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The role of purifying selection in the origin and maintenance of complex function

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ABSTRACT

Fitness contribution alone should not be the criterion of 'function' in molecular biology and genomics. Disagreement over the use of 'function' in molecular biology and genomics is still with us, almost eight years after publicity surrounding the Encyclopedia of DNA Elements project claimed that 80.4% of the human genome comprises "functional elements". Recent approaches attempt to resolve or reformulate this debate by redefining genomic 'function' in terms of current fitness contribution. In its favour, this redefinition for the genomic context is in apparent conformity with predominant experimental practices, especially in biomedical research, and with ascription of function by selective maintenance. We argue against approaches of this kind, however, on the grounds that they could be seen as non-Darwinian, and fail to properly account for the diversity of non-adaptive processes involved in the origin and maintenance of genomic complexity. We examine cases of molecular and organismal complexity that arise neutrally, showing how purifying selection maintains non-adaptive genomic complexity. Rather than lumping different sorts of genomic complexity together by defining 'function' as fitness contribution, we argue that it is best to separate the heterogeneous contributions of preaptation, exaptation and adaptation to the historical processes of origin and maintenance for complex features.

1. Introduction

Publication of the results of the *Encyclopedia of DNA Elements* (ENCODE) project (ENCODE, 2004; 2012) was accompanied by claims that 80.4% of the human genome comprises "functional elements", sparking a host of scientific and public debate. Initial criticisms focused on the implausibility of ENCODE's criteria for inclusion of parts of the genome as "functional elements" (Graur et al., 2013), and the project's failure to distinguish between distinct concepts of function, inviting criticism that they had equivocated between mere genomic phenotype and a richly evolutionary – though more difficult to prove – notion of function as selected effect (Brunet & Doolittle, 2014; Doolittle, 2013; Doolittle et al., 2014; Kellis et al.,. 2014a,b). It remains a problem that their results did not demonstrate that most "functional elements" contribute to organismal fitness, even currently. Brzovíc and Sustar (2020) recently offered an attempt to resolve the ENCODE controversy by providing a concept of function that strikes a balance between evolutionary considerations and the methodology actually used by ENCODE. Their account is the principle focus here.

Continuing debate over the implications of the findings by ENCODE investigators have focused on experimental or computational ways to assess function as fitness contribution, with interspecies sequence conservation (generally *much* below 80%) being taken as the most rigorous (Ecker et al., 2012; Doolittle, 2013; Graur et al., 2013; Kellis et al., 2014a; Ponting, 2017, but see; Omer et al., 2017). Such conservation is assumed to show that a DNA segment has been under what is usually called "negative selection" or "purifying selection", and so is fitness-contributing. That is, conservation indicates that alterations to a trait are detrimental to fitness and have been weeded out by natural selection, at least since the time of the relevant species' common ancestor. It is tempting, particularly in a context of continuing ambiguity, to stipulate that maintenance under purifying selection for performance of some effect is all that 'functionality' requires in genomics. There have been several attempts at this, the most carefully reasoned in our view being that of Brzovíc and Sustar (2020). They propose "weak etiological monism" (WEM) as the concept of function most

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appropriate for molecular biology and genomics and as a way to resolve the ENCODE debate. Similarly, Germain et al. (2014) show that much molecular biology and genomic research is biomedically oriented, and for many purposes cares little about a trait's history. Indeed, that a trait is (or might be inferred to be) under purifying selection is generally all that comparative molecular biology and genomic researchers *can* conclude. Still, we suggest that defining function in terms of present effects in such a way as to make origins irrelevant would be a retrograde step in our understanding of genomic complexity.

Brzovíc and Sustar (2020) make a distinction between weak etiological monism (WEM) and strong selected effect (SSE) etiological accounts, specifying this according to fitness effects. We argue (Sec.2, see also Linquist et al., 2020) that this distinction is best analyzed by two features of the evolutionary explanation for a trait: past positive selection *for* its effects (origin) and present purifying selection *against* loss of those effects (maintenance). We endorse such a distinction and argue here that concepts of function such as WEM, requiring only purifying selection, are too permissive and should not be adopted. We first argue (Sec.3) that WEM is a fundamentally non-Darwinian account in which positive natural selection does not have a necessary creative role to play in complex adaptation. We then show that WEM fails to adequately distinguish many traits that likely arose neutrally but whose elimination may now be deleterious. This is because treating fitness contribution as a proxy for function glosses over the distinction between function and effect, and between adaptation, exaptation and preaptation (Sec.4). Many complex traits have a mixed etiology, being explained in part by adaptive processes and drift, but also exaptation and preaptation (Sec.5). We examine three cases—Syncytin, RNA virus defense in old world monkeys, and the spliceosome—to show in detail what is neglected by narrowly focusing on fitness contribution, and propose a general conceptual framework, a triangular simplex, as a way of parsing these (Sec.6). Though we emphasize the practical difficulty in distinguishing between positive and purifying selection for any complex trait, we see such a distinction as being fundamental to making sense of biology, genomic biology in particular (Dobzhansky, 1973; Griffiths, 2009; Linquist et al., 2020).

2. Defining SSE and WEM

Concepts of function based on fitness contribution are meant to capture that a number of genomic structures—so called, "functional DNA elements" as designated by ENCODE—have origins which do not necessarily depend on a history of past positive selection for their current (or necessarily any) effect. Accounting for such structures is indeed a lofty goal, as they have in large part been neglected by biologists and philosophers of biology. We discuss Brzovíc and Sustar's (2020) recent approach here. These authors distinguish two concept of function, Strong Selected Effect (SSE) and Weak Etiological Monism (WEM), as follows,

> [T]he strong version [SSE] is, in fact, the standard SE account that ascribes functions to traits that were selected for; that is, they contributed to differential survival and reproduction of organisms bearing them. A weak version [WEM], instead, does not require that there was selection for the functional trait. What suffices is a contribution to the containing organism's and its ancestors' fitness. —Brzovíc and Sustar (2020).

To make it clear that both SSE and WEM are selection-based concepts of function, we present the distinction in terms of positive and purifying selection, as follows. A trait T with current effect E has E as its *strong etiological* (SSE) function iff T both originated by positive selection for E *and* is either still undergoing such positive selection or is maintained by purifying selection for E. (If T was once but is no longer under selection of either type, it is what we would call a "relic".) Likewise, E is the *weak etiological* (WEM) function of T iff T is now and was for some time in the past under purifying selection for the maintenance of E, regardless of whether positive selection explains its origin or distribution. The "strength" of these concepts is in their order of implications. SSE implies WEM, which in turn implies that a trait has an effect in some more inclusive biological system, that is to say has a Causal Role (CR) function, since nothing can be said to have a function if it has ceased to, or never did, have any biological effect. But the reverse implications do not hold (Fig. 1).

