

1 **Title:** Polymorphism and the Predictability of Evolution in Fisher's Geometric Model

2 Sandeep Venkataram, Diamantis Sellis, Dmitri A. Petrov

3 *Department of Biology, Stanford University, Stanford, CA 94305*

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6 **Corresponding Author:**

7 Dmitri A. Petrov

8 Stanford University Department of Biology

9 371 Serra Mall Stanford CA 94305

10 (650) 736 1169

11 dpetrov@stanford.edu

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## ABSTRACT

13 Predicting the future evolutionary state of a population is a primary goal of evolutionary  
14 biology. One can differentiate between forward and backward predictability, where forward  
15 predictability is the probability of the same adaptive outcome occurring in independent  
16 evolutionary trials, and backward predictability is the likelihood of a particular adaptive  
17 path given the knowledge of the starting and final states. Most studies of evolutionary  
18 predictability assume that alleles along an adaptive walk fix in succession with individual  
19 adaptive mutations occurring in monomorphic populations. However, in nature, adaptation  
20 generally occurs within polymorphic populations, and there are a number of mechanisms  
21 by which polymorphisms can be stably maintained by natural selection. Here we  
22 investigate the predictability of evolution in monomorphic and polymorphic situations by  
23 studying adaptive walks in diploid populations using Fisher's geometric model, which has  
24 been previously found to generate balanced polymorphisms through overdominant  
25 mutations. We show that overdominant mutations cause a decrease in forward  
26 predictability and an increase in backward predictability relative to diploid walks lacking  
27 balanced states. We also show that in the presence of balanced polymorphisms, backward  
28 predictability analysis can lead to counterintuitive outcomes such as reaching different final  
29 adapted population states depending on the order in which mutations are introduced and  
30 cases where the true adaptive trajectory appears inviable. As stable polymorphisms can be  
31 generated in both haploid and diploid natural populations through a number of  
32 mechanisms, we argue that natural populations may contain complex evolutionary histories  
33 that may not be easily inferred without historical sampling.

34

## INTRODUCTION

35 Predicting evolution is one of the fundamental challenges of evolutionary biology (reviewed  
36 in DE VISSER and KRUG (2014)). This question became particularly prominent with  
37 Gould's famous thought-experiment on "replaying the tape of life" (GOULD, 1990). Gould  
38 wondered whether we would regenerate the observed evolutionary history of the world if we  
39 reset our evolutionary history to any point in the past and let evolution retake its course  
40 from there. More generally, we can ask whether it is possible to predict the path or the  
41 final destination of the evolutionary process from a given starting point. It is also possible,  
42 however, to ask whether we can reconstruct the true evolutionary trajectory given the final  
43 adapted state (WEINREICH *et al.*, 2006). This distinction between types of predictability is  
44 rarely made (however see NOURMOHAMMAD *et al.* (2013) and SZENDRO *et al.* (2013)), so  
45 we formalize the methods for studying predictability and utilize these distinctions to study  
46 the impact of polymorphism on the predictability of evolution.

47 **Forward predictability of evolution:** We define forward predictability as the  
48 probability of observing a particular future evolutionary outcome from a known starting  
49 state. Previous experimental evolution studies have generally (but not always) focused on  
50 the forward predictability of evolution. This type of analysis can be done at a number of  
51 levels, including the predictability of overall fitness changes, phenotypic shifts and different  
52 levels of genotypic changes (pathways, genes, and individual mutations).

53 For example, FEREA *et al.* (1999), COOPER *et al.* (2003) and FONG *et al.* (2005) evolved  
54 independent replicates of microbes and observed similar changes in gene expression and  
55 growth rate in the evolved clones. A large study of 145 parallel long-term experimental  
56 evolutions with *Escherichia coli* grown at elevated temperature showed that the same  
57 genes and pathways were repeatedly targeted for mutations in independent populations  
58 (TENAILLON *et al.*, 2012) as did a study of 40 replicate *Saccharomyces cerevisiae* batch

59 culture evolutions (LANG *et al.*, 2013) and a study that sequenced clones from 10 replicate  
60 evolutions for each of 13 different genetic backgrounds (KRYAZHIMSKIY *et al.*, 2014).  
61 TENAILLON *et al.* (2012) also observed a high degree of parallel evolution at the level of  
62 individual nucleotides, but nucleotide level parallelism was rarely observed by LANG *et al.*  
63 (2013). HERRON and DOEBELI (2013) evolved *E. coli* under multiple carbon sources and  
64 repeatedly observed the evolution of two distinct ecotypes with differential ability to grow  
65 on each carbon source. By sequencing independent replicate clones of both ecotypes, they  
66 found the same genes, and sometimes the same exact mutations invading these replicate  
67 populations and differentiating the ecotypes. These studies suggest that evolution is indeed  
68 forward predictable to a surprising degree.

69 Repeated evolution has been observed at both the genetic and morphological levels in  
70 natural systems as well (reviewed in STERN (2013)). KVITEK *et al.* (2008) showed that  
71 highly divergent yeast strains isolated from oak trees had similar growth rates across a  
72 panel of diverse growth conditions. Studies of *Anolis* lizards in the Caribbean show  
73 repeated independent adaptive radiations into similar niches across the islands (LOSOS,  
74 1998). In addition, a study of the adaptive radiation of cichlid fish in Lake Tanganyika  
75 showed convergent morphological evolution when the skeletal morphology of the various  
76 species was compared to their phylogeny (MUSCHICK *et al.*, 2012).

77 **Backward predictability of evolution:** In addition to Gould's thought-experiment, one  
78 can study predictability in a historical manner. Given the current state, we can try to  
79 predict the ancestral state or the evolutionary path that resulted in the current state of the  
80 study system. We call this backward predictability, as it requires us to look backward in  
81 time. For example, one can try to predict exactly how corn or rice became domesticated  
82 from one or more wild ancestors (MATSUOKA *et al.*, 2002; MOLINA *et al.*, 2011), identify  
83 the ancestral species that gave rise to Darwin's Finches (DARWIN, 1872; SATO *et al.*, 2001),

84 or reconstruct the ancestral state of a particular protein (ORTLUND *et al.*, 2007).

85 Alternatively, if we already know the ancestral state, we can try to predict the particular  
86 order of mutations or phenotypic states that led to the evolution of the current state.

87 WEINREICH *et al.* (2006) conducted a seminal study of backward predictability in this  
88 sense, using a combinatorially complete reverse genetic study design pioneered by  
89 MALCOLM *et al.* (1990). WEINREICH *et al.* (2006) reconstructed every possible  
90 combination of five mutations in the beta-lactamase gene in *E. coli* which are known to  
91 lead to high levels of resistance to the drug rifampicin. They then assayed each genotype's  
92 resistance to the drug, which they used as a proxy for fitness. Using this data, they  
93 determined the fitness changes involved in every step of each of the  $5! = 120$  possible  
94 mutational paths that converts the wild-type genotype to the resistant five-mutant  
95 genotype. A mutational path was deemed viable if fitness monotonically increased with  
96 every step, that is, there were no mutations along the path that decreased resistance to the  
97 drug.

