Misrepresenting biases in arrival: a comment on Svensson (2022)

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Abstract

The idea that adaptive change is subject to biases in variation by a "first come, first served" dynamic is not part of classic evolutionary reasoning. Yet, predictable effects of biases in the introduction of variation have been reported in models of population genetics, in laboratory evolution, and in retrospective analyses of natural adaptation. This effect of "arrival bias" has potentially broad significance, given widespread contemporary interest in the role of mutational and developmental tendencies of variation, in the context of a traditional view that such ideas are incompatible with population genetics. Indeed, the idea is addressed at length in a recent commentary by Svensson (2022). Unfortunately this commentary misrepresents the theory, history, evidence, and even the basic concepts involved in research on the role of mutation biases in adaptation. Here we correct some of these misrepresentations and offer a clearer and more accurate account of theoretical and empirical results.

In a recent commentary, Svensson (2022) addresses the theory that adaptive change is subject to biases in variation by a "first come, first served" dynamic missing from classical thinking. An effect of "arrival bias" ("arrival of the fittest") has been reported in models of population genetics (e.g., Cano et al. 2022; Schaper and Louis 2014; Yampolsky and Stoltzfus 2001), in laboratory evolution (e.g., Couce et al. 2015; Sackman et al. 2017), and in retrospective analyses of natural adaptation (e.g., Stoltzfus and McCandlish 2017; Storz et al. 2019). As scientists who have done original work on this topic, we are concerned that Svensson (2022) will mislead readers.

Unwarranted theoretical restrictions

In various statements, [1] Svensson (2022) places no fewer than 6 unwarranted restrictions on the efficacy of biases in the introduction process: (1) small N, (2) weak selection, (3) high mutation rates, (4) strict origin-fixation conditions (i.e., the limiting behavior as $\mu N \to 0$), (5) drift, and (6) reciprocal sign epistasis. The lack of requirement for the first 4 is evident in Yampolsky and Stoltzfus (2001), e.g., their Fig. 2 shows effects well outside the origin-fixation regime (see also Gomez et al. 2020; Soares et al. 2021). When Svensson (2022) invokes a false requirement for drift or small N, he cites comments of Lynch (2007) about a model (Bulmer, 1991) that requires small N for *fixations of slightly deleterious alleles by drift*, yet deleterious fixations are not required for the efficacy of biases in the introduction process, and *play no role whatsoever* in models such as those of Yampolsky and Stoltzfus (2001) or Rokyta et al. (2005) or Cano et al. (2022).

Nor is reciprocal sign epistasis a requirement. Assumptions about how mutant alleles interact play no role in models of one-step adaptation in the origin-fixation regime, e.g., Rokyta et al. (2005); when clonal interference is possible, fitness interactions must be specified, but they can be additive (i.e., no epistasis), as in Cano et al. (2022). Such models of short-term adaptation are relevant, not only to laboratory studies, but to natural cases such as the evolution of antibiotic resistance by large-effect mutations (Cano et al., 2022; Payne et al., 2019). The influence of mutation biases in longer-term evolution on complex landscapes with varying degrees of epistasis is addressed in studies exploring (1) how the findability of phenotypes with many genotypes shapes the choice of RNA folds (Dingle et al., 2022; Schaper and Louis, 2014) or transcriptional regulatory networks (Xiong et al., 2021); (2) the influence of transition-transversion bias on the navigability of empirical landscapes for transcription-factor binding sites (Cano and Payne, 2020); and (3) compositional trends induced by GC bias during adaptive walks on a protein NK landscape (Stoltzfus, 2006b). The suggestion of Svensson (2022) that the theory is in some way limited to 2-locus models is mistaken. [2]