Part of the objection to ENCODE's declaration that 80.4% of the human genome comprises "functional elements" is that the effects on fitness or even on phenotypes beyond the molecular were not tested. Thus all activities in the largest ellipse in Fig. 1 *could* have been included, though no doubt some measures were discounted that would have made the genome entirely "functional elements", such as whether a given element was replicated (Graur et al., 2013). Perhaps no one would defend such a broad usage of 'function' encompassing any biochemical effect whatsoever. However, earlier criticism of ENCODE highlighted just how close the definition of "functional elements" came to embracing anything with a mere causal role (Doolittle, 2013). More sensibly, Brzovíc and Sustar's (2020) definition of WEM is meant to ascribe functionality to traits whose effects are explicable in terms of present (and likely past) *purifying selection,* though without requiring prior positive selection. No doubt, this is all that much current comparative genomic and experimental research shows or indeed can show. Inferences about the past cannot be more than inferences to the best explanation, and inference to present purifying selection alone is less onerous than inferring past positive selection as well. The former can in principle be demonstrated experimentally or by sequence conservation, the latter only tentatively concluded from comparative data.

But reliance on purifying selection alone poses two problems. First, it permits a fundamentally non-Darwinian (as we construe it below) account of biology, in which positive natural selection need play no role in the origin of function. Second, even when allowing for the importance of selection, it deems as functional many traits that likely arose neutrally, not obviously being "for" anything in the way that eyes might be said to be "for seeing", or hearts "for

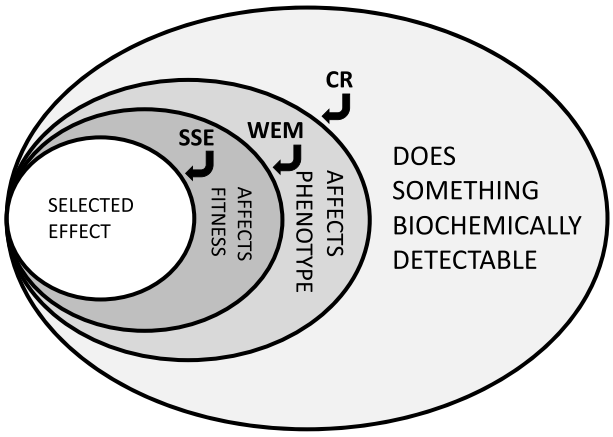
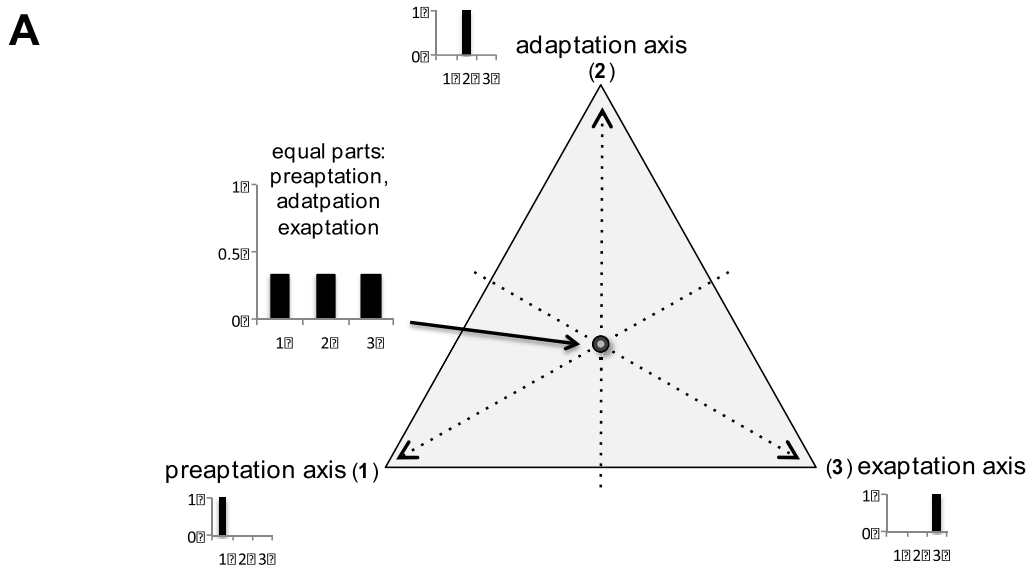


Fig. 1. Overlap of strong selected effect (SSE), weak etiological monism (WEM), and causal role (CR) accounts of function (fitness-neutral phenotypes beyond molecular, such as gene sequence or blood type) subsumed by properties detectable by methods like those of the ENCODE project (1).



