**The Scope of Natural Selection:**

**MITTENS-Validated Case Studies in Local Adaptation**

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# Abstract

The MITTENS framework (Mathematical Impossibility of The Theory of Evolution by Natural Selection) establishes quantitative constraints on achievable fixations based on generation time, the selective turnover coefficient (*d*), and empirically observed fixation rates. While MITTENS demonstrates a 158,000-fold shortfall for macro-evolutionary divergence (e.g., human-chimpanzee), critics might argue that local adaptation represents an intermediate test case. Here we examine four well-documented examples of local adaptation: beach mouse pigmentation, stickleback armor reduction, peppered moth melanism, and warfarin resistance in rats. In every case, the required genetic changes involve 1–3 fixations—precisely the scale MITTENS predicts natural selection can accomplish. Using taxon-appropriate parameters and, where available, empirically measured selection coefficients, we show that all four cases pass MITTENS constraints. The peppered moth case is particularly instructive: MITTENS predicts 0.66 achievable fixations, implying the allele should reach high frequency but not fix—exactly what was observed before selection reversed. These results confirm that natural selection operates effectively within its proper scope while remaining incapable of the million-fold extrapolation required for macro-divergence. The boundary is not philosophical; it is mathematical.

**Keywords:** MITTENS, local adaptation, fixation, selective turnover coefficient, parallel evolution, natural selection

# 1. Introduction

The MITTENS framework establishes that natural selection, operating under empirically derived constraints, can accomplish approximately 126 fixations on the human lineage since divergence from chimpanzees—against a requirement of 20 million (Day & Athos 2025a). This 158,000-fold shortfall is not a modeling artifact; it emerges from three independently validated constraints: (1) the selective turnover coefficient *d* ≈ 0.45 for humans, derived from ancient DNA time series; (2) the fastest observed fixation rate of 1,600 generations from the *E. coli* Long-Term Evolution Experiment; and (3) the Bernoulli Barrier, which forecloses parallel fixation as an escape hatch.

A natural question arises: if MITTENS precludes macro-divergence, what about local adaptation? Organisms manifestly *do* adapt to local environments—beach mice match their substrate, freshwater sticklebacks lose their armor, moths track industrial pollution. If MITTENS is correct, these cases should fall within its predicted scope. If they do not, the framework fails.

We therefore subjected four canonical examples of local adaptation to MITTENS analysis. The results are unambiguous: every case involves 1–3 fixations, and every case passes. This is not a rescue of MITTENS; it is a confirmation of its boundary conditions. Natural selection accomplishes exactly what the mathematics predicts—no more, no less.

# 2. Methods

## 2.1 The MITTENS Framework

MITTENS calculates achievable fixations as:

*Achievable = (Nominal Generations × d) / Gf*

where Nominal Generations = Timeline / Generation Time, *d* is the selective turnover coefficient, and Gf is the number of generations required per fixation.

The selective turnover coefficient quantifies how overlapping generations impede allele frequency change. In a discrete-generation model, the entire breeding population turns over each generation (*d* = 1). In real populations, individuals from previous cohorts persist, diluting the effect of selection. For species with high mortality and minimal generational overlap, *d* approaches 1; for long-lived species with substantial overlap, *d* may be 0.5 or lower.

The baseline Gf = 1,600 derives from the *E. coli* LTEE, where 25 beneficial mutations fixed over 40,000 generations under strong selection (Good et al. 2017). This is the fastest fixation rate ever directly observed. Using it for all taxa is generous to the standard model.

However, when empirical selection coefficients are available for a specific case, Gf should be calculated directly:

*Gf ≈ (2/s) × ln(2Ne)*

For typical selection coefficients (*s* ≈ 0.01–0.05), this yields Gf in the range of 800–4,000 generations. For strong selection (*s* > 0.1), Gf compresses to 100–200 generations. We use empirically measured *s* values where available; otherwise, we default to Gf = 1,600.

## 2.2 Terminological Note: Fixation and Substitution

Throughout this paper, we use “fixation” to refer to the process by which a new allele replaces the ancestral allele throughout a population. In sexually reproducing organisms, this is equivalent to what molecular evolutionists call a “substitution”—a fixed difference between species. Unlike asexual organisms, where a beneficial mutation can sweep to fixation without displacing other lineages, sexual reproduction requires that every substitution transit through the same population bottleneck: the allele must increase from initial frequency to 100% in a breeding population subject to recombination, segregation, and generational turnover.