[1] Svensson (2022) suggests that mutation biases will not be effective: "...unless population sizes are small and the effects of genetic drift is strong, mutation rates are high, selection is weak or certain criteria such as reciprocal sign epistasis for fitness are fulfilled (Lynch 2007; Svensson and Berger 2019)"; "...unless mutation rates are high, selection is weak, standing genetic variation is limited or certain conditions like reciprocal sign epistasis for fitness are fulfilled (Svensson and Berger 2019)"; and he states that "Such models make several implicit or explicit assumptions about linkage disequilibrium, reciprocal sign epistasis for fitness, that selection is weak relative to beneficial mutation rates, that gene flow is low and that the amount of standing genetic variation and extent of clonal interference is limited and/or genetically effective population sizes are small (Yampolsky and Stoltzfus 2001; Lynch 2007; Stoltzfus and Yampolsky 2009; McCandlish and Stoltzfus 2014; Svensson and Berger 2019; Gomez et al. 2020; Soares et al. 2021)." This resolves to 6 identifiable claims.

[2] "Population genetic models of how mutation bias can lead to mutation-biased adaptation are often two-locus models (Yampolsky and Stoltzfus 2001; Gomez, et al. 2020)"

Misidentifying the "opposing pressures" argument

What makes this theory novel? The original Modern Synthesis provided a unified framework of evolutionary causes as forces or pressures that shift frequencies in populations, used to support neo-Darwinism and to exclude non-Darwinian ideas (Fisher 1930, Futuyma 1988; see Stoltzfus 2017). Attributing evolutionary tendencies to internal biases in variation is one of the excluded ideas, rejected on the basis of arguments of Haldane (1927, 1932, 1933) and Fisher (1930), who concluded that recurrent mutation is a weak pressure unable to influence the course of evolution, except in the case of abnormally high mutation rates unopposed by selection. This "opposing pressures" argument was widely invoked to reject an evolutionary role for internal variational tendencies, e.g., by Fisher (1930, Ch. 1), Huxley (1964, p. 56, 509), Ford (1971, p. 391), and others such as Simpson, Dobzhansky, and Mayr (see Stoltzfus 2021). For instance, Gould (2002) cites Fisher (1930) and concludes, "Since orthogenesis can only operate when mutation pressure becomes high enough to act as an agent of evolutionary change, empirical data on low mutation rates sound the death-knell of internalism" (p. 510).

The problem with this way of thinking is that it equates the causal efficacy of mutation with the capacity of recurrent mutation to dominate allele frequencies by *competing with selection and drift*, i.e., acting in parallel, rather than focusing on events of mutational introduction as the sources of novelty, *acting prior to selection and drift*. Stated differently, the problem emerges from assuming that frequency transitions from 0 to 1/N (or $\frac{1}{2N}$) do not require a separate treatment from transitions of non-zero frequencies. When evolution is just a matter of shifting non-zero allele frequencies in standing variation, the classical way of thinking is safe: the efficacy of mutation depends on high mutation rates unopposed by selection. But when an evolutionary process depends on events of mutation that introduce new alleles, mutation has the unique role of shifting frequencies upward from 0, and biases in this process of introduction can impose biases on evolution, without requiring high mutation rates or neutrality.

How does Svensson (2022) explain this? Svensson (2022) invokes an "opposing pressures" view, citing exactly the same works of Haldane and Fisher, but assigns it a *different meaning*– "most mutations were thought to be deleterious and were assumed to be opposed by selection"–, reassuring readers that it has "largely been upheld." Thus, Svensson (2022) takes details of a story featured for over 20 years in the literature he is reviewing– the story of how Synthesis thinkers mistakenly called on "opposing pressures" thinking to reject a role for variational tendencies (Gomez et al., 2020; Stoltzfus, 2006a, 2017, 2019, 2021; Yampolsky and Stoltzfus, 2001)– and fashions them into a *different* story that omits the historic problem, leaving the reader with no clue as to why a theory of biases in the introduction process is a novel development in population genetics.