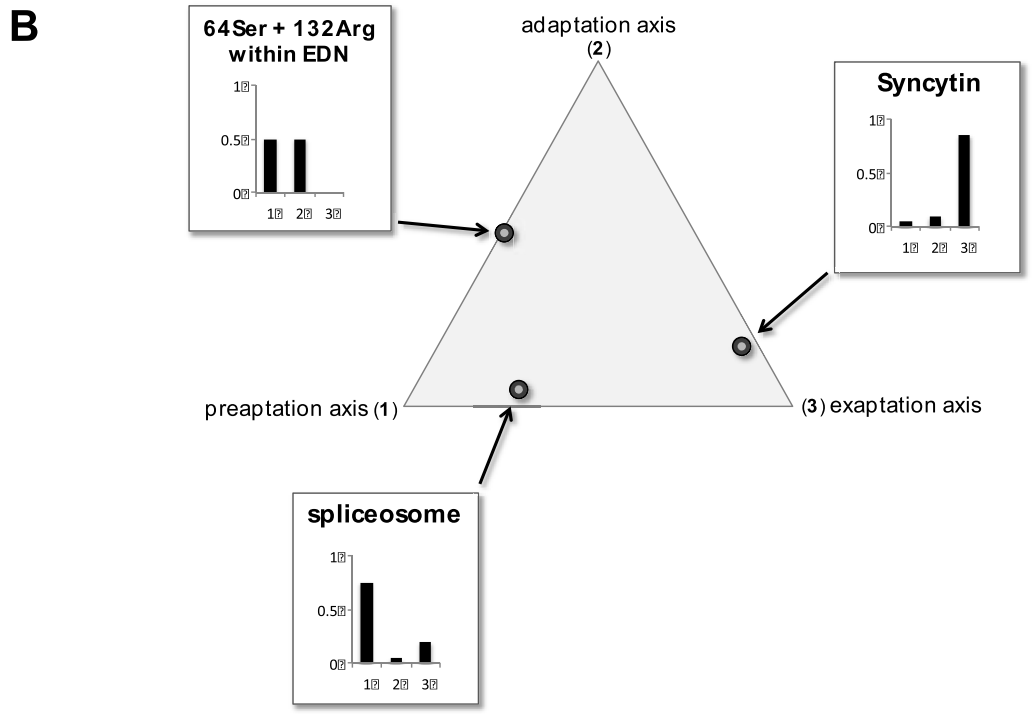


Fig. 2. The historical simplex is a triangular diagram that depicts the contribution of preaptation, adaptation, and exaptation to a trait currently maintained by purifying selection. Each point in the diagram represents a unique mixture of three historical processes that sum to a constant value (1.0). The coordinate axes for preaptation, adaptation, and exaptation are plotted in panel **A**. The center point of the simplex (coordinates: 1/3 adaptation, 1/3 exaptation, 1/3 preaptation) is plotted for reference. Note that the three vertices of the triangle, labeled 1, 2 and 3 in **A** and **B**, represent the three points of "pure" preaptation (1,0,0), adaptation (0,1,0), and exaptation (0,0,1), respectively. Panel **B** depicts proposed coordinates for three real examples; the amino acids for antiviral activity in Old World monkey EDN, the syncytin protein, and the eukaryotic spliceosome. Histograms give the relative proportion of preaptation, adaptation, and exaptation for each case. Proportions might be best assessed in terms of fractions of nucleotide positions fixed as a consequence of each process.

pumping blood". We discuss these problems in turn, before discussing an experimental issue at the heart of this conceptual disagreement: that positive and purifying selection can only be properly distinguished historically. The right way to account for the distinct contributions of positive and purifying selection is, we argue, not to lump these into a single notion of 'function' but to explicitly separate them on historical grounds. To facilitate this, we then propose an historically-based graphical way of parsing the diverse historical contributions of selection to genomic structures (Fig. 2). Our terminology agrees in general with that of Linquist et al. (2020) who proceed quite differently but, like us, seek to disambiguate "origin" and "maintenance" functionality. However, we add that some fitness-contributing traits are best seen as historically "maintained" not only by "purifying selection" but also by episodes of "positive selection" that repair drift-induced damage to function. Often, we think, these are conflated.

3. Why fitness contribution alone is non-darwinian

Maynard Smith (1969) wrote that "the major task of any evolutionary theory" is "to explain adaptive complexity, i.e. to explain the same set of facts which Paley used as evidence for a Creator". Darwin – and now "neoDarwinists" – did this by invoking the iterated operation of natural selection. When selection and mutation together create such adaptive complexity, it is through successive fixation of ever more fit genetic variants, moving up a fitness peak in some "adaptive landscape", by the action of what is normally called "positive" or "directional" selection, driven by some positive "selection coefficient". "Adaptation" is both a description of such upward movement and a description of its product. It is a core commitment of Darwinism, we think, that complex adaptations help organisms survive and reproduce, and that these were shaped at least in part by past positive selection, incremental in its operation. We Darwinians think of our eyes as complex adaptations arising through the successive fixation of mutations conferring better and better vision, and our hearts as optimized organs for pumping blood, evolved stepwise over time. Even if we do not see evolution as progressive, but simply as tracking environmental change, it is *positive selection* that does the tracking.

However, we don't need to be Darwinists in this sense to think that some traits will be maintained by purifying selection in the future, and are in principle so maintained now. A non-Darwinist can just as well predict that blinding of an individual would make it more difficult for them to find mates and produce progeny, and would presumably have done so in the recent past. This is just what it means to say that vision is (and was recently) maintained by "purifying selection". Indeed, perhaps a majority of people on this planet do not think as Darwinians do, believing instead – as did Paley (1802) – that the adaptedness (or aptness, in Gould and Vrba's [1982] language) of living things is evidence that they were intelligently designed or created, "creatures" in the literal sense. For this majority, "adaptedness" need not imply "adaptation" by positive natural selection. Of course traits that originated by intelligent design might still be maintained thereafter by purifying selection, and so have been under such selection since the time of creation until now. After all, an intelligent creator creating all species in their current form could scarcely be imagined to have intended that blinding be harmless, regardless of when this creation occurred.

Thus, requiring a history of purifying selection does not forbid intelligent design, however far in the past this history reaches. Paley would have believed that God made eyes so we might see. And if Paley were a contemporary molecular biologist, he might well have understood that the designer used genes to accomplish His purposes. So, if maintenance by purifying selection now and even some time into the past is all that is required as a definition of function, and it is the fitness of an organism's various functions to the requirements of its environment (Maynard Smith's "adaptive complexity") that we want to explain, then we have not really come up with an alternative to what Paley believed. Importantly, if we rely only on purifying selection as a criterion of function, then we have not made any risky predictions about the *causes* of functional complexity (Thornton, 2019). Our concept of 'function' is then no longer of service to Maynard Smith's "major task" of evolutionary theory. To *be* Darwinists, at least in our sense of that word, we need to explain the *origin* of traits as well as the processes that keep (and have kept) them in place. If "etiology" is taken to be about *origins,* then in that sense WEM is not etiological.

Nevertheless, studying the effects of altering or deleting particular cellular or genomic components (most often simply assuming that fitnesses would thereby be reduced) is what most molecular biologists and genome scientists do, most of the time, while using interspecies sequence conservation as evidence for past purifying selection. Germain et al. (2014) similarly argue for a weaker account of function on the basis of its relevance to practice, writing that "ENCODE's controversial claim of functionality should be interpreted as saying that 80% of the genome is engaging in relevant biochemical activities and is very likely to have a causal role in phenomena deemed relevant to biomedical research". Indeed, one needn't be a Darwinian to investigate biochemical activities with medical significance—for that project, etiological concepts of function can be mostly dispensed with. Where the aim is biomedicine, we endorse this interpretation of ENCODE. Indeed Kellis et al. (2014b) write that "the (ENCODE) catalog and similar data are important foundations for understanding the DNA elements and molecular mechanisms underlying human biology and disease". We agree and do not question the utility of such an effort.

Perhaps defining 'function' in such a way as to discomfit non-Darwinians should not be our goal, Maynard Smith notwithstanding. Moreover, if most cellular components that are currently maintained by purifying selection *were* created by reiterated positive selection for the effect that they now have, the distinction between WEM and SSE would be largely irrelevant. Many molecular biologists and genomicists do operate as if they believed this (for extensive critiques, see Lynch, 2007; Koonin, 2016). From such an adaptationist or panadaptationist perspective (Gould and Lewontin; 1979; Wilkins and Godfrey-Smith; 2009), the SSE ellipse in Fig. 1 would be nearly coextensive with the WEM ellipse, and ENCODE's mistake was only a too permissive conflation of "potential medical relevance" and "function". But we do not think this is correct. Not all components currently maintained by purifying selection were created by positive selection, and even some highly complex features acquired through multiple fixation events were not so created. Our reasons for this claim and arguments for the necessity of disambiguating purifying and positive selection are discussed next.