98 WEINREICH *et al.* (2006) found that only 18 of the 120 possible paths were viable,  
99 suggesting high backward predictability of evolution. In contrast, KHAN *et al.* (2011)  
100 performed an analysis of five adaptive mutations from experimentally evolved bacterial  
101 lineages using identical methodology and found that a majority of the orders were viable.  
102 Finally, FRANKE *et al.* (2011) studied backward predictability in all subsets of two to six  
103 mutations in an empirical eight-locus system and found that the number of viable paths  
104 varied widely for a given subset size. For example, they observed both zero and nine viable  
105 paths (out of 24 possible) in different four-locus subsets. The varying degrees of backward  
106 predictability found in these different systems does not yet allow us to draw general  
107 conclusions, and the laborious nature of the experiments makes it challenging to study  
108 more than a few mutations at a time. In addition, without knowing the true order in which

109 the mutations arose in the population, it is unclear how accurate backward predictability  
110 analysis actually is.

111 **Predictability in Fisher's Geometric Model:** Overall, there seems to be no consensus  
112 on whether evolution is backward predictable using the method of WEINREICH *et al.*  
113 (2006). It is also unclear how forward and backward predictability are correlated with each  
114 other. In principle, one would want to conduct forward evolution and then conduct  
115 backward predictability analysis on the same system to understand their relationship.  
116 However such studies would be extremely laborious, and given the disparate answers  
117 coming out of different experimental systems, a large number of independent experiments  
118 in many systems would need to be conducted to give a convincing answer.

119 Another difficulty in experimental evolution studies of predictability are practical  
120 limitations in sampling adaptive mutations. As most studies can only afford to sample a  
121 few adapted individuals from a given experiment, mutations must be at high frequency to  
122 be observed and a common assumption is that each of these mutations fixed in the  
123 population in succession (GILLESPIE, 1983, 1984; ORR, 2002; WEINREICH *et al.*, 2006;  
124 KHAN *et al.*, 2011; FRANKE *et al.*, 2011). However, we know that mutations can be  
125 maintained in a polymorphic state by a number of mechanisms. These include negative  
126 frequency-dependent selection (LEVIN *et al.*, 1988; ISERBYT *et al.*, 2013), spatial and  
127 temporal fluctuations in selection (RAINEY and TRAVISANO, 1998; KASUMOVIC *et al.*,  
128 2008; SALTZ and NUZHIDIN, 2014) and heterozygote advantage (also called overdominance,  
129 TAKAHATA and NEI (1990)). Polymorphisms can also be present in an unstable form  
130 through clonal interference (DESAI and FISHER, 2007; HERRON and DOEBELI, 2013;  
131 KVITEK and SHERLOCK, 2013; LANG *et al.*, 2013). The presence of functionally  
132 consequential polymorphisms in a population can in principle significantly alter  
133 predictability analysis as the selective effect of a new mutation may be dependent on other

134 alleles segregating in the population (fitness epistasis). Many of these polymorphisms are  
135 either lost by the end of the experiment or are not observed in the sampled adapted  
136 individuals, leading to incorrect inferences of predictability. Additional complications can  
137 arise when estimating predictability as mutations can occur in multiple backgrounds in a  
138 given population, so the likelihood of each mutation occurring in a particular background  
139 also has to be taken into account, as well as any epistatic interactions the mutation has  
140 with the rest of that background.

141 Due to the challenges of isolating sufficient numbers of independent adaptive mutations  
142 from experimental populations to study predictability, we utilize a simulation-based  
143 approach to study the impact of polymorphisms on forward and backward predictability.  
144 We employ Fisher's geometric model (FGM, FISHER (1930)), which is a well-studied (ORR,  
145 1999, 2005) phenotypic model that treats individuals and alleles as a phenotype that is a  
146 vector in coordinate space with a fitness that is determined by the distance of the  
147 individual's phenotype from a predefined optimal phenotype using a gaussian function  
148 (Figure 1a). SELLIS *et al.* (2011) showed that adaptive mutations in diploid FGM  
149 simulations are frequently overdominant if the mutations are sufficiently large in phenotypic  
150 space, resulting in balanced polymorphisms. Such overdominant mutations are stable but  
151 can be driven out of the population by subsequent adaptive mutations. As we are  
152 interested in the interaction between balanced polymorphic states and the predictability of  
153 evolution, we select the distribution of mutational effects such that some evolutionary  
154 trajectories contain overdominant mutations, generating stable polymorphisms, and others  
155 do not. We then compare both types of trajectories to understand how polymorphisms  
156 influence predictability. We conclude that the presence of polymorphic states has a  
157 substantial qualitative effect on the predictability of evolution, such that at least in this  
158 model, forward and backward predictability are inversely correlated.



159

## METHODS

160 **Simulations:** We model adaptive walks in diploid populations with Wright-Fisher  
161 simulations using Fisher's geometric model (FGM) as in SELLIS *et al.* (2011). In FGM,  
162 alleles are represented as a vector in n-dimensional phenotype space (Figure 1a). The  
163 simulations use code modified from Sellis et al. to allow for more than 2 dimensions. We  
164 perform 10,000 replicate simulations with population size  $N = 5,000$  for 10,000  
165 generations. We explore two models, one with two dimensions and one with 25 dimensions.  
166 We partition our adaptive walks into those that do and those that do not contain  
167 overdominant mutations to study the impact of balanced states on predictability. For the  
168 remainder of our analysis, we identify the most frequent allele in each simulated population  
169 at the end of 10,000 generations of evolution and study the mutations present on that  
170 allele. We limit our analysis to studying the first five mutations of each adaptive walk and  
171 ignore simulations with fewer than 5 mutations in order to control for the length of the  
172 adaptive walk when studying predictability.

173 **Forward Predictability Analysis:** We calculate the forward predictability of the  
174 adaptive trajectory using two metrics. In both of these metrics, we only consider  
175 homozygous phenotypes. Our first metric, maximum pairwise distance, considers pairs of  
176 adaptive walks. We compute the maximum of the phenotypic distances between the  
177 observed single mutant phenotypes of the two adaptive walks, the double mutant  
178 phenotypes, the triple mutant phenotypes etc. Our second metric measures the maximal  
179 deviation from the optimal trajectory. For each adaptive walk, we compute the maximal  
180 phenotypic distance of any encountered (homozygous) phenotype from the line segment  
181 connecting the ancestral phenotype and the optimum.

