasispecies model (Eigen et al. 1989; Wilke 2005; Nowak 2006; Cerf and Dalmau 2018), an effect of high mutation rates which impedes populations to reach and/or reside on a fitness landscape peak, and disperse them over the sequence space. However, critical robustness differs from the latter for its focus on the minimal level of phenotype resistance to mutations that permits adaptation, irrespective of what causes this robustness (Wagner 2005; Green et al. 2017), rather than on the maximum permissible mutation rate to avoid an *error catastrophe*, i.e. the loss of the favourable genotype(s) through mutation (Bull et al. 2005).

In Reidys et al. (2001) and Takeuchi et al. (2005) a phenotypic error threshold was discussed in terms of the minimum permissible replication accuracy per base (q_{min}) with respect to a parameter (λ_m or λ, in the notation of the two articles, respectively) that represents the fraction of selectively neutral neighbours (one point mutation apart) of any given genotype. Reidys et al. (2001) showed that a rather low degree of mutational neutrality can increase the error threshold unlimitedly, whereas Takeuchi et al. (2005), whose formulation does not adopt some of the assumptions of Reidys et al.'s model (e.g., on the number of substitutions per replication), showed that the increase of the error threshold due to mutational neutrality is limited. However, although both contributions focus on evolutionary dynamics at the phenotypic level, their analyses maintain the implicit assumptions of the original (genotype-based) quasispecies model, i.e. relatively short sequences (like those of RNA molecules and virus genomes), high selection coefficients and a single-peak fitness landscape. Here, these assumptions are relaxed by adopting a definition of robustness that does not coincides with λ (in fact ρ corresponds to the overlooked parameter Λ in Takeuchi et al. 2005), and a definition of adaptation that is not limited to the possibility for the phenotype that displays the highest replication rate to be maintained in a stationary equilibrium. Robustness, as defined here, simply stems from considering genotypes and phenotypes as two distinct (although connected) spaces of organismal variation, with no need to further modelling either mutation patters or details of the genetic architecture. This is therefore more suitable for discussing the role of robustness at the organismal level in the whole tree of life.

Critical robustness turns out to be directly dependent on mutation probability and inversely dependent on selection coefficient. These relationships, in combination with the observed values of these parameters in a majority of organisms, converge to explain the fact that in most biological cases, a sizable level of robustness is required. On the basis of the operational definition of genotype adopted here, where the genotype includes the whole genome of the organism (*omigenic model*; Boyle et al. 2017), the relevant mutation rate is that of the whole genome per generation. These values obviously tend to be sizably higher than the mutation rate of single genes. In multicellular eukaryotes this in the order of several mutations per genome per generation (Drake et al. 1998). As for the selection coefficient, this can vary extensively, depending on the taxon, population, season, life stage, and many other factors. However, it is widely accepted that selection coefficients tend to be relatively small in nature (Orr 2005). For example, experimental measurements of s usually span between 10^{-4} and 10^{-1} (Tamuri et al. 2012; Nielsen and Yang 2003; Mathieson and McVean 2013). Small selection coefficients are also generally assumed in most evolutionary models (e.g., Tachida 2000; Kingsolver et al. 2001; Wild and Traulsen 2007; Wu et al. 2010). Using representative real data on the mutation rates per base pair per generation (μ; (Drake et al. 1998; Denver et al. 2004)) and genome size (G; Drake et al. 1998), one can easily get a rough estimation of the mutation probability per genome per generation (η_g) under standard Binomial distribution of point mutations as $\eta_g = 1 - (1 - \mu)^G$. Considering a selection coefficient of $s_p = 0.1$, which represents a large, challenging value for our model, we can calculate ρ_c for different kinds of organisms using Eq. (11). ρ_c values are typically high for RNA viruses ($\rho_c = 0.89$; $G = 10^4$; $\mu = 10^{-4}$) and pluricellular eucaryotes (*C. elegans, D. melanogaster, M. musculus, H. sapiens*; $\rho_c = 0.86$

to 0.91; $G = 10^8$ to 10^{10}; $\mu = 10^{-8}$), but result to be negative for DNA viruses ($\rho_c = -23$; $G = 8 \times 10^4$; $\mu = 5 \times 10^{-8}$) and both prokaryote and eukaryote unicellulars (*E. coli, S. cerevisie, N. crassa*; $\rho_c = -35$ to -30; $G = 5 \times 10^6$ to 4×10^7; $\mu = 7 \times 10^{-11}$ to 2×10^{-10}). As we have defined robustness as a probability, ρ_c values ≤ 0 indicate that no robustness is required in these cases. However, for smaller and more common selection coefficients ($s_p < 0.01$) or in consideration of the fact that during a stressful condition (and thus adaptation) viruses and unicellular organisms can experience augmented mutation rates (from three to ten-fold the basal; Drake et al. 1998; Galhardo et al. 2007; Foster 2007), ρ_c values tends to get positive in all cases. For instance, for a bacterium like *E. coli*, in a stressful condition with a ten-fold mutation rate ($\mu = 5 \times 10^{-9}$), and a still large selection coefficient of $s_p = 0.01$, the minimum level of robustness required is $\rho_c = 0.60$.

These results, which represent an attempt to formally include phenotypic robustness in the more inclusive framework of adaptive dynamics, are in agreement with the pervasiveness of robustness at different levels of biological organization, from molecules to whole organisms (e.g., Rennell et al. 1991; Edwards and Palsson 2000; Sinha and Nussinov 2001; Giaever et al. 2002; Raser and O'Shea 2005; Raj et al. 2006; White et al. 2013; Vachias et al. 2014; Fares 2015; Félix and Barkoulas 2015; Klingenberg 2019). Phenotypic robustness qualifies as a key feature of the organism genotype–phenotype map, a major quantitative determinant of biological system's ability to adapt and, in the end to evolve.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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