4. Neutral evolution can generate non-adaptive genomic complexity

Some fraction of genomic complexity arises by neutral processes. At the most fundamental level, this is because variation is often introduced in ways that do not initially affect organismal fitness. The dynamics of such neutral variation can drive the evolution of genomes through the trait space of complexity. Canonical cases are those of the neutral theory of molecular evolution (Kimura, 1983) and the zero force evolutionary law (Brandon & McShea, 2020; McShea & Brandon, 2010; McShea et al., 2019). Of course, some fraction of genomic complexity is also deleterious, but deleterious traits, too, can be fixed when effects are mild or

populations are small (Lynch, 2007; Ohta, 1973). In Graur et al.'s (2015) terminology, the genome has both "junk" (which is non-functional and inert) and "garbage" (non-functional and deleterious). This, perhaps, is unremarkable when admitting interplay between drift and selection (see sec.5–6). What is generally overlooked, however is that genomic structures of both varieties, neutral and even in sum mildly deleterious, can be maintained by purifying selection after they arise.

Examples of initially neutral but selectively maintained complexity diverge widely, there is no currently accepted canon, and the idea itself has both many origins and many names: epistatic ratchets (Bridgham et al., 2009), contingency and entrenchment (Shah et al., 2015; Starr et al., 2018), Constructive Neutral Evolution (CNE; Covello & Gray, 1993; Stoltzfus, 1999; Brunet & Doolittle, 2018) and subfunctionalization (Force et al., 1999). Though these ideas differ substantively in parts, they share the claim that a fortuitous interaction or unselected "promiscuous activity" (call it trait T) of a cellular component can, through some change to the environment or epistatic interactions, become subject to purifying selection. Such fortuitous interaction or activity can be seen as an "excess capacity" that the organism later becomes "dependent" on (Stoltzfus, 1999), as a "pre-suppressor" which allows mutations that would otherwise be detrimental to occur in another component (Brunet & Doolittle, 2018; Covello & Gray, 1993; Stoltzfus, 1999), or as an "historically permissive" (Starr et al., 2017) mutation that is later "entrenched" by epistasis. Often this condition persists once it occurs, since the accumulation of additional "dependencies" or "entrenchments" strengthen the intensity of purifying selection (Shah et al., 2015; Stoltzfus, 1999), and this becomes increasingly difficult to reverse because the historical order of mutations matters for fitness. After just a few evolutionary steps the probability of re-tracing the exact neutral path that would allow the evolutionary "undoing" of each accumulated dependency becomes exceedingly small. Importantly, the simple accumulation of changes during a neutral "walk" can increases the selective constraints associated with T and thereby necessitate a history of purifying selection dating back to the original stochastic event (change in environmental or epistatic interaction) that caused T to become subject to purifying selection without prior adaptive evolution. In recent years, new exemplary cases featuring single protein molecules have been developed. For instance, Starr et al. (2018) show that most substitutions in Hsp90 were "contingent on prior epistatic substitutions and/or entrenched by subsequent changes". These substitutions were not driven by positive selection, as simplistic evolutionary reasoning would have had it.

An abstract model of neutral emergence of complexity involves the interaction of two proteins, A and B. Let us assume in this case that A and B are products of unlinked neutrally-arising loci, fixed by drift at approximately the same time, though the relevant features of this example are the same if (perhaps more realistically) either or both are initially fixed by selection. Suppose that A and B have some fortuitous interaction that prevents further mutations in either locus from having a deleterious effect, perhaps this interaction is *binding* such that both are *stabilized* (or *solubilized, insolubilized, folded,* etc.). Once this interaction leads to stabilization of A and B, the accumulation of mutations in either locus is "pre-suppressed by", "permitted by", or "contingent on" that stabilization; i.e., the interaction of A and B allows population fixation of otherwise intolerable mutations that destabilize the individual proteins. After several such destabilizing mutations are fixed, the binding of A to B has become "essentialized", "locked in" or "entrenched". That is, absent this interaction, the descendent forms of A or B would be individually unstable, possibly wreaking havoc in the cellular environment and subject to purifying selection (see Harms and Thornton; 2013, and references therein).

A simultaneous deletion of both A and B might be neutral (or slightly advantageous, given the population genetic burden of maintenance) but is, all things considered, the *least likely outcome* when the genes encoding A and B are not genetically linked. What is more likely is that the independent deletion of either locus A *or* locus B would be selected against, allowing the further accumulation of otherwise destabilizing mutations in both. The process of evolving new fitness dependencies can continue almost indefinitely when the epistatic nature of the interaction between A and B is combinatorially complex (Starr et al., 2017; Stoltzfus, 1999). That is, a complex interaction that originated for no adaptive reason can be perpetuated by selection against its loss, even if it is assumed that the interacting components A and B initially had no effect on organismal phenotype, let alone positive fitness effects. Although the co-evolving sequences of A and B might not be highly conserved, their joint presence would be.

If positive effects *are* assumed — say A was selected for, and became dependent on B — then B would have become essential for the performance of A's activity though CNE, regardless of any function B might have independently had. And though subsequent environmental change might mean that an organism was in sum disadvantaged simply by having to maintain A + B, it could be difficult to show this because of other evolved dependencies (of some trait C on A + B, for instance). Any real cell or organism is only incompletely modular, and likely all complex structures are sub-optimal products of evolutionary tinkering, which creates unnecessary complexity (Denise et al., 2020; Jacob, 1977). A favourite example of CNE proponents is the spliceosome, whose current complexity seems to offer no advantage in speed or accuracy over the group II introns that presumably gave rise to it (Cavalier-Smith, 1991; and see Sec.6.0). Yet many of its tinkered components are now maintained by purifying selection, regardless of origin.

Had Gould and Vrba been aware of such processes at the time they wrote "Exaptation — a missing term in the science of form" (1982), we believe they would have been more strict in separating out the varieties of exaptations. In their now famous carving of the conceptual terrain, everything with some "current use" (maintained by purifying selection and functional according to WEM) is an "aptation", and aptations are then subdivided into "adaptations" and "exaptations". There were two further subcategorizations of "exaptation" entertained by Gould and Vrba, one which we will hereafter call 'exaptation' and a second we call 'preaptation'. An exaptation is a trait T that was shaped by positive selection for some effect E, but now has another effect E′ as its "current use", that is, it is only now under positive or purifying selection for E'. Its current use differs from its reasons for origin. In contrast, a preaptation is a trait T that was not under selection for any effect whatever (was previously a "non-aptation"), but is now under selection for some current use. The A and B components in our example above could both be such, each having the "current use" of stabilizing the other.