MITTENS treats substitutions as fixation events because, in sexual populations, they are. The 35 million fixed differences between humans and chimpanzees represent 35 million such transits—not 35 million neutral passengers that accumulated without population-level replacement. Neutral theory does not exempt these differences from the generational constraints that govern allele frequency change; it merely posits that drift rather than selection drove them. Either way, each substitution required population-wide allele replacement.

## 2.3 Case Selection

We selected cases meeting the following criteria: (1) well-documented genetic basis with identified causal loci; (2) known timeline for the adaptation; (3) available demographic parameters to estimate *d*; and (4) verified phenotypic change in natural populations. We excluded cases with ambiguous genetic architecture (e.g., Darwin’s finches) or uncertain timelines (e.g., cavefish).

The four cases span vertebrates and invertebrates, timelines from decades to millennia, and selection regimes from moderate to extreme. If MITTENS fails on any of them, the framework is falsified.

# 3. Results

## 3.1 Florida Beach Mice (*Peromyscus polionotus leucocephalus*)

Gulf Coast beach mice inhabit the white sand dunes of Florida's barrier islands, where pale coloration provides camouflage against avian predators. Mainland ancestors are dark-colored. Santa Rosa Island formed approximately 7,000 years ago as post-glacial sea levels stabilized, providing a maximum timeline for the adaptation (Steiner et al. 2007).

The genetic basis is well characterized. A single amino acid change in *Mc1r* (Arg65Cys) explains 10–36% of color variation (Hoekstra et al. 2006). Regulatory changes at the *Agouti* locus contribute additional variance. Minor contributions from *Kit* or other loci may exist but are not required for the phenotype. The total genetic requirement is 2–3 fixations.

For *Peromyscus*, generation time is approximately 1 year, and the high predation mortality characteristic of small rodents yields *d* ≈ 0.85. Using Gf = 1,600:

*Achievable = (7,000 × 0.85) / 1,600 = 3.7 fixations*

Against a requirement of 2–3 fixations, MITTENS predicts success with a margin of 1.2–1.9×. The prediction matches observation: beach mice are effectively fixed for the light phenotype.

## 3.2 Stickleback Armor Reduction (*Gasterosteus aculeatus*, Eda Locus)

Marine three-spined sticklebacks possess bony lateral plates protecting against predation. When marine fish colonize freshwater lakes—a process that occurred repeatedly as glaciers retreated 10,000–15,000 years ago—they evolve reduced plate number. This transition has occurred independently in dozens of lakes worldwide.

The genetic basis is remarkably simple. A single T→G base pair change in an enhancer element of the *Eda* gene accounts for >75% of variance in plate number (Colosimo et al. 2005; O'Brown et al. 2015). The low-plated allele exists as standing variation in marine populations at 1–4% frequency; freshwater colonizers do not require de novo mutation.

Using a midpoint timeline of 12,500 years, generation time of 2 years (sexual maturity at 1 year, mean reproductive age approximately 2 years), and *d* ≈ 0.80 reflecting high juvenile mortality:

*Achievable = (6,250 × 0.80) / 1,600 = 3.1 fixations*

Against a requirement of 1 fixation, MITTENS predicts success with a margin of 3.1×. Budget utilization is only 32%. The prediction matches observation: the low-plated Eda allele has fixed in freshwater populations worldwide.

The parallel evolution of armor reduction across independent lakes is itself evidence for MITTENS. If many genetic paths could achieve the phenotype, different lakes should fix different mutations. Instead, the same *Eda* allele fixes repeatedly—precisely because so few targets can achieve the phenotype within fixation constraints.

## 3.3 Peppered Moths (*Biston betularia*, *carbonaria* Morph)

The peppered moth is the textbook example of rapid adaptation. Prior to industrialization, the light-colored *typica* form predominated in Britain. As industrial pollution darkened tree bark with soot, the dark *carbonaria* morph rose from <1% to approximately 95% frequency in polluted regions within 50–80 years (van't Hof et al. 2016). Following the Clean Air Acts of 1956 and 1968, *carbonaria* declined as pollution decreased.

The genetic basis was identified in 2016: a single transposable element insertion of 21,925 bp in the first intron of the *cortex* gene (van't Hof et al. 2016). The mutation is dated to approximately 1819. The genetic requirement is 1 fixation.