182 **Backward Predictability Analysis:** We compute backward predictability on adaptive  
183 walks of exactly five mutations. We calculate the probability of all possible mutational

184 orders for the given set of mutations in a manner similar to WEINREICH *et al.* (2006), but  
185 generalized to allow balanced states as the experimental protocol of WEINREICH *et al.*  
186 (2006) assumes that every mutation along each mutational order fixes in succession. We  
187 summarize the set of possible mutational orders for a given set of mutations through the  
188 effective number of trajectories statistic, which we define as

189 
$$\frac{1}{\sum p^2}$$

190 where  $p$  is the probability of each mutational order possible for a given set of mutations. If  
191 no mutational order is viable (has nonzero probability), the effective number of trajectories  
192 is defined to be 0. Please see the Supplementary Methods for full methodological details.

## RESULTS

193

194 We explore the predictability of evolution in the framework of Fisher’s geometric model  
195 (FGM) of adaptation. In FGM, alleles are represented as vectors in coordinate space, with  
196 individuals having a phenotype that is the average of the phenotypes of their constituent  
197 alleles. Mutations are vectors that modify the phenotype of an allele, and fitness is a  
198 gaussian function of the distance of the individual’s phenotype from the optimal phenotype  
199 (which we define as the origin).

200 In order to focus on the effect of polymorphic states on the predictability of evolution, we  
201 choose a parameter regime that generates simulations both with and without overdominant  
202 mutations after a number of trial simulations with various parameter values. We perform  
203 10,000 replicate simulations of adaptation under FGM in diploids with  $N = 5000$   
204 individuals. Mutational magnitudes are drawn from an exponential distribution with mean  
205  $= \frac{1}{2}$  and the population is initiated at two units from the optimum. The mutation rate is  
206  $5 * 10^{-6}$ , which results in a mutation-limited regime (significantly less than one mutation  
207 per generation as  $2 * N * \mu = 0.05$ ), in order to minimize the generation of polymorphic  
208 states by clonal interference so that we can focus on only those polymorphic states  
209 generated by overdominant mutations.

210 We conduct our simulations using an FGM of two dimensions, and show that our  
211 qualitative results also hold at 25 dimensions. In the 25 dimension regime, we need to  
212 rescale our mutational magnitude mean to 5 in order to obtain a sufficient number of walks  
213 with five mutations over our 10,000 generation simulations for statistical analysis. For all  
214 of our statistical analyses, we consider only those mutations that are present on the most  
215 frequent allele at generation 10,000. Such mutations are typically the only ones available  
216 for analysis in a natural system. We additionally limit our analysis to studying the first  
217 five mutations of each adaptive walk, and ignore simulations with fewer than five mutations

218 in order to compare adaptive walks of equal lengths. We partition the resulting  
219 five-mutation adaptive walks into those that do (n=4975, 1548 in simulations with two and  
220 25 dimensions, respectively) and do not (n=1251, 10) contain overdominant mutations to  
221 study the impact of balanced polymorphisms on the predictability of evolution. The  
222 presence of overdominant mutations in an observed five-mutation adaptive trajectory is  
223 detected by the observation of a set of alleles during the FGM simulation that are capable  
224 of being maintained as a balanced polymorphism (KIMURA, 1956). For details, please see  
225 the Supplementary Methods.

226 **Predictability of Adaptive Walks:** We first consider the forward predictability of  
227 phenotypic paths, which we define as the tendency of independent adaptive walks to  
228 explore similar portions of phenotypic space. The ability of adaptive walks with  
229 overdominant mutations to explore a larger phenotypic space compared to walks without  
230 overdominance ( $\alpha$ -dip vs  $\gamma$ , Figure 1a) should lead to lower predictability of the phenotypic  
231 intermediates along the adaptive walk, which is confirmed by visual inspection of our  
232 simulations (Figure 1b,c) and is consistent with the findings of SELLIS *et al.* (2011).

233 We quantify forward predictability by measuring the distribution of maximal phenotypic  
234 distances between pairs of independent adaptive trajectories. Pairs of walks with  
235 overdominant states are, on average, 40% further apart than walks without overdominant  
236 mutations and are therefore less forward predictable (Figure 2, Kolmogorov-Smirnov test  
237  $p \ll 10^{-10}$ ). We also measure forward predictability as the maximal phenotypic distance of  
238 each observed trajectory from the optimal trajectory - the vector from the ancestral  
239 phenotype to the optimal phenotype. We observe that the presence of overdominant  
240 mutations in a walk increases the average distance from the optimal trajectory by 5%  
241 (Figure 3, Kolmogorov-Smirnov test  $p \ll 10^{-10}$ ), again suggesting that overdominant  
242 mutations decrease forward predictability.

243 We then study backward predictability in a manner similar to WEINREICH *et al.* (2006).  
244 As before, we limit our analysis to adaptive walks of exactly five mutations, which is  
245 comparable to many recent experimental studies of backward predictability (WEINREICH  
246 *et al.*, 2006; KHAN *et al.*, 2011; FRANKE *et al.*, 2011). Backward predictability analysis  
247 requires knowledge of the five mutations that occurred during the FGM simulations and  
248 computes the likelihood of every possible order of those five mutations in generating the  
249 observed adapted five-mutation allele (e.g. see WEINREICH *et al.* (2006) Figure 2). In  
250 order to conduct this analysis, we compute the probability of every possible path to the  
251 five-mutant state by successively introducing each of the five mutations into the population  
252 and assessing the probability of each of these mutations to successfully invade the  
253 population (see Supplementary Methods). Although we artificially constrain the available  
254 phenotypes to only those generated by combinations of the five mutations under  
255 consideration, this analysis is a model for studying predictability in situations where there  
256 are only a few possible adaptive mutations, such as the drug resistance mutations used by  
257 Weinreich *et al.* We compute the effective number of adaptive trajectories for each  
258 adaptive walk, with a higher number suggestive of a lower backward predictability.

259 The results of our backward predictability analysis are shown in Figure 4. We find that in  
260 contrast to forward predictability, overdominant states decrease the effective number of  
261 paths (and thus increase backward predictability) in a walk by 30%, on average  
262 (Kolmogorov-Smirnov test  $p \ll 10^{-10}$ ). In other words, conditional on reaching a particular  
263 five-mutant state, it is more probable that independent trials of a walk that experienced at  
264 least one overdominant state will use the same mutational order in repeated trials relative  
265 to a walk without overdominant states. We also utilize the mean path divergence of  
266 LOBKOVSKY *et al.* (2011) to study backward predictability and find that overdominant  
267 states resulted in walks that were 10% less divergent (and thus more backward  
268 predictable), on average (Kolmogorov-Smirnov test  $p \ll 10^{-10}$ ).

269 **Multiple End States:** In addition to studying the probability of a given mutational order  
270 in our backward predictability analysis, we also study the adapted population state that  
271 results from each viable mutation order. In particular, we observe that when mutations are  
272 introduced in different orders, the population encounters different intermediate alleles,  
273 resulting in instances where the final adapted five-mutant allele can balance against  
274 different intermediate alleles depending on the order in which the mutations were  
275 introduced into the population. We also observe instances where walks that did not  
276 experience balanced states in the FGM simulations generate balanced states when  
277 introduced in a different order.