Gould and Vrba's (1982) project was to encourage biologists to distinguish between current utility, defined as *enhancing current fitness,* and historical genesis. This much is accomplished by their ex/adaptation distinction. Their typology is also, at the same time, a distinction between "functions" and "effects". In their table taxonomizing aptations, "function" is the usage ascribed to adaptations, while "effect" is ascribed to exaptations (of both types). One way to look at accounts of 'function' that equate it with aptness is as an explicit definitional disagreement with the canon of usage captured, correctly we think, by Gould and Vrba. In an effort to include neutrally-arising variation, Brzovíc and Sustar (2020) define as "functional trails" what Gould and Vrba defined as "effects".

> [W]e argue that the set of *functional traits is much wider than the set of traits selected for* at some point in the evolutionary past. Take, for instance, *genomic traits that perform some useful activity* for the organism … or cases where a trait has arisen through a neutral process and fortuitously started to contribute to the organism's fitness. –(Brzovíc and Sustar, 2020) (emphasis added).

Redefining "functional traits" as those with "useful activities", might be benign, provided equivocation is meticulously avoided, but it is hard to do this. In discussing the degree of purifying selection required of WEM-functions, Brzovíc and Sustar, like Gould and Vrba, intend an analysis restricted to presently or previously apt traits.

> In our account … it is not necessary for [a trait] to be so important that a dysfunction of that trait would lead to the organism's death, but rather for it *to increase the organism's fitness* to such a degree that the trait will not be wiped out by purifying selection. That is, the trait is supposed to make a positive contribution to the way the organism deals with its environment. [ibid, emphasis added]

One problem here is the equation of traits that will not be "wiped out by purifying selection" with those that "increase the organism's fitness". Admittedly, a mutant selected against by purifying selection exhibits *decreased* fitness vis-à-vis the fixed allele. But the notion of increasing an organism's fitness is too easily taken to imply a history of incremental positive selection in the sense of the stepwise climbing of a fitness landscape. We discuss the roles of purifying and positive selection in trait maintenance, and fitness landscapes, in detail in the next section. For instance, we speculate (also in more detail below) that the eukaryotic spliceosome, with more components even than the ribosome, arose largely through CNE. Thus, although many of the spliceosome's individual components might now be maintained by purifying selection, this does not mean that individually they increased "the organism's fitness", nor that collectively these components "increase the organism's fitness", if we were to compare an intron-rich contemporary eukaryote to its intronless (or group II intron-invaded) progenitor. Similar arguments can be made for many other genomic products of CNE (see Sec.6).

Many have argued that fitness makes sense only in relative terms and in real populations, so Gould and Vrba's aptness definition ("the general, static phenomenon of being fit" (Gould & Vrba, 1982)) is at issue (for example, Wilson, 2004). The scope of the comparison across time, and number of relevant components, affects our judgements of relative fitness and of the granularity of 'function'. Significantly, a trait might be maintained by purifying selection on many of the genes required for its formation or performance now, and thus appear to be "apt" relative to contemporary mutants, even though many steps leading to its current form did not enhance fitness relative to immediate ancestors. Moreover, for such traits generated by CNE, if selection had been presented with a situation where variation was in the presence or absence of the fully realized complex trait, its absence would have had the selective advantage.

Seen as more than an attempt to temper ENCODE's overestimates without imposing all of the constraints of an SE account of function, accounts like WEM have the laudable aim of embracing a larger class of genomic traits and accommodating actual practice in molecular biology and genomics. But actual practice is biased by conflation, not only of current utility and reasons for origin, but also of purifying and positive selection.[1] Also laudably, requiring fitness contribution for 'function' points to the need for a "next step" of empirical assessment of the fitness effects of "functional elements", ENCODE's logical next step (Kellis et al., 2014a). Nonetheless, without an account of the origin and maintenance of fitness-contributing traits, these accounts will still conflate the consequences of contingent entrenchment or CNE with those of adaptation.

Put another way, "the adaptationist programme" and "panadaptationism" critiqued by Gould and Lewontin (1979) are still at work in genomics and molecular biology, making the accommodation of practice by theory a misstep. Adaptationism holds that "natural selection [is] so powerful and the constraints upon it so few that direct production of adaptation through its operation becomes the primary cause of nearly all organic form, function, and behaviour" (Gould and Lewontin, 1979). Lynch, Koonin and others (Koonin, 2016; Lynch, 2007; Agren, 2016) have argued that this attitude is still the default in much molecular biology and genomics. Indeed, it was an apparent confirmation of this mind-set that there was so much public enthusiasm (see for instance Carey, 2015) for the claimed debunking of "junk DNA". Weak etiological accounts are a genuine improvement on this. They are not adaptationist, but they are still "aptationist", and for reasons discussed above, unclear as to what that means.

The extent to which CNE and like neutral processes have created structures and processes that are now maintained by purifying selection is unknown and hard to know, but in any case such components and processes are not Darwinian adaptations as we would define them. WEM, given the adaptationist bias of many in the field, encourages conflation. To quote D'Arcy Thompson, such investigators are "wont to liken the course of organic evolution not to the straggling branches of a tree, but to the building of a temple, divinely planned" (Thompson, 1961). Excluding the alternatives to adaptation takes work, and indeed, the onerous task of assessing the fitness effects of most of the elements identified by ENCODE has hardly begun (Luo et al., 2020). Addressing potential non-adaptive processes in the case of each trait invoked by proponents of CNE is also hard—a detailed consideration of even just the spliceosome according to all adaptationist alternatives would be a *magnum opus* of a task (Vosseberg & Snel, 2017; and see section 6.0). But this is work that must be done before identifying maintained complexity with functionality in the sense of adaptation, as in Maynard Smith's challenge. We identify some of the issues involved in sections 5 and 6.

Since it is difficult to disentangle CNE from adaptation, further discussion of the neutral emergence of complexity and the role of natural selection – both what is called "positive" and what is called "purifying" – in the maintenance of fitness will be made easier by restricting our discussion to abstract models. In the following section we contrast adaptive evolution and maintenance selection in the context of fitness landscapes. Then we follow this (section 6.0) with a presentation of empirical examples of complex origin and maintenance stories for genomic complexity.