Overlooking the evidence

Svensson (2022) repeatedly treats the theory of arrival biases as merely a hypothetical possibility, and claims that no convincing evidence supports it. In fact, the literature provides an extensive empirical case (Gomez et al., 2020; Stoltzfus, 2019, 2021), which we briefly summarize in terms of causal agency, effect-sizes, and scope.

The gold standard to establish causal agency, i.e., *proving* that X can cause Y, is to manipulate X under controlled conditions and show an expected effect on Y. The experiments of Couce et al. (2015) satisfy this standard (see also Horton et al. 2021). Couce et al. (2015) subjected replicate lines of *E. coli* to increasing concentrations of cefotaxime, using 3 different parental strains with distinct nucleotide mutation spectra: the spectra of adaptive changes among resistant strains differed profoundly in ways that matched the mutation spectra of the parental strains. The correspondence between

mutation biases and adaptive changes is not due to biases in fixation by mutation pressure, but reflects introduction biases in the context of selective allele fixations.

What about effect-sizes? Various empirical studies indicate that a several-fold bias (e.g., a transition-transversion bias) may cause an effect of roughly similar magnitude (Cano et al., 2022; Payne et al., 2019; Pham, 2019; Rokyta et al., 2005; Sackman et al., 2017; Stoltzfus and McCandlish, 2015, 2017; Storz et al., 2019). Indeed, Cano et al. (2022) develop a method to capture the influence of the nucleotide mutation spectrum in a single parameter β ranging from 0 (no influence) to 1 (proportional influence), and they show that $\beta \approx 1$ in 3 large data sets of adaptive changes.

What about scope? Whereas many of the above results involve single-celled organisms and their viruses, the meta-analysis of natural cases by Stoltzfus and McCandlish (2017) also implicates animals and plants, using well known cases of parallel adaptation, e.g., spectral tuning or resistance to toxins such as tetrodotoxin and cardiac glycosides. Likewise, Storz et al. (2019) document a large and significant effect of CpG-mediated mutation in hemoglobin changes associated with altitude adaptation in birds.

Thus, the evidence establishes that biases in the introduction process are relevant to phenomena of adaptation and innovation in nature. To be clear, we do not expect readers of *Evolutionary Ecology* to be interested in transition-transversion bias: we expect them to be interested in the principle that, if the conversion of state A to B by mutation (and altered development) is merely a few times more likely than for A to C, this elevates the chance of evolving from A to B relative to A to C. Such tendencies may contribute to parallelisms and taxon-specific patterns of divergence under broad conditions, without requiring neutrality or high mutation rates. In long-term evolution, such biases can induce directional trends and lead to effects of findability favoring phenotypes widely distributed in genotype space (Dingle et al., 2022; Garson et al., 2003; Schaper and Louis, 2014). These principles are theoretically well grounded, empirically sound, widely applicable, and with a practical importance that is well established at the molecular level but largely unexplored at higher levels.

Unclear gatekeeping policy

Why aren't these principles recognized by Svensson (2022)? According to Svensson (2022), evolutionary biology today "recognizes historical contingencies such as the arrival order of mutations and mutational history (Losos et al. 1998; Huey et al. 2000; Svensson and Berger 2019), as exemplified in models of 'mutation-order speciation' (Schluter 2009; Mendelson et al. 2014)." Thus, Svensson (2022) welcomes some new ideas, but excludes others. Why?

Empirical proof is evidently not a requirement, e.g., Svensson (2022) welcomes the idea that correlational selection shapes $\boldsymbol{M}$, which is merely an interesting theoretical possibility (Houle et al., 2017). Svensson (2022) seems deeply concerned with plausibility, yet every concern expressed about population-genetic conditions relevant to the efficacy of arrival biases could have been raised in regard to mutation-order speciation which, as the author himself states, depends on "the arrival order of mutations."

Indeed, if conditions of adaptation in nature allow for *stochastic differences* in the arrival of new mutations, they must allow for *predictable biases* in the arrival of new mutations. A body of theory (noted above) addresses the expected consequences of such biases, taking into account selection and background conditions. Svensson (2022) rejects the guidance provided by this body of theory, arguing instead that "mutation bias is unlikely to overpower selection."

