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Adaptive Landscapes in Fungicide Resistance: Fitness, Epistasis, Constraints and Predictability

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INTRODUCTION

In the simplest cases, fungicide resistance evolves when a single mutation, conferring a high level of resistance with negligible fitness costs, emerges and is selected in a pathogen population. However, some fungicides such as the triazoles have proven more durable in the field precisely because this simplest resistance scenario has not occurred. Through the evolutionary viewpoint of adaptive landscapes, combined with functional genetic tools to investigate the effects of mutations individually and in different combinations, it is possible to better understand the evolutionary trajectories available under fungicide selection.

ADAPTIVE LANDSCAPES

An adaptive landscape is a way of visualising evolutionary possibilities, in which the horizontal plane is a two-dimensional representation of genotype space, and the vertical axis represents fitness. Genotypes with higher fitness form peaks, whereas less fit genotypes form valleys (Wright 1932).

A smooth adaptive landscape represents cases with a single optimum or adaptive peak, in which fitness decreases with greater genetic distance from that optimum. On such a landscape, a lineage starting at any point will be under selection to climb that adaptive peak (Figure 1a).

However, in other cases, the adaptive landscape may be more rugged, with multiple local optima forming multiple peaks separated by valleys. On such a landscape, natural selection will drive a lineage uphill from its current position, which, depending upon the starting point, may be climbing the highest adaptive peak towards the global optimum, or may result in the lineage becoming trapped on a local adaptive peak, from which the global optimum could only be reached by crossing an adaptive valley (Figure 1b).

Adaptive landscapes were generally considered as a metaphor, or hypothetical model. However, in recent years, there has been growing interest in reconstructing empirical fitness landscapes. (Martin & Wainwright 2013) represented morphometric data on the horizontal surface, with fitness based on survival rates and growth measurements. (Khan, Dinh et *al*. 2011) show a mutational network on the horizontal plane, comprising all possible pathways from the parental genotype to an experimentally-evolved genotype with five mutations, with fitness measured directly based on growth in culture. (Weinreich, Delaney et *al*. 2006) also present a complete mutational network of intermediate genotypes, but in this case the vertical axis represents resistance to an antibiotic.

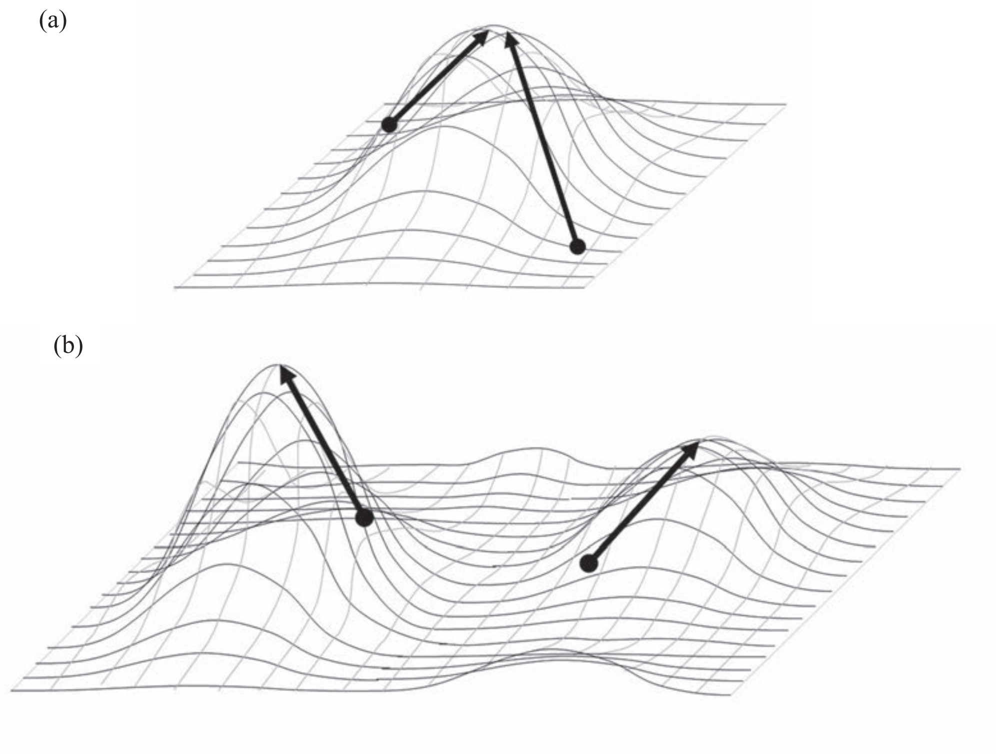

Figure 1 Hypothetical adaptive landscapes. (a) Smooth adaptive landscape with a single peak: positive selection from any starting point will lead to the single, global peak. (b) Rugged adaptive landscape with multiple peaks: depending on the starting point, a lineage may climb the global adaptive peak, or be confined to a local peak.