278 We find that 53% of all walks have at least two different end population states containing  
279 the final adapted allele, with a maximum of 19 different population states for a single set of  
280 five mutations. We also find that the presence of overdominant mutations in the FGM  
281 simulation has a significant effect on whether there are multiple end states observed. The  
282 presence of an overdominant mutation in the observed walk increases the frequency of  
283 multiple end states from 30% to 60%. Our results suggest that adaptation occurring in the  
284 same genetic background, in response to the same selection pressure and using the same  
285 mutations, can result in significantly different final population states depending on the  
286 historical order in which the adaptive mutations occurred.

287 **Qualitative categorization with regard to backward predictability:** We analyze  
288 our backward predictability results to discern qualitative categorizations of our simulations.  
289 We find four broad categorizations of simulations: 1) simulations whose backward  
290 predictability reconstructions of the five-mutant allele by introducing the mutations in the  
291 order observed in the FGM simulation generate no balanced states, 2) those  
292 reconstructions that do generate balanced states, 3) reconstructions where the order of  
293 mutations that was observed in the simulation was impossible to reconstruct due to

294 deleterious intermediate states during the reconstructions and 4) reconstructions where  
295 every possible order of mutations was impossible due to deleterious intermediate states  
296 (which is a subset of category 3).

297 We observe 2326, 3898, 89 and 5 simulations in each of these four categories, respectively.  
298 We can further separate these categories by conditioning on our original definitions of  
299 whether or not a simulation contained an overdominant intermediate state (i.e. whether  
300 there was a set of alleles that could be maintained in a stable balanced state at any point  
301 during the FGM simulation before the 5-mutant state reached 5% frequency). We find  
302 1187, 62, 2 and 0 simulations in each of these four categories, respectively, among the  
303 simulations that we had previously identified as not containing overdominant intermediate  
304 states while we observe 1139, 3836, 87 and 5 simulations in each of these four categories,  
305 respectively, among simulations that we had previously identified as containing  
306 overdominant intermediate states.

307 The presence of backward predictability reconstructions where the observed order (and in a  
308 few cases, every order) of mutations is impossible is surprising. We hypothesize that this is  
309 due to the presence of adaptive alleles that are generated and stably maintained during a  
310 walk that are transient and do not survive until the end of the simulation. We call these  
311 “hidden alleles”, as they are hidden from almost all modern experimental studies of  
312 adaptation. Lack of knowledge of hidden alleles appear to decrease the computed  
313 probability of the true adaptive path observed in the FGM simulations, and in extreme  
314 cases, can make the true path impossible to reconstruct. Visual inspection of adaptive  
315 trajectories that are unable to be successfully reconstructed confirms this intuition (Figure  
316 5). Backward predictability reconstructions that incorporate all mutations present at  $\geq 1\%$   
317 frequency at any point in the simulation, regardless of whether the mutation was present  
318 on the allele sampled at the end of the simulation, can successfully reconstruct the

319 observed adaptive trajectory of this previously impossible evolutionary outcome,  
320 confirming the necessity of hidden alleles for the viability of the observed adaptive  
321 trajectory in these instances.

322 We then compare the forward and backward predictability metrics described above on the  
323 different categories of simulations. In particular, we compare the simulations that were  
324 initially defined as not containing overdominant states at any point to those that did not  
325 have balanced states in the backward predictability analysis but did have balanced states  
326 during the FGM simulation. We find no significant difference between these sets of  
327 simulations by any of our predictability metrics (maximum pairwise distance, maximum  
328 distance from optimal trajectory and effective number of paths Kolmogorov-Smirnov test  
329  $p > 0.05$ ). This result suggests that the signal in our predictability metrics is being driven  
330 by the presence of balanced states between intermediate alleles along the adaptive  
331 trajectory to the five-mutant allele rather than a general feature of observing balanced  
332 states in our simulations as a whole.

333 **High Dimensionality:** In our implementation of Fisher's Model, balanced states arise  
334 when mutations are overdominant. The presence of additional phenotypic dimensions,  
335 which seems realistically plausible from observed rates of pleiotropy (DUDLEY *et al.*, 2005;  
336 ALBERT *et al.*, 2008), increases the frequency of overdominant mutations (SELLIS *et al.*,  
337 2011). However, this concordantly decreases the fitness advantage of the average new  
338 beneficial mutation, decreasing the number of adaptive mutations that successfully invade  
339 the population over our 10,000 generation FGM simulations. To study the impact of high  
340 dimensional landscapes on predictability, we conducted simulations using 25 dimensions  
341 with a mean mutation size of 5. The increase in mean mutation size relative to our original  
342 two dimensional simulations is necessary to generate a sufficient number of walks containing  
343 at least 5 mutations within 10,000 generations. We again partitioned the simulations into



344 those with ( $n = 1548$ ) and without ( $n = 10$ ) overdominant mutations at any point of the  
345 FGM simulation before the time when the five-mutant allele reached 5% frequency.

346 We observe the same qualitative results in 25 dimensions as in 2 dimensions (see  
347 Supplementary Figures 1-4). In general, it appears that our conclusions about  
348 predictability of adaptive walks do not depend on the dimensionality of the system, and  
349 only on the presence of overdominant mutations in the adaptive walk.

## DISCUSSION

350  
351 In this study, we explored the predictability of evolution using Fisher’s geometric model.  
352 We distinguished between forward and backward predictability, where forward  
353 predictability measures the likelihood of the same or a similar adaptive trajectory  
354 occurring in independent evolutions, while backward predictability measures the likelihood  
355 of a particular order of adaptive mutations given the ultimate adapted state. We knew  
356 from prior work that diploids frequently generate overdominant mutations under Fisher’s  
357 geometric model (SELLIS *et al.*, 2011), so we studied predictability using walks with and  
358 without overdominant mutations to understand the impact of balanced polymorphisms on  
359 predictability.

360 We found that simulations without overdominant mutations are more forward predictable  
361 than simulations with overdominance, while the reverse is true for backward predictability.  
362 The anti-correlation between forward and backward predictability can be intuitively  
363 understood by considering the the nature of adaptation in Fisher’s geometric model. In  
364 walks without overdominant mutations, mutations are confined to within  $\gamma$  (Figure 1a),  
365 leading to high forward predictability. There is minimal opportunity for deviation from the  
366 optimal trajectory, and most of the adaptive mutations that occur during these walks have  
367 similar direction vectors to the optimal trajectory. Therefore, regardless of the order of  
368 mutations, each step will move the population closer to the optimum, making most of the  
369 trajectories viable, and resulting in low backward predictability. The reverse is true in  
370 walks with overdominant mutations, which explore a much larger portion of phenotypic  
371 space ( $\alpha_{dip}$ ). Overdominant mutations tend to overshoot the optimum and are frequently  
372 followed by compensatory mutations. The larger amount of phenotypic space explored  
373 generates lower forward predictability, while the high frequency of compensatory mutations,  
374 and thus the importance of the order in which the mutations are introduced, results in high  
375 backward predictability. While Fisher’s geometric model is a useful tool to consider

376 adaptation under phenotypic stabilizing selection, further work is required to determine the  
377 extent to which this anti-correlation is generalizable to biological systems. Nevertheless,  
378 the anti-correlation we observe between forward and backward predictability highlights the  
379 importance of distinguishing between types of predictability in future studies.