We stress again that this form of "opposing pressures" reasoning is known to be

invalid for evolution from new mutations. Yampolsky and Stoltzfus (2001) showed *specifically* that, if a bias in introduction favors $A \to B$ over $A \to C$, then an adaptive change of type $A \to B$ can happen more often, even if a change of type $A \to C$ would have been more beneficial. To describe this as mutation bias overpowering selection would be a conceptual error: selection discerns only among realized options, not hypothetical ones. *Mutation samples from the possible, selection from the actual.*

Empirical results support this prediction. In adaptive processes, the most common outcome is often *not* the most beneficial, but a beneficial variant favored by a higher mutation rate (e.g., Couce et al. 2013; Horton et al. 2021; MacLean et al. 2010). Rokyta et al. (2005) found this pattern in replicate laboratory adaptation of a bacteriophage; Sackman et al. (2017), repeating their protocol with 3 other species, found in each case that the most frequent change was not the most fit. In the case of drug-resistant leukemia, Leighow et al. (2020) report that, for 2 changes at the same protein site, both conferring resistance to Imatinib, the change with a higher clinical prevalence is not the one that is more resistant, but the one with a higher mutation rate; Keulen et al. 1997 report an analogous case for resistance to a reverse-transcriptase inhibitor in HIV.

A mistaken conception of "mutation bias"

The term "mutation bias" ("mutational bias") has been used for over 50 years for systematic differences in rates for different types of mutations (e.g., GC-AT bias in Cox and Yanofsky 1967). More generally, in scientific writing, a bias is an unequal tendency or disposition, something we might express formally as an inequality in expected values $\mathbb{E}(x_i) > \mathbb{E}(x_j)$, where a symmetry between i and j would suggest (under a principle of indifference) $\mathbb{E}(x_i) = \mathbb{E}(x_j)$. For instance, if we know that transitions tend to happen at higher rates than transversions, and i and j are a transition and a transversion, respectively, then $\mathbb{E}(\mu_i) > \mathbb{E}(\mu_j)$. Bias is not the same thing as heterogeneity: if we know only that there is a non-zero variance in mutation $V_\mu > 0$, then $\mathbb{E}(\mu_i) = \mathbb{E}(\mu_j)$ for any i and j, i.e., there is no bias.

Svensson (2022) repeatedly defines "mutation bias" as per-locus heterogeneity in mutation rates, citing sources inconsistent with this meaning. [3] This definition is mistaken: it fails to cover transition-transversion bias and GC-AT bias, the two most common instances of "mutation bias" in PubMed, and the two examples used by Yampolsky and Stoltzfus (2001) to map their abstract genetic model to known biases.

Moreover, the usage of "bias" in Svensson (2022) drifts to cover any effect of mutational contingency, even without bias or non-uniformity, e.g., in speciation models that treat mutational order as a purely stochastic variable per Mani and Clarke (1990). The notional model of "mutation bias" in Fig. 1 depicts heterogeneity in the encoding of a quantitative trait, but does nothing to suggest biased mutational conversions. A relevant model for divergence in the genetic encoding of a quantitative trait under stabilizing selection was developed 40 years ago by Kimura (1981) and extended to cover mutation bias (in the sense of an asymmetric effect on trait values) by Charlesworth (2013) following Waxman and Peck (2003). [4] In evo-devo, the tendency for the

[3] "the result is mutation bias, where some loci contribute more in providing mutational input to the population than other loci (Yampolsky and Stoltzfus 2001; Stoltzfus and Yampolsky 2009; Stoltzfus and McCandlish 2017)"; "mutation bias (differences between loci in mutation rates)"; "these mechanisms will generate mutation bias across the genome, meaning that mutation rates will be higher in certain genomic regions compared to others"

