**The Real Rate of Molecular Evolution**

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## **Abstract**

Balloux and Lehmann (2012) demonstrated that the neutral substitution rate depends on population size under the joint conditions of fluctuating demography and overlapping generations. Here we derive an independent closed-form expression for the substitution rate in non-stationary populations using census data alone. The formula generalizes Kimura’s (1968) result k = μ to non-constant populations. Applied to four generations of human census data, it yields k = 0.743μ, confirming Balloux and Lehmann’s finding and providing a direct computational tool for recalibrating molecular clock estimates.

One of the foundational results of the Neutral Theory of molecular evolution (Kimura 1968; Kimura and Ohta 1971) states that the rate of allele substitution at neutral loci equals the mutation rate, independent of population size. The derivation is concise: in a diploid population of *N* individuals, 2*N*μ new neutral mutations arise per generation, each with fixation probability 1/(2*N*). The product is simply μ.

*k* = 2*N*μ × 1/(2*N*) = μ

This cancellation of *N* provides the theoretical foundation for the molecular clock (Zuckerkandl and Pauling 1962) and has been universally applied to calibrate divergence times across taxa for fifty-seven years.

In 2012, Balloux and Lehmann showed that this cancellation fails when two biologically ubiquitous conditions hold simultaneously: overlapping generations and fluctuating population size. Using a Markov chain framework for age-structured populations with discrete demographic states, they demonstrated that the number of newborns entering the population in a given demographic state does not correspond to the inverse of population size when adults from previous cohorts survive. Their key equation expresses the substitution rate as *k* = μ Σj*p*j*n*j*π*j, where *p*j is the stationary probability of demographic state *j*, *n*j is the expected number of newborns, and *π*j is the fixation probability. The terms *n*j and *π*j cancel only when every individual in the population is a newborn—the discrete-generation assumption. As both overlapping generations and population size fluctuations are the norm in natural populations, Balloux and Lehmann concluded that their observation “may be relevant for understanding variation in substitution rates within and between lineages.”

The Balloux-Lehmann framework is analytically general but requires specification of demographic transition probabilities and survival parameters. It was never applied to empirical census data for any species. In subsequent work, Lehmann (2014) argued that the substitution rate reduces to μ when measured in units of “average generation time” using the “effective mutation rate in newborns.” This redefinition rescues *k* = μ at the cost of changing what *k* measures—a move from census-observable quantities to effective parameters that cannot be independently verified.

Here we derive an independent closed-form expression for the neutral substitution rate in non-stationary populations. Our formula takes census population sizes directly as input, requires no estimation of survival probabilities or demographic transition matrices, and produces a numerical result from observable quantities. We confirm the Balloux-Lehmann finding through a different mathematical route and provide the first empirical application to real demographic data.

**The Hidden Assumption**

Kimura’s cancellation requires that the *N* governing mutation supply (the 2*N*μ term) is the same *N* governing fixation probability (the 1/2*N* term). In the Wright-Fisher model with discrete, non-overlapping generations, this is guaranteed: the entire population is replaced each generation, so the population producing mutations is identical to the population in which those mutations compete for fixation.

In real populations, generations overlap. Mutations that arose in a population of size *N*i now compete for fixation in a population of size *N*t ≠ *N*i. The *N* in the numerator and the *N* in the denominator are not the same number. The cancellation fails, and *k* ≠ μ.

This point was identified by Balloux and Lehmann (2012) in their abstract formulation. We now derive the consequence arithmetically.

**Derivation**

Consider a population observed at time *t* with census size *N*t. Mutations currently segregating in this population arose in previous generations *i* = 0, 1, 2, …, each with census size *N*i at the time of origin.

For each generation *i*:

(1) Mutation supply: *M*i = 2*N*iμ new neutral mutations per site.

(2) Current frequency: Each mutation, if present, exists as one copy among 2*N*t gene copies, giving frequency 1/(2*N*t). For mutations only a few generations old in populations of billions, the single-copy assumption is well justified: the probability of a second independent copy or of drift to multiple copies in fewer than four generations is negligible.

(3) Cohort contribution: The substitution rate contributed by generation *i*’s mutations is:

*k*i = *M*i × 1/(2*N*t) = 2*N*iμ × 1/(2*N*t) = μ*N*i/*N*t (1)

When *N*i = *N*t for all *i* (constant population), *k*i = μ for every cohort—Kimura’s special case. When *N*i < *N*t (growing population), *k*i < μ for every cohort that predates the current generation.

(4) Population-weighted average: The total substitution rate is the weighted mean across all contributing cohorts, weighted by each cohort’s mutation supply:

*k*t = Σ(*M*i × *k*i) / Σ*M*i = (μ/*N*t) × (Σ*N*i² / Σ*N*i) (2)

Equation (2) is the Real Rate of Molecular Evolution (RRME). It reduces to *k* = μ if and only if every *N*i = *N*t: Σ*N*i²/Σ*N*i = *gN*²/*gN* = *N* = *N*t, where *g* is the number of contributing generations. For any growing population, Σ*N*i²/Σ*N*i < *N*t, so *k* < μ. For any shrinking population, *k* > μ. The departure is systematic, predictable, and one-directional for any sustained demographic trend.

**Worked Example: Human Population, 1950–2025**

We apply the RRME to four generations of known human census data, using the standard human per-site per-generation mutation rate μ = 1.2 × 10−8 (Kong et al. 2012) and a generation time of 25 years.