380 In natural populations, stable polymorphisms can be due to overdominance or other types  
381 of balancing selection, such as negative frequency dependent selection (LEVIN *et al.*, 1988;  
382 ISERBYT *et al.*, 2013), and spatially or temporally variable selection (RAINEY and  
383 TRAVISANO, 1998; KASUMOVIC *et al.*, 2008; SALTZ and NUZHDIN, 2014). Transient  
384 functional polymorphisms at intermediate frequencies can also be generated via clonal  
385 interference (DESAI and FISHER, 2007; HERRON and DOEBELI, 2013; KVITEK and  
386 SHERLOCK, 2013; LANG *et al.*, 2013). Both frequency dependent selection and clonal  
387 interference can occur in both haploid and diploid populations. Our work shows that the  
388 presence of polymorphisms in the population, regardless of source, significantly complicates  
389 analysis of adaptive trajectories, and these complications must be considered in all natural  
390 systems.

391 One such complication is the existence of simultaneous mutational lineages, which can  
392 result in hidden alleles (i.e. alleles that are not present at the end of the evolution) and  
393 transient population states that nevertheless significantly impact the future course of  
394 evolution. Ignoring hidden alleles can significantly modify the inferred backward  
395 predictability, and in extreme cases, can incorrectly suggest that the true order of  
396 mutations is impossible. Different orders of mutations can also generate different sets of  
397 heterozygous genotypes and different end population states, requiring the consideration of  
398 the state of the entire adapted population rather than the presence of a particular adapted  
399 allele.

400 Polymorphic states also drastically increase the number of possible adaptive paths. In  
401 systems where adaptation proceeds through sequential fixation, one only needs to consider  
402 the fitness of the  $2^n$  possible genotypes relative to the ancestral background for an  
403  $n$ -mutation system. This is the methodology used in the experimental backward  
404 predictability studies of WEINREICH *et al.* (2006), KHAN *et al.* (2011) and FRANKE *et al.*  
405 (2011). However, in regimes where polymorphic states are frequently generated, the fitness  
406 of an invading mutation can vary depending on the alleles already present in the  
407 population. Within each adaptive trajectory, every mutation along the trajectory needs to  
408 be introduced into the prior population at low frequency on every available allele and  
409 tracked until the frequency of the new mutation has been stabilized in order to establish  
410 that the mutation is truly beneficial. Such a study would be extremely laborious, and to  
411 our knowledge, has never been conducted in any system.

412 **Experimental Implications:** In an experimental setting, high forward predictability  
413 means it is likely that the same set of mutations will be generated in independent adaptive  
414 walks, which make the probabilities generated through backward predictability analysis  
415 meaningful for predicting future events. This can occur by either a small mutational target  
416 size such as mutations that cause resistance to drugs, or a large mutational input into the  
417 population which makes rare but extremely beneficial mutations dominate the adaptive  
418 process (e.g. DESAI and FISHER (2007); KVITEK and SHERLOCK (2011); GERSTEIN *et al.*  
419 (2012); PENNINGS (2012)). A study in FGM also suggests that a multi-locus FGM where  
420 each locus only influences a subset of the independent phenotypic dimensions (restricted  
421 pleiotropy) also promotes forward predictability, which the authors call parallel evolution  
422 (CHEVIN *et al.*, 2010). Despite the large number of replicates required to achieve statistical  
423 significance, experimentally determining forward predictability has been shown to be  
424 feasible.

425 On the other hand, the possibility of hidden alleles makes accurate estimates of backward  
426 predictability impossible in both natural and artificial experimental systems. Since we do  
427 not have access to hidden alleles from natural populations, it is impossible to accurately  
428 compute the backward predictability of the adaptive walk leading to the current  
429 population state. Studying backward predictability using forward evolutions and constant  
430 sampling is equally infeasible. Even if we could sample every mutation that rises to  
431 reasonable frequency in a population, almost all of these mutations will be lost, and there  
432 may be far too many to determine the subset which are non-neutral. As mentioned above,  
433 there is also the problem of combinatorially many adaptive walks possible for even a few  
434 mutations, making complete experimental analysis of even a five mutation system  
435 extremely challenging. As others have mentioned, sampling a few high-fitness mutations  
436 and conducting backward predictability experiments may not generate a correct  
437 representation of the probability of any particular adaptive walk, as there may be  
438 alternative adaptive peaks (WEINREICH *et al.*, 2006). Additionally, there is the possibility  
439 of adaptation and potential epistatic interactions at sites not under consideration, and  
440 spatial or temporal fluctuations in selection pressures can further complicate accurate  
441 assessments of backward predictability in natural systems, and calls into question the  
442 accuracy of reconstructed ancestral states.

443 Finally, the impact of hidden alleles on evolutionary trajectories depends on the rate at  
444 which stable polymorphic states are generated. RAINEY and TRAVISANO (1998), for  
445 example, observed adaptive radiation by niche construction in every replicate evolution  
446 experiment they conducted. Under these conditions, we may expect hidden alleles to be  
447 frequent in a large evolving population. The adapted state of natural populations may thus  
448 experience a strong historical dependence on transient mutations that are eventually lost  
449 and impossible to sample, decreasing the forward predictability of evolution and making  
450 the inference of backward predictability impossible. The rate at which polymorphic states

451 are generated in natural systems and potential differences between types of polymorphic  
452 states and their impact on forward and backward predictability should be further explored  
453 to improve our understanding of the predictability of evolution.