5. The evolutionary details of selective origin and maintenance of function

The contribution of selection to both the evolutionary origin *and* maintenance of a trait is a perennial source of confusion. In this section we set apart the notions we find most important for assessing the etiology of complex genomic traits, namely: purifying

[1] Proponents of CR definitions of function (e.g. Cummins and Roth 2009) have argued against such underlying bias and advocates of SE definitions in particular recognize such conflation/equation as a common failing.

and positive selection, selective maintenance and origin, and fitness landscapes. Distinguishing WEM and SSE requires a clear distinction between past positive selection for a function (required by SSE) and present purifying selection against loss of a function (required by both SSE and WEM). It is not widely appreciated, however, that selective maintenance of a trait involves complex evolutionary dynamics that include both purifying selection against maladaptive mutations and continual repair of function by positive selection.

Positive and purifying selection. Positive selection refers to a process whereby the proportion of individuals having a beneficial trait increases over time within a population. The increase of a beneficial trait due to natural selection is determined by the fitness difference between the beneficial trait and other variants of the trait (denoted s). Consider two alleles A1 and A2 within a population, A2 being selected against or outcompeted by A1. The sign of the selection coefficient, s, is determined by the direction of comparison. Here, selection against A2 is identical to selection for the alternative A1; the effect of positive selection is that the beneficial trait increases in frequency. Thus a meaningful distinction between purifying and positive selection can only be made when there is external information about the historical order in which A1 and A2 were first introduced into the population.

For example, when some maladaptive A2 arises by mutation within a population previously fixed for A1, then selection at the time of A2's origin is said to be acting *against* its fixation in the population. This scenario is commonly called purifying (or negative) selection because it is easiest to think of selection against fixation of A2 in terms of $-s$. Alternatively, if beneficial A1 arises by mutation in a population previously fixed for A2, then selection at the time of A1's origin can be thought of as driving it to fixation. This scenario is commonly referred to as positive selection because it is easiest to think of selection for fixation of A1 in terms of $+s$—though it is, with respect to population genetic change, the same process as negative selection against A2. A situation in which A1 comprise 99% of alleles and A2 makes up 1% might either indicate positive selection just prior to fixation of the favored allele (A1) or the transient appearance of less favored variants (possibly 'revertants'), likely soon to be purified away. Although there is an historical truth here, without further information bearing on this history, empirical evidence about these allele frequencies and fitness effects cannot tell these situations apart.

Selective maintenance. It is easy to understand how purifying selection, by acting to prevent the fixation of a new deleterious mutation, plays a central role in the maintenance of function. However, drift cannot be ignored since all finite populations are subject to evolution by drift as well as by natural selection. This means that maladaptive alleles will periodically be fixed by drift. Thus, fixation of new mutations by positive selection that either reverses a deleterious mutation, or restore fitness through positive epistatic effects, is essential to the selective maintenance of a trait. When restoration is through secondarily-occurring epistatic effects, the results would count as "suppression", but this would be indistinguishable from the "pre-suppression" that is CNE. Only knowing the history could allow a decision, although for large populations CNE explanations involving pre-suppression seem more reasonable, since there need be no transient period of reduced fitness. Interestingly, selective maintenance has characteristic dynamics such that, over a long timescale, any decreases in fitness due to drift are balanced by restorative actions of positive selection (Hartl & Taubes, 1996; Jones et al., 2016; Sella & Hirsh, 2005). This equilibrium state has been referred to as non-adaptive shifting balance (Jones et al., 2019).

Selective origin. SSE only ascribes a function to a trait if it evolved by positive selection. Since positive selection is also crucial to selective maintenance of function, the distinction between SSE and WEM therefore depends on a precise understanding of the dynamics and history of selective origin and selective maintenance. The selective origin of a trait (adaptation) involves fixation of one, or a sequence of, beneficial mutations that move a population to some new optimal state and is not an equilibrium process. Evolution towards the new optimum involves an excess of fitness-enhancing positive selection, as compared to non-adaptive shifting balance (Jones et al., 2016; dos Reis, 2015).

Fitness landscapes offer useful insights into the how the dynamics associated with selective origin differ from subsequent maintenance (Jones et al., 2016; Wright, 1932). A new optimum in trait design or function can be represented as a peak, not yet occupied by the population, on a landscape where elevation represents fitness. Adaptive evolution is depicted as up-slope evolution towards that peak. Such directional evolution requires that fixation of fitness *enhancing* changes brought about by positive selection is more effective than drift at moving the population on the landscape. Once at the peak, the population becomes subject to selective maintenance with no net change in location. We might say that "fitness enhancing" has become "fitness contributing", insofar as movement off the peak would be selected against in large populations. Drift can, on occasion, move the population off the peak by fixation of a mildly deleterious mutation but, when the trait is under selective maintenance, such changes are eventually repaired by positive selection for a new mutation (thereby returning it to the peak). As long as conditions do not change, a consequence of selective maintenance is that the traits' central tendency on the landscape will coincide with the peak, which is what we perceive over evolutionary time as selective constraint (required by both SSE and WEM). If the fitness landscape shifts, as it will either through environmental change or epistatic effects, then there may again be an excess of such fitness-enhancing positive selection.

Any distinction between positive and purifying selection is historical—it depends on the actual history of how a mutant arose and the processes operative at that time—and thus can only be indirectly determined. Because of CNE, not all "fitness contributing" traits were historically "fitness enhancing". Exceptions can perhaps be made when evolutionary events take place during the period of our observation, as they sometimes do during experimental evolution, e.g. in research programs such as that of Richard Lenski (Good et al., 2017). But for the human traits of interest to ENCODE and those of molecular biology and genomics broadly speaking, distinguishing between these processes is an extraordinarily difficult task.

Below, we examine the details of three cases that are good examples of traits with a mixed heritage of evolutionary processes. We suggest that these fit best not within a weak etiological framework of fitness contribution, but within the triad of types of fitness effects described by Gould and Vrba (1982). Indeed, the histories of these complex traits are so mixed that they can hardly be assigned to one aptive category to the exclusion of the other two. To remedy this, we present their historical contributions to fitness effects within a simplex, where individual complex traits appear between the extremes of adaption, exaptation and preaptation (Fig. 2).

6. Most organismal traits have evolved through a complex mixture of historical processes

A common critique of Darwinian theory is that the "incipient stages" of many complex traits cannot effectively serve their *current function* (Mivart, 1871). Although the intended anti-Darwinian conclusion (that such traits could not be the products of an

evolutionary process) is incorrect, the implication that intermediate stages could negatively affect current function is correct. Certainly many complex adaptations result from the sub-optimal cobbling together (tinkering) of components evolved to perform different functions. The tension here arises from failure to recognize that the majority of complex traits cannot be explained with simplistic evolutionary histories, the most egregious form of which is often called pan-adaptationism, as noted above (Cummins & Roth, 2009). That pan-adaptationism remains a challenge to modern biology is understandable, and several authors (Graur et al., 2013; Lynch, 2007; Koonin, 2016; Gould & Lewontin, 1979; Agren, 2016) have hinted at some of the extraneous social and cultural forces that encourage it. The reason we address it here, internal to evolutionary theory, is that the historical sequence of events that explain the origin and maintenance of a trait is often a complex mixture of processes of adaption, exaptation and preaptation.[2] Reconstruction of evolutionary history is an exceptionally difficult task, but it is one that must be pursued, if we are to make sense of anything in biology (Dobzhansky, 1973, but see; Griffiths, 2009). To assist with the task of disentangling heterogenous evolutionary origins, we offer a graphical framework for mapping the relative contribution of adaptation, exaptation and preaptation.