This case has empirically measured selection coefficients from mark-recapture studies: *s* ≈ 0.15–0.30 in polluted woodlands (Kettlewell 1973; Cook 2003). Using *s* = 0.20 and Ne = 50,000:

*Gf = (2/0.20) × ln(100,000) = 10 × 11.5 = 115 generations*

With a timeline of 80 years (80 generations for this univoltine species) and *d* ≈ 0.95 (annual life cycle, complete turnover):

*Achievable = (80 × 0.95) / 115 = 0.66 fixations*

**This is the most instructive result in our analysis.** MITTENS predicts 0.66 achievable fixations—the allele should reach high frequency but *not fix*. What happened? The *carbonaria* allele reached approximately 95–98% frequency in polluted areas but never achieved complete fixation. When selection reversed after pollution reduction, the allele declined—something that would not have occurred had fixation been complete.

MITTENS does not merely predict pass/fail outcomes. It predicts *trajectories*. The peppered moth case confirms that allele frequency dynamics follow the quantitative constraints the framework specifies.

## 3.4 Warfarin Resistance in Rats (*Rattus norvegicus*, Vkorc1)

Warfarin was introduced as a rodenticide in 1950. By 1958, resistant rat populations were reported in Scotland. Resistance spread rapidly wherever warfarin was deployed, and at least seven independent resistance mutations have been documented worldwide (Rost et al. 2004; Pelz et al. 2005).

The genetic basis is simple: single missense mutations in *Vkorc1*, which encodes the vitamin K epoxide reductase enzyme that warfarin inhibits. Different populations carry different mutations (Tyr139Cys, Tyr139Ser, Leu128Gln, etc.), but each confers resistance through a single amino acid change. The genetic requirement is 1 fixation per population.

Warfarin is essentially lethal to susceptible individuals in treated areas, yielding extreme selection coefficients (*s* ≈ 0.50–1.0). Using the conservative estimate *s* = 0.50 and Ne = 10,000:

*Gf = (2/0.50) × ln(20,000) = 4 × 9.9 = 40 generations*

With a timeline of 70 years, generation time of 0.33 years (approximately 3 generations per year), and *d* ≈ 0.90 (very high wild mortality, minimal generational overlap):

*Achievable = (210 × 0.90) / 40 = 4.7 fixations*

Against a requirement of 1 fixation per population, MITTENS predicts success with substantial margin.

As with sticklebacks, the parallel evolution of resistance through independent mutations at the same gene supports MITTENS. The *Vkorc1* gene is hit repeatedly because few other genetic paths can confer resistance within the available fixation budget. Seven independent origins at the same locus is not coincidence; it is constraint.

## 3.5 Summary

Table 1 summarizes the MITTENS analysis for all four cases.

**Table 1. MITTENS analysis of local adaptation case studies.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Case | Timeline | *G*f | *d* | Eff. Gen. | Req'd | Achievable | Result |
| Beach Mice | 7,000 yr | 1,600 | 0.85 | 5,950 | 2–3 | 3.7 | **PASS** |
| Sticklebacks | 12,500 yr | 1,600 | 0.80 | 5,000 | 1 | 3.1 | **PASS** |
| Peppered Moths | 80 yr | 115\* | 0.95 | 76 | 1 | 0.66 | **PASS†** |
| Warfarin Rats | 70 yr | 40\* | 0.90 | 189 | 1 | 4.7 | **PASS** |

\*Selection-adjusted Gf using empirical *s* values. †Predicted high frequency without fixation; observed ~95% then decline.

# 4. Discussion

## 4.1 The Scope of Natural Selection

All four cases pass MITTENS constraints. This is not surprising—it is predicted. Natural selection can accomplish small-scale adaptations involving 1–3 fixations over ecological timescales. The mechanism is real. It is observable. It is quantifiable.

What natural selection cannot accomplish is the extrapolation from 1–3 fixations to 20 million. The gap is not one of degree; it is one of kind. Local adaptation operates in a regime where achievable fixations meet or exceed requirements. Macro-divergence operates in a regime where achievable fixations are 158,000-fold short of requirements. These are not points on a continuum; they are separated by a boundary that MITTENS defines precisely.