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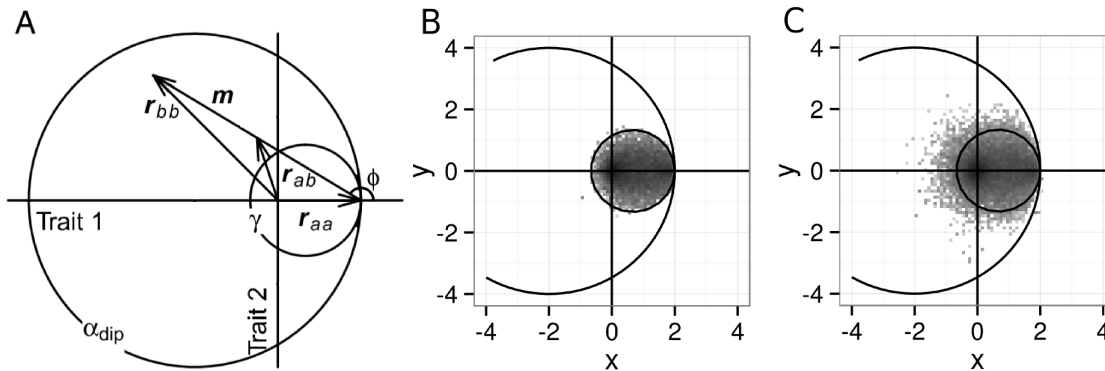
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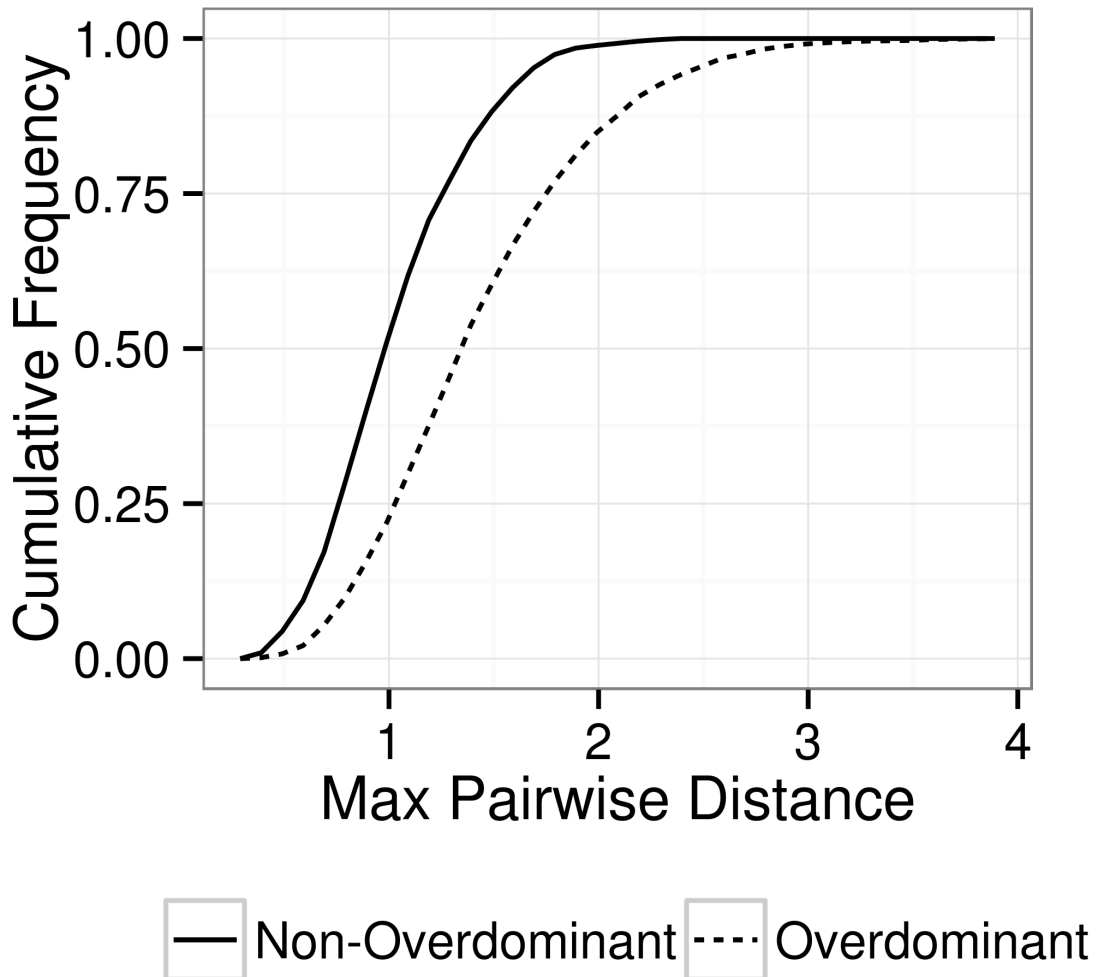
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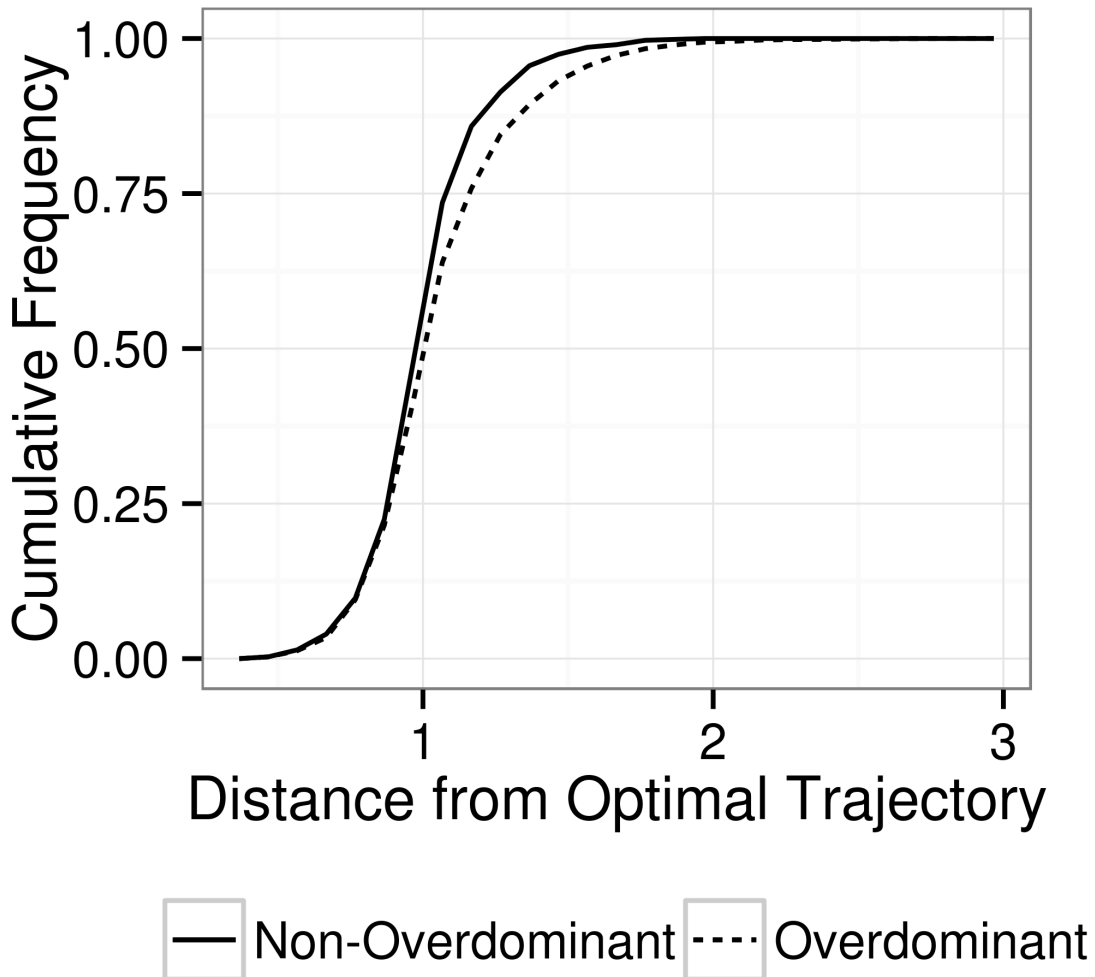
576 **Figure 1. Fisher's geometric model description and confirmation of accurate**  
 577 **separation of simulations into those with and without overdominant mutations.**