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| --- | --- | --- | --- | --- |
| **Generation** | **Year** | ***N***i **(billions)** | ***N***i**²** | ***k***i**/μ** |
| 0 | 1950 | 2.5 | 6.25 | 0.305 |
| 1 | 1975 | 4.0 | 16.00 | 0.488 |
| 2 | 2000 | 6.1 | 37.21 | 0.744 |
| 3 | 2025 | 8.2 | 67.24 | 1.000 |
| **Sum** |  | **20.8** | **126.70** |  |

**Table 1.** Human census population by generational cohort, 1950–2025. *k*i/μ = *N*i/*N*t is the substitution rate contributed by each cohort relative to μ.

Applying Equation (2): Σ*N*i²/Σ*N*i = 126.70/20.8 = 6.091 billion.

*k* = μ × 6.091/8.2 = **0.743μ** (3)

The neutral substitution rate for the human population in 2025, computed from known census data, is 74.3% of the mutation rate—not 100%. The 25.7% shortfall is not an approximation error or a boundary effect; it is the mathematical consequence of computing *k* from real population sizes rather than assuming constant *N*. Any species whose population has grown over the relevant timescale will show the same pattern: *k* < μ, with the magnitude of the departure depending on the rate and duration of growth.

**Relationship to Balloux and Lehmann (2012)**

The RRME and the Balloux-Lehmann framework arrive at the same conclusion through different mathematical routes. Balloux and Lehmann model the substitution rate using Markov chain demographic transitions with explicit survival probabilities, showing that the product *n*j*π*j fails to cancel when generations overlap in a changing population. The RRME derives the same departure from *k* = μ by tracking mutation supply and current frequency across cohorts using census data alone.

The two approaches are complementary. The Balloux-Lehmann framework is analytically more general, accommodating arbitrary demographic state spaces and survival schedules. The RRME is computationally direct: it requires only census population sizes at generational intervals and produces a numerical substitution rate without intermediary parameters. The convergence of two independent derivations strengthens the conclusion that *k* = μ is a special case of a more general relationship, not a universal law.

Lehmann’s (2014) subsequent work argued that the substitution rate equals μ when measured in “units of average generation time” using the “effective mutation rate in newborns.” This redefinition does not dispute the census-measurable result. It demonstrates that one can construct an effective time unit in which *k* = μ holds by definition—which is mathematically legitimate but biologically circular, as the effective time unit is not independently observable. The RRME is expressed in calendar generations and census-observable population sizes, quantities that require no theoretical framework to measure.

**Implications**

The RRME has three immediate consequences.

First, molecular clock calibrations that assume *k* = μ systematically overestimate the rate of neutral substitution in any species with a history of population growth. Since most species used in molecular clock studies—primates, mammals, birds—have experienced substantial population size changes over the timescales relevant to divergence, published divergence dates require recalibration. The direction of the error is always the same: the clock runs slower than assumed, and true divergence times are longer than reported.

Second, the effective population size *N*e inferred from genetic diversity under the assumption *k* = μ absorbs the error introduced by the incorrect substitution rate. When *k* < μ but the model assumes *k* = μ, the back-calculated *N*e must be smaller than the true effective size to compensate. This may partially explain the persistent and well-documented discrepancy between census population size and inferred *N*e in large mammals (Frankham 1995; Palstra and Ruzzante 2008).

Third, the RRME predicts an acceleration of the molecular clock in declining populations (*k* > μ when *N*i > *N*t). This may contribute to the elevated substitution rates observed in species that have undergone recent population bottlenecks—a pattern noted empirically (Ho et al. 2005; Soares et al. 2009) and attributed by Balloux and Lehmann (2012) to the demographic mechanism formalized here.

**Anticipated Objections**

*The fixation probability at birth is 1/(2N*i*), not 1/(2N*t*).* This objection holds only in the Wright-Fisher model with discrete, non-overlapping generations. In that model, the entire population present at the time a mutation arises will be replaced before fixation can occur, so the relevant *N* is the one at origin. In overlapping-generation populations, the mutation competes for fixation against a gene pool that includes survivors from previous generations carrying alleles sampled from a different frequency distribution. The relevant *N* for computing current frequency—and therefore current fixation probability—is the current *N*t, not the historical *N*i. For a strictly neutral allele at frequency *p* in the current population, the fixation probability is *p*, regardless of what the population size was when the allele first appeared.

*The substitution rate resets each generation.* This is the discrete-generation assumption itself. In a Wright-Fisher population, the gene pool is completely replaced each generation, so each generation’s mutations compete only against their contemporaries. In any population with overlapping generations, this is false. Mutations from generation 0 coexist in the 2025 gene pool with mutations from generations 1, 2, and 3. Their frequencies are diluted by the growth of the population around them. The substitution rate at any time *t* depends on the accumulated history of mutation supply across all cohorts still contributing to the gene pool.

*The per-generation-time measurement resolves the discrepancy.* Lehmann (2014) showed that measuring in “units of average generation time” using “effective mutation rate in newborns” restores *k* = μ. However, this restoration depends on redefining the time unit to absorb the very effect being measured. The RRME is expressed in calendar generations and census population sizes—quantities that can be independently verified without theoretical assumptions. Any “correction” that restores *k* = μ by redefining the measurement unit has not resolved the discrepancy; it has renamed it.

**Conclusion**

Balloux and Lehmann (2012) proved that the neutral substitution rate depends on population size when generations overlap and demography fluctuates. We have independently derived a closed-form expression—the RRME—that produces the same result from census data without requiring specification of demographic transition probabilities. Applied to human data, both approaches confirm that *k* < μ in growing populations. The celebrated identity *k* = μ is a special case valid only for the idealized conditions of the Wright-Fisher model: constant population size and discrete, non-overlapping generations. Both conditions are violated in every natural population of interest. The correction is straightforward, requires only census data, and yields a number that differs from μ by a magnitude sufficient to affect molecular clock calibrations and *N*e inference across all taxa with overlapping generations and non-constant population sizes.

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