The plot in Fig. 2A represents all possible contributions of three historical processes to the "aptness" of a trait currently maintained by purifying selection – traits that have "maintenance functions". Note that all elements under purifying selection at present – that demonstrably contribute to fitness or would be inferred to do so by contemporary molecular and genomic methods – would fall somewhere in this triangular simplex. The history of a maintained trait can be described as a single point within the unit simplex of historical scenarios. The simplex has three axes, one for each historical process. The center of the simplex is a trait that has an exactly balanced history; such a trait is explained by equal parts adaptation, exaptation and preaptation. The three vertices represent the points of "pure" adaption, exaptation or preaptation. All other points within the simplex describe a more complex origin and/or maintenance.

The distinction between origin function and maintenance function demanded by Linquist et al. (2020) maps to our simplex: all traits having a "maintenance function" are included in the simplex: those having as well an "origin function" would be found towards the "exaptation" or "adaptation" axes of our simplex – near the former if evolved for a separate effect than that now maintained, near the later in the simple cases in which both are the same. Any currently useful trait matching Brzovíc and Sustar's WEM must lie somewhere within the simplex, and more complex features are more likely to be found nearer the center. The historical details of each case will depend on the complexity and scope of the individual trait in question. We illustrate this below with three examples.

6.1. Syncytin - primary exaptation of fusogenic viral genes followed by secondary adaptation for mammalian placentation

Mammals have been subject to continuous infection by retroviruses (with reverse transcription of their genome into the mammalian host) for millions of years. Sometimes, a retrovirus inserts its genome as a DNA copy into a host germline cell, and the retrovirus becomes "endogenous"; all host offspring subsequently inherit the viral genome, and horizontal infection of new cells is no longer required. Between 8 and 10% of the genomes of humans and mice are composed of endogenous retrovirus (ERV) genes, with the vast majority having been rendered non-functional via accumulation of mutations (Gifford & Tristem, 2003). However, some ERVs have retained the ability to replicate and spread from within the host germline whereas others have been coopted to serve a function for the host. *Syncytins* are a well-known class of ERV genes that were captured from infectious retroviruses and are now essential to the development and maintenance of the mammalian maternal-fetal interface (Haig, 2012).

A defining feature of live-bearing mammals is the formation of organs for mediating exchanges between the mother and a growing embryo (placentation) and suppression of the mother's immune system. An envelope gene (*env*) captured from an ERV in an ancestral Therian was the likely prerequisite for the evolution of placentation, including permissive immune tolerance of a developing embryo (Dupressoir et al., 2012). Independent of this ancient event, there have been multiple subsequent captures of *env* proteins from ERVs throughout mammalian history, leading to repeated evolution of *syncytin* proteins in primates (Mi et al., 2000), rodentia (Dupressoir et al., 2005), lagomorpha (Heidmann et al., 2009), and carnivora (Cornelis et al., 2012) lineages. In each case, the ancestral immunosuppressive and fusogenic functions of *env* were coopted, respectively, for embryonic immune tolerance and for structural development of placentation (Dupressoir et al., 2012).

Each instance of *syncytin* evolution from an *env* gene is an independent exaptation event. The frequency of independent exaptations throughout mammalian history suggests that capture of an *env* gene avoids suffering the fitness cost of evolving mammalian genes through various incipient stages to meet the immunosuppressive and fusogenic needs of placentation. It is noteworthy that, despite mammalian common ancestry, there is considerable structural diversity among lineages in placentation (Dupressoir et al., 2012). Panadaptationism explains this diversity as "designed" by natural selection to fit the unique needs of each lineage. However, this diversity is better explained as the contingent constraints arising from independent exaptation of different *env* genes. Because each *env* gene was unlikely to be perfectly suited to mammalian placentation, we expect some degree of fine-tuning by natural selection after each exaptation event (Gould & Vrba, 1982 call this secondary adaptation). For this reason mammalian *syncytin* proteins fall close to, but not exactly at, the exaptation vertex in Fig. 2B. In this view of evolution (in contrast to simplistic pan-adaptationism) the immunosuppressive and fusogenic functions of *syncytins* are exaptations for the mammalian maternal-fetal interface. Some additional within-lineage genetic changes of *syncytins* are secondary adaptations that fine-tune the architecture of the feto-maternal interface in different lineages. Interestingly, we need to collapse levels of selection to keep the simplex two dimensional. That is, the original adaptations favored the differential replication or survival of ERV's, not of their hosts. We could imagine another simplex, below the first, in which genome-level (as opposed to organism level) adaptations, preaptations and exaptations, all pertaining to TEs as units of selection, are displayed. Similarly, at a level above that shown in Fig. 2 we might construct a simplex with similar axes for selection at the species level (Jablonksi, 2007). Exaptations and preaptations, as we understand them, might occur across levels.

6.2. Old world monkey EDN - preaptation and adaptation of a complementary amino acid pair for anti-viral activity

A gene duplication occurred approximately 31 million years ago in an ancestral Old World anthropoid primate that produced the ECP-EDN gene family (Cornelis et al., 2012; Rosenberg et al., 1995). Having two gene copies permitted one gene (EDN) to evolve

[2] Linquist et al. (2020) have recommended the word "function" always be preceded by "origin" or "maintenance" or, in the case of genuine SE functions, both.

substantially increased RNAase activity (selectively maintained in all extant old world monkeys and hominids) (Zhang & Rosenberg, 2002). EDN proteins are expressed within eosinophilic leukocytes, where they contribute to a 13-fold increase in the ribonucleic activity of eosinophil-derived neurotoxin (Zhang and Rosenberg, 2002). The enhanced capacity to digest viral RNA leads to reduced infectivity of certain RNA viruses, which is a positive fitness effect (Domachowske et al., 1998; Rugeles et al., 2003). Through ancestral amino acid state reconstruction, site-directed mutagenesis, and laboratory investigations, two physically interacting sites within the EDN gene (positions 64 and 132) have been extensively characterized with respect to their effect on RNAase activity and viral infectivity (Zhang & Rosenberg, 2002).