578 **(A)** Modified from Figure 2A Sellis et al 2011. Two orthogonal axes represent independent  
 579 character traits. Fitness is determined by a symmetrical Gaussian function centered at the  
 580 origin. Consider a population initially monomorphic for the wild-type allele  $r_{aa}^{\vec{}}$ . A  
 581 mutation  $m$  gives rise to a mutant phenotype vector  $r_{bb}^{\vec{}} = r_{aa}^{\vec{}} + \vec{m}$ . The phenotype of the  
 582 mutant heterozygote assuming phenotypic codominance ( $h = 1/2$ ) is  $r_{ab}^{\vec{}} = r_{aa}^{\vec{}} + \vec{m}/2$ . The  
 583 different circles specify the areas in which mutations are adaptive (i.e. successfully invade  
 584 the population,  $\alpha_{dip}$ ) and replacing (i.e. fix in the population,  $\gamma$ ) in diploids. **(B)** Density  
 585 plot of all phenotypes of homozygous individuals observed in the adaptive walks of FGM  
 586 simulations that do not contain overdominant mutations. Note that all observed  
 587 phenotypes lie within  $\gamma$ , as all mutations must be replacing and not balancing in this group  
 588 of simulations. Circles denote  $\alpha_{dip}$  and  $\gamma$  as described in (A). **(C)** Homozygous phenotypes  
 589 for simulations that do contain overdominant mutations. Note that a large number of  
 590 phenotypes lie outside of  $\gamma$ , as expected for overdominant mutations, confirming that we  
 591 are correctly separating walks with and without overdominant mutations. When comparing  
 592 B and C, we observe that simulations with overdominant mutations are less forward  
 593 predictable than those without such mutations.



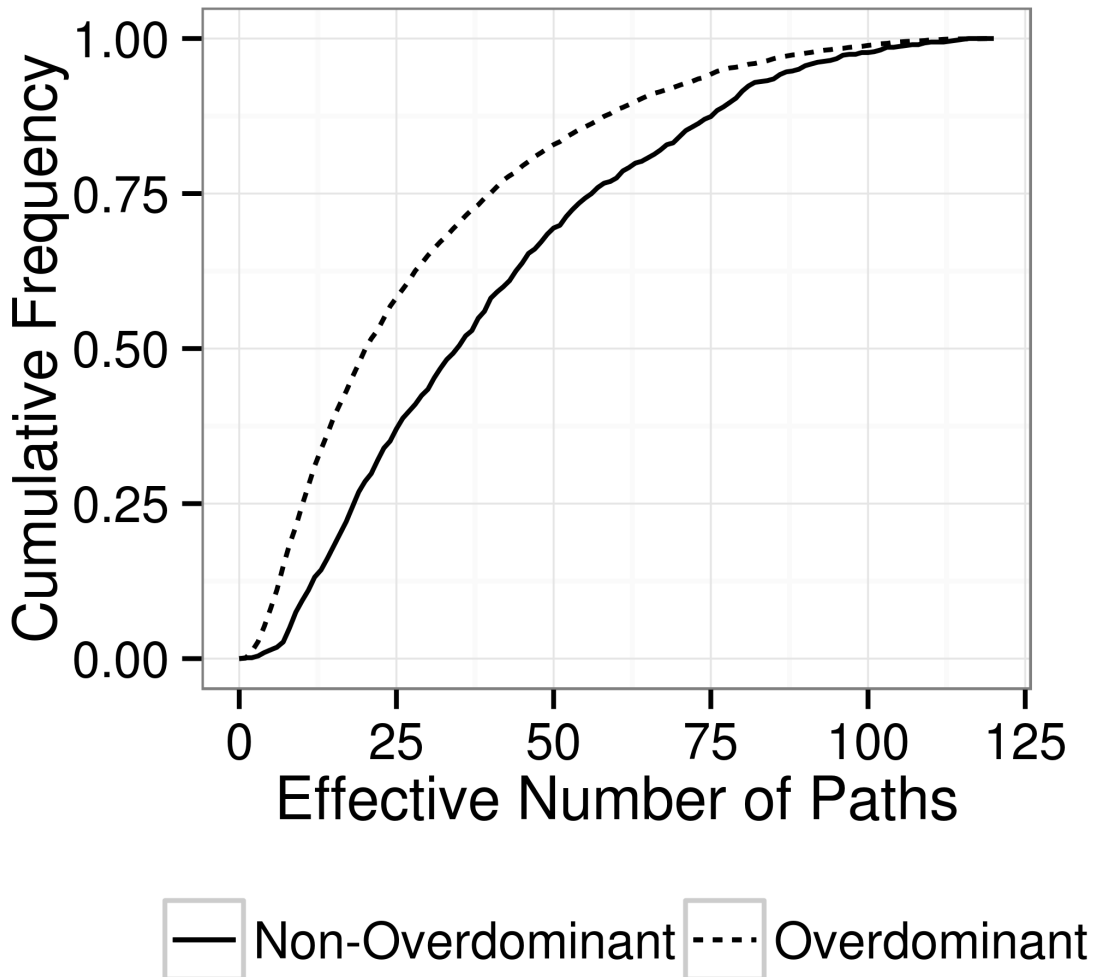
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595 **Figure 2. Overdominant mutations decrease forward predictability by 40%**  
596 **using the maximum pairwise distance metric.** Shown are the cumulative  
597 distributions of the maximum phenotypic distance between independent pairs of adaptive  
598 walks, excluding the ancestral state. This is a measure of the phenotypic forward  
599 repeatability of independent walks on the same evolutionary landscape. The maximum  
600 phenotypic distance in simulations without overdominant states is significantly less than in  
601 simulations with such states (Kolmogorov-Smirnov test  $p \ll 10^{-10}$ ).