A potential objection deserves explicit address: polygenic adaptation can shift phenotypes without fixing individual alleles. This is true but irrelevant to the present argument. Polygenic adaptation explains how populations track shifting optima through coordinated small frequency changes at many loci. It does not explain fixed genomic differences. The 35 million SNPs distinguishing humans from chimpanzees are not frequency shifts hovering at intermediate values—they are fixed at 100% in one species and 0% in the other. Every one required population-wide allele replacement. Polygenic phenotypic tracking and genomic divergence through fixation are distinct phenomena; conflating them obscures rather than resolves the constraint problem.

## 4.2 Parallel Evolution as Evidence for Constraint

Three of our four cases exhibit parallel evolution: the same gene mutates independently in multiple populations facing similar selection pressures. Sticklebacks fix the same *Eda* allele in dozens of lakes. Rats develop resistance through at least seven independent *Vkorc1* mutations. Beach mice carry the same *Mc1r* mutation found in other light-substrate *Peromyscus* populations.

This parallelism is often cited as evidence for the power of natural selection. We suggest the opposite interpretation: it is evidence for *constraint*. If many genetic paths could achieve a phenotype, different populations should fix different mutations. The repeated targeting of the same genes indicates that few paths exist within the achievable fixation budget. Evolution takes the same route because that route is one of the only ones available.

## 4.3 The Peppered Moth as Trajectory Test

The peppered moth case deserves special emphasis. MITTENS predicted 0.66 achievable fixations—a number less than 1. This means the allele should rise to high frequency but fail to fix. The observed trajectory matched this prediction precisely: the *carbonaria* allele reached approximately 95% frequency, then declined when selection reversed.

Had the allele fixed at 100%, it would have remained at 100% regardless of subsequent selection changes (absent countervailing selection strong enough to drive it back down from fixation). Its failure to fix, followed by its decline, is direct evidence that MITTENS correctly models allele frequency dynamics—not just endpoint predictions, but the trajectory itself.

## 4.4 Use Empirical Values When Available

The baseline Gf = 1,600 from the LTEE is a calibration, not a constant. It represents fixation dynamics under typical selection in the fastest-evolving system ever directly observed. When empirical selection coefficients are available—as for peppered moths and warfarin resistance—they should be used to calculate case-specific Gf values.

This is not special pleading. It is proper methodology. The MITTENS framework provides the structure; empirical data populate the parameters. When data are unavailable, we use conservative defaults. When data are available, we use the data. In both scenarios, the predictions match observations.

## 4.5 Comparison with Macro-Scale Failure

The contrast between local adaptation and macro-divergence is stark:

|  |  |  |
| --- | --- | --- |
|  | Local Adaptation | Human–Chimp Divergence |
| Fixations required | 1–3 | 20,000,000 |
| MITTENS achievable | 0.66–4.7 | ~126 |
| Ratio (achievable/required) | 0.66× to 4.7× | 0.0000063× |
| Outcome | **SUCCESS** | **158,000× SHORTFALL** |

Local adaptations succeed because they require what natural selection can deliver. Macro-divergence fails because it requires 158,000 times more than natural selection can deliver. The four cases examined here are not evidence that natural selection can accomplish anything beyond its scope. They are evidence that it accomplishes exactly what its scope permits—and nothing more.

# 5. Conclusion

We subjected four canonical examples of local adaptation to MITTENS analysis. All four passed. Beach mice, sticklebacks, peppered moths, and warfarin-resistant rats achieve their adaptations through 1–3 fixations—precisely the scale MITTENS predicts natural selection can accomplish.

The peppered moth case is particularly instructive: MITTENS predicted 0.66 achievable fixations, implying high frequency without fixation. The observed trajectory—rise to ~95%, then decline—matched this prediction exactly. MITTENS models dynamics, not just outcomes.

Parallel evolution in sticklebacks, rats, and beach mice supports the framework: the same genes are targeted repeatedly because few genetic paths can achieve the phenotype within fixation constraints. This is evidence for constraint, not for unbounded creative power.

The boundary between local adaptation and macro-divergence is not philosophical. It is mathematical. On one side, achievable fixations meet or exceed requirements. On the other, a 158,000-fold shortfall. Natural selection operates effectively within its scope. The claim that it can extrapolate beyond that scope—to millions of fixations over millions of years—finds no support in the cases examined here or anywhere else.

The mechanism is real. The scope is bounded. MITTENS defines the boundary.

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