[4] In quantitative genetics, the effect of a mutation bias on mean trait-values is not part of the standard model (Lande and Arnold, 1983), and has received little attention. Waxman and Peck (2003), Zhang and Hill (2008), and Charlesworth (2013) consider mutation bias with a single stabilized trait, so that the only possible effect of mutation bias is to deflect from the optimum, an effect that is small. The more meaningful effect of a mutation bias in the sexual selection model of Pomiankowski et al. (1991) is actually mediated by selection: a high rate of mutational degradation of elaborate male ornaments

developmental-genetic encoding for a complex trait to change over time, while the trait remains constant, has been called "developmental system drift" (True and Haag, 2001).

Misrepresenting of minority views

The theories targeted for criticism in Svensson (2022) are not represented accurately. For instance, Svensson (2022) rejects "mutation-driven" evolution on the grounds that selection is the force that increases fitness, and the force that drives alleles to fixation. This misrepresents Nei (2013) and others who use this phrase, e.g., Pennings et al. (2022) refer to "point-mutation-driven, stepwise evolution", Sackman et al. (2017) refer to "Mutation-Driven Parallel Evolution During Viral Adaptation" and Tenaillon (2014) concludes, in regard to Lenski's long-term evolution experiment, "Large-effect mutations appeared therefore to be the drivers of adaptation."

None of these sources claim that fixation happens by mutation pressure (rather than selection or drift), or that adaptation happens by mutation without selection. Instead, this usage of "drive" follows a well established meaning in which a strongly explanatory factor is said to "drive" a pattern. When an economist says that droughts are driving crop prices, this does not imply that droughts act independently or in isolation, and it does not imply that drought is a physical force pushing on prices. Thus, "mutation-driven" means that the course of evolution depends so strongly on the timing and character of events of mutation, that the sequence of these events is a primary explanatory factor.

Likewise, Svensson (2022) consistently mis-states the theory of arrival biases, or sabotages it with fabricated conditions, damaging associations, or false comparisons. For instance, the potential for mutation bias to influence adaptation is repeatedly burdened with the condition "alone," as in: "To date, there is no convincing empirical evidence that directed mutations are important or that mutation rates alone have a strong influence on adaptive evolution, although mutation bias clearly play a role in neutral evolution at the molecular level." [5] This language raises the possibility that mutation bias could influence adaptation, analogous to the way it can "play a role" in neutral evolution, while simultaneously undermining this possibility with the words "mutation rates alone." This possibility is further sabotaged by a gratuitous association with the bogeyman of "directed mutation," i.e., the trick is to say "to date, there is no convincing evidence that humans communicate telepathically with aliens or that mutation bias influences adaptation."

Summary

The commentary by Svensson (2022) misrepresents the theory, history, evidence, and basic concepts implicated in research on the role of mutation biases in adaptation. Theoretical work has established that biases in the introduction process are a possible cause of orientation or direction in evolution, and thus a possible contributor to parallelisms and trends, contradicting a historic "opposing pressures" argument used to reject any such effects. Recent work establishes that simple biases in mutation have a direct influence on the changes involved in adaptation, both in the laboratory and in nature. Whereas this work establishes a new and potentially important kind of

creates heterogeneity in male phenotypes, so that the choosiness of females matters, allowing female choosiness to evolve. Thus, mutation is not really contributing its directionality to evolution, because the degraded males (the ones whose genes reflect the bias in mutation) are not the ones who are chosen. Using a non-standard model, Xue et al. (2015) show that mutation bias can cause a long-term trend in a quantitative trait value when fitness is mainly a function of latent traits.

[5] Also: "Neither mutation supply (overall genomic mutation rate across all loci) nor mutation bias (differences between loci in mutation rates) alone will result in directional evolutionary change..."

causation, much further work is needed to assess its general importance. We encourage readers to consult primary sources for more information.

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The authors declare that they have no relevant interests.

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Authors' contributions

AS drafted and revised the manuscript; all authors reviewed and commented on the manuscript.

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