602

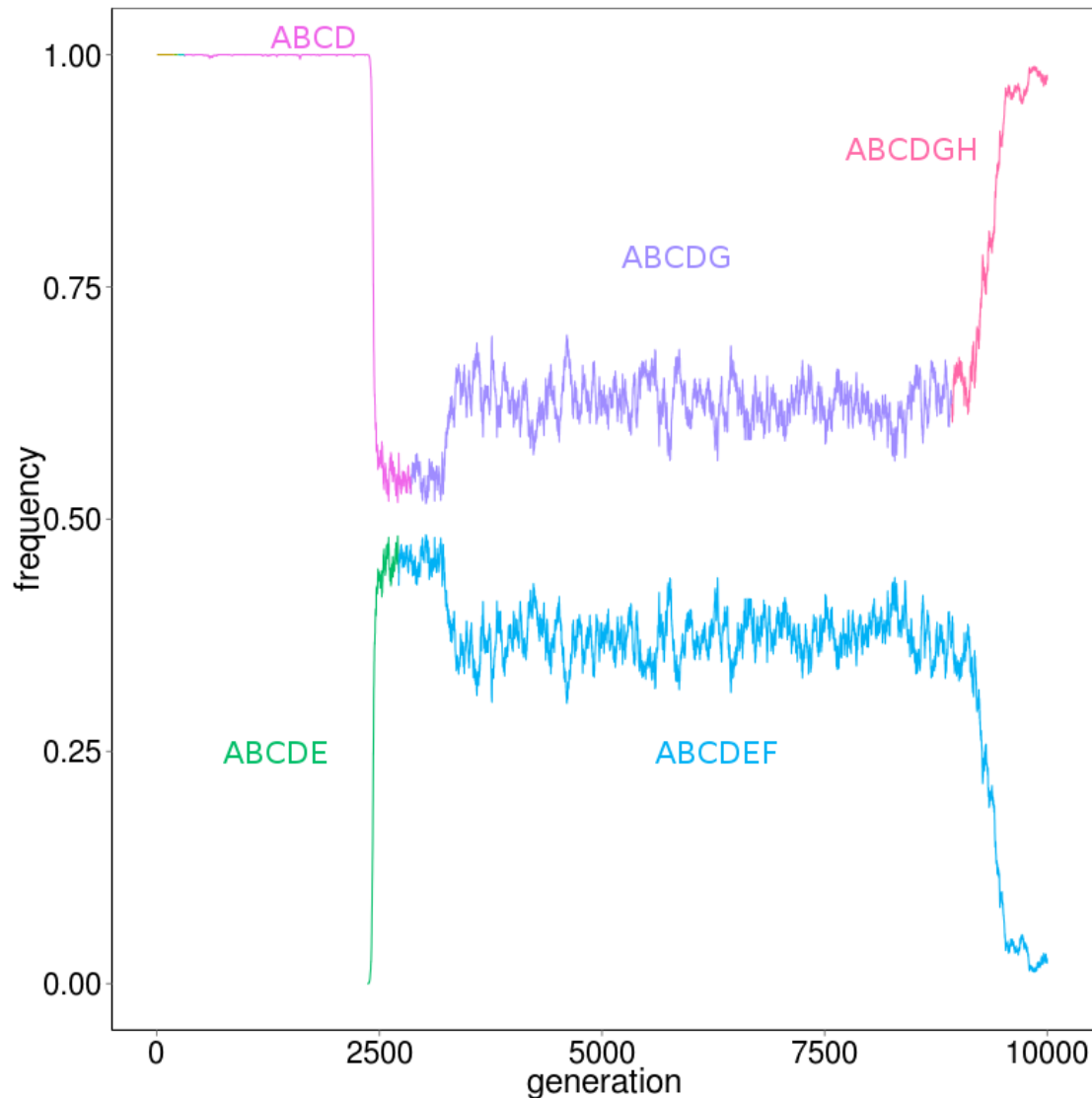
603 **Figure 3. Overdominant mutations decrease forward predictability by 5% using**  
604 **the maximum distance from the optimal trajectory metric.** Shown are the  
605 cumulative distributions of the maximum distance from the optimal trajectory of adaptive  
606 walks. This is a measure of the phenotypic forward predictability of walks. The maximum  
607 distance from the optimal trajectory in simulations without overdominant mutations is  
608 significantly less than those with such mutations (Kolmogorov-Smirnov test  $p \ll 10^{-10}$ ).



609

610 **Figure 4. Overdominant mutations increase backward predictability by 30%**  
611 **using the effective number of paths metric.** Shown are the cumulative distributions  
612 of the effective number of paths for adaptive walks with five mutations. This is a metric of  
613 backward predictability of evolution. Each mutation is introduced into the ancestral  
614 background in every possible order, and the number of viable mutational orders, weighted  
615 by their probabilities, determines the effective number of paths. The effective number of  
616 paths in simulations without overdominant mutations is significantly greater than in  
617 simulations with such mutations (Kolmogorov-Smirnov test  $p \ll 10^{-10}$ ).





618

619 **Figure 5. Example simulation with a hidden allele where the observed most**

620 **frequent allele was impossible to reconstruct by our method to compute**

621 **backward predictability.** The frequency of the two mutational lineages that reached at

622 least 1% frequency in the population are shown throughout the 10,000 generations of the

623 simulation. The main lineage, ending with allele ABCDGH, is at high frequency at the end

624 of the simulation, while the minor lineage, ending with allele ABCDEF (a “hidden allele”)

625 is at low frequency at the end of the simulation.

626 In the simulation, four mutations initially fix in quick succession, resulting in allele ABCD

627 fixed in the population. At this point, mutations causing balanced polymorphisms result in  
628 branched mutational lineages. Mutation E is the first mutation to occur on allele ABCD,  
629 generating a balanced polymorphism between alleles ABCD and ABCDE and allowing  
630 both alleles to be stably maintained in the population at intermediate frequency. Mutation  
631 F then quickly occurs on the background of allele ABCDE, generating allele ABCDEF  
632 which also balances with allele ABCD. Mutation G then occurs on the background of allele  
633 ABCD generating allele ABCDG soon afterwards, which balances with allele ABCDEF.  
634 Finally, mutation H occurs on allele ABCDG generating allele ABCDGH, which  
635 outcompetes all other alleles and is nearly fixed by the end of the simulation.

636 In our backward predictability reconstructions, we consider only the first five mutations of  
637 the most frequent allele at the end of the simulation, that is, we consider only mutations A,  
638 B, C, D and G as these were the first five mutations on allele ABCDGH. In attempting to  
639 reconstruct this observed order of mutations, we find that we can successfully introduce  
640 mutations A, B, C and D in order, but mutation G, which results in allele ABCDG, is not  
641 beneficial if allele ABCD is the only other allele in the population (data not shown).  
642 Therefore, the true order of mutations is impossible to reconstruct in this case when only  
643 sampling allele ABCDGH at the end of the simulation. However, if we also consider  
644 mutations E and F, we are able to successfully reconstruct the intermediate steps of the  
645 observed adaptive trajectory, suggesting that the presence of allele ABCDEF is necessary  
646 for allele ABCDG to be beneficial (data not shown).