FITNESS AND FUNCTIONAL CONSTRAINTS

Under fungicide selection, increasing resistance will generally confer greater fitness, and therefore MIC or EC_{50} may be used as the vertical axis for adaptive landscapes.

However, the increase in fitness due to resistance may be offset by other fitness costs, such as reduced growth rate, sporulation or pathogenicity (Lalève, Fillinger et *al*. 2014), reduced competitiveness in mixed culture (Almughrabi & Gray 1995), or increased temperature sensitivity (Ma, Yoshimura et *al*. 2003). In such cases, the shape of the adaptive landscape will vary according to the presence and dose of the fungicide, and trade-offs may be environment-dependent. Greater fitness costs will result in a higher minimum selective dose at which the resistance mutation becomes beneficial, and may result in back-selection towards sensitivity in the absence of the fungicide.

The majority of cases of fungicide resistance result from target site mutations. Therefore, the available mutational possibilities are limited by the need to maintain target site function, and so the evolution of resistance is subject to functional constraints (Cools, Hawkins et *al*. 2013). For example, microtubule function requires the correct balance of tubulin multimer binding and disassembly, which is altered by some MBC-resistance mutations in β-tubulin (Oakley and Morris 1981). In the azole target site, CYP51, some mutations result in loss of sterol demethylase function (Cools, Parker et *al*. 2010).

EPISTASIS

In addition to the effects of individual mutations on both resistance and protein function, in cases where a single mutation does not confer complete field resistance, interactions between mutations must also be considered. If mutations do not interact, their effects should be additive: their combined effect is equal to the sum (or product, for fold-changes) of their individual effects. Where the combined effects differ from pure additivity, this constitutes epistasis (Table 1).

Magnitude epistasis is a difference in scale of fitness benefits or costs, which may be positive (synergism) or negative (diminishing returns), whereas sign epistasis is a difference in whether a mutation is beneficial or deleterious depending on other mutations present (Table 1). Sign epistasis, and especially reciprocal sign epistasis, can result in rugged adaptive landscapes (Poelwijk et *al*. 2007).

Examples of sign epistasis in fungicide resistance include the G143A substitution in the presence or absence of an intron following codon 143 in the *cytochrome b* gene (Grasso, Palermo et *al*. 2006), and the I381V substitution in *Zymoseptoria tritici* CYP51 in the presence or absence of other mutations such as alterations at codons 459-461 (Cools, Parker et *al*. 2010). Functional genetic tools, such as heterologous expression or homologous gene replacement, combined with site-directed mutagenesis, make it possible to dissect multi-mutation haplotypes and elucidate the impact and interactions of each mutation on resistance and fitness.

EVOLUTIONARY ACCESSIBILITY AND PREDICTABILITY

In addition to functional constraints reducing the total available mutational space, there are limits to the mutational space accessible from a given point. The most common mutations are single-nucleotide substitutions, with multiple genetic changes expected to accumulate in a step-wise fashion.

An evolutionarily accessible pathway to a multi-mutation genotype is one in which fitness increases at every step, with each additional mutation (Poelwijk et *al*. 2007).

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Table 1 Epistatic interactions between mutations.

Epistasis type	Fitness effect		
	Mutation A	Mutation B	Mutations A+B
None (Additive)	a	b	= a + b
Magnitude: positive	a	b	> (a + b)
Magnitude: negative	a	b	< (a + b)
Sign	+ -	b b	< b > b
Reciprocal sign	+ -	+ -	- +

Where the transition between two genotypes, such as a local and global optimum, requires multiple changes including the crossing of a fitness valley, this is likely to be rarer, and may depend on larger genetic changes such as multiple mutations, genome rearrangements, gene duplication or recombination, or a change or relaxation in selective pressure.

In contrast, any single-step pathway to an adaptive peak is inherently accessible. Therefore, where a single mutation results in effectively complete resistance without significant fitness costs, it will be accessible from any starting point in the absence of mutations causing sign epistasis.

PREDICTABILITY: CONCLUSIONS

Evolutionary outcomes will be more predictable if the adaptive landscape is smooth, with a single adaptive peak accessible from any starting point, and simple, conserved functional constraints.

Evolution will be less predictable if the adaptive is rugged, with multiple local peaks and fitness valleys making the global optimum inaccessible from some starting points; with fitness costs in the absence of fungicide selection, or different resistance patterns among different fungicides, causing the landscape to shift over time; and complex, background-specific functional constraints with widespread epistasis. However, increased understanding of the underlying fitness landscape and the functional constraints involved could improve the predictability in such cases.

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