The enhanced RNase activity of EDN is due to having a Serine (Ser) at position 64 and an Arginine (Arg) at position 132 (Zhang & Rosenberg, 2002). Phylogenetic reconstruction of the history of amino acid evolution at these two sites revealed two high-probability scenarios. In one scenario, the first substitution was from Arg to Ser at site 64 (Arg64→Ser) and the second was from Thr to Arg at site 132 (Thr132→Arg). The alternative scenario is simply the reverse order: Thr132→Arg occurred first and Arg64→Ser occurred second. Both scenarios were characterized and, surprisingly, the first substitution always yielded *a reduction* in RNAase activity (scenario 1: 46% reduction; scenario 2: 21% reduction) compared to the ancestor. Given a mechanistic link between RNAase activity and antiviral activity, both incipient stages of EDN evolution presumably conferred lower fitness for its current function. Hence, neither could have been fixed by natural selection for antiviral activity. The accepted explanation is that the first substitution, regardless of the scenario, was fixed by neutral genetic drift. After such a chance fixation, natural selection acted to fix a change at a second site to confer the RNAase activity required for potent anti-viral function.

Since the novel anti-viral activity depends on the interaction of amino acids at two sites, "aptness" is determined by the joint effect of two amino acids (64Ser and 132Arg). We cannot know the true evolutionary history of these two sites, but whatever it was, the first of the two required changes is a preaptation for enhanced RNAase activity. Only the second could have been an adaptation thereafter. Thus this trait (64Ser and 132Arg within EDN) is exactly 50% preaptive and 50% adaptive, and consequently falls on the unique point in Fig. 2B that specifies those values and 0% exaptation.

6.3. The eukaryotic spliceosome - pervasive preaptation of complex interactions for maintenance of mRNA splicing

The eukaryotic spliceosome appears to have evolved from an ancestral form of self-splicing mRNA that contained group II introns (Cavalier-Smith, 1991). Unlike self-splicing mRNA, the spliceosome depends on a host of auxiliary proteins to initiate and carry out mRNA splicing. Its evolution is hard to reconcile with a simplistic adaptationist view, as there seems to be no immediate benefit to constructing such complex and costly cellular machinery (which must later be dissembled) merely to accomplish the same function as their group II ancestors. To be sure, complex spliceosomes facilitate the subsequent evolution of tissue-specific and alternative controls, but surely complexity did not first arise so that later it might be useful for such purposes (Vosseberg & Snel, 2017). It may be that complexity confers evolvability and thus that among surviving clades those with complex features are disproportionately represented. But such "future use" does not provide an origin story.

Sharp's original and largely supported "five easy pieces" scenario (Sharp, 1991), in which the five canonical snRNAs arose as fragments of originating intact group II introns has been broadly confirmed (Haack & Toor, 2020; Smathers & Robart, 2019), although the story is often told in an adaptationist fashion. But in his original exposition of CNE, Stoltzfus (1999) suggested that this neutral mechanism might have played a substantial role (see also Lukes et al., 2011; Vosseberg & Snel, 2017). Instead of comprehensive construction of spliceosomal complexity by natural selection (which would suffer from the problem of low fitness for incipient stages of evolution) they invoke a neutral process whereby chance interactions between mRNA and RNA-binding proteins pre-suppress otherwise stability-degrading mutations. These chance interactions effectively increase the space of the system for evolution by genetic drift. New mutations that were deleterious prior to the pre-suppressive interactions (and were selected against) can now become fixed by drift. Once evolution begins to explore such an expanded neutral space it will likely never return to the earlier state. Subsequent removal of an interaction between an mRNA and an RNA-binding protein would also remove suppression of the otherwise deleterious mutations that were fixed by drift. In such a system, complexity inevitably increases over time without any necessary change or enhancement of function.

Although pre-suppression opens-up neutral space for evolution of spliceosome complexity, it does not prevent purifying selection from maintaining the original function. Any negative effects on mRNA splicing will be strongly selected against. Thus, the "aptness" that we perceive in the immense complexity of the eukaryotic spliceosome arose by neutral evolution that successively increased the intensity of purifying selection to maintain the accumulated pre-suppressive molecular interactions. When any trait of the system (fixed genetic changes or increases in complexity) that did not arise by natural selection becomes subject to maintenance forces acting on the existing function (i.e., mRNA splicing) that trait becomes a preaptation. Thus, the spliceosome may be largely a preaptation for maintaining stable interactions with mRNA and carrying out splicing. However, it does not fall at the vertex for pure preaptation (Fig. 2B). The prior capacity of its proteins to bind RNA are exaptations, and it seems likely that at least some lineage specific fine-tuning was accomplished by secondary adaptations. The important conclusion is that natural selection did not design the entire spliceosome (or necessarily even most of its components) for its current function.

7. Conclusion

Against the backdrop of ENCODE's sense of 'functional DNA element', and the diversity of weakened or ambiguous senses of 'function' available throughout biochemistry and biomedicine, there is a widely-held belief that the SE account of function is too strong, limiting, or "overly restrictive" (Brzovíc & Sustar, 2020), perhaps to the point of being inappropriate. Nonetheless, weakening the SE notion of function specifically in service of genomics and biomedical practice opens a pandora's box of clashing intuitions about the meaning of functional designations and the importance of evolutionary history in grounding claims throughout biology. We argue that equating 'biological functions' with 'traits having fitness effects' flattens our ontology of aptations, blending "functions," "effects," adaptations, exaptations and preaptations together, and likely including traits that are overall deleterious. This comes both at the cost of the by-now proven utility of these distinctions and Darwinian commitments that make biology make sense.

Teasing out explanations of origin is not an easy task and may even at times be fundamentally epistemically limited by the availability of historical evidence. Nonetheless, as the examples detailed above indicate, evolution is capable of rich diversity in its genesis of complexity and this, we argue, should not be overlooked

in service of weaker or more permissive terminology for bioscience. These examples also reveal some of the perils of different accounts of function—confusions that we hope to set straight by appreciating and appropriately representing the mixed history of complex traits.

ENCODE's initial characterization of "functional elements", basically a CR account, lumped all historical diversity within the simplex (Fig. 2) together with other knock-on effects, which may not even be subject to purifying selection. But accounts of function, like WEM, that equate it with fitness contribution or being subject to purifying selection also lump together diverse evolutionary kinds. A sufficiently strong etiological account of function should distinguish the diversity represented within the historical simplex. To do otherwise constrains the way we think and talk about the evolution of function, thereby limiting the hypotheses we can examine and, as was made clear by the public reception of ENCODE's claims, also thereby lowering our epistemic standards for rejecting them.

Author statement

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