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Lamarckian or not, CRISPR-Cas is an elaborate engine of directed evolution

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I appreciate the interest of many biologists and philosophers of biology in my discussion of the conceptual and philosophical aspects of CRISPR-Cas (Koonin 2018) and their insightful comments. Perhaps, not surprisingly, the primary topic that is addressed in 4 of the 6 commentaries is my proposition that the evolutionary changes mediated by the CRISPR-Cas systems are of the Lamarckian character. It is interesting that two of these commentaries (Wideman et al. 2018; Woolley et al. 2018) object (strenuously, in the latter case) to the characterization of the CRISPR-Cas systems as engines of Lamarckian evolution, whereas the other two (Jablonka 2018; Veigl 2018) largely concur with my view and extend it. Perhaps, this also should have been anticipated because Lamarckian evolution is a perpetually controversial subject, and as aptly noticed by Jablonka, many biologists have a "knee-jerk" reaction to the very name "Lamarck". In this brief response, I first address the two "anti-Lamarckian" commentaries, then, even more briefly, turn to the two "pro-Lamarckian" ones, and finally, discuss the remaining two comments that deal with other aspects of my paper.

The central point of Wideman and colleagues is that CRISPR-Cas is a mechanism of directed mutation not a mechanism of Inheritance of Acquired (adaptive) Characters (IAC). First, I should note that reading the comments of Wideman and colleagues (as well as parts of the other Commentaries), one might be left with the impression that, in my own discussion, I did not include the requirement of adaptive nature of the inherited characters among the criteria of Lamarckian evolution. However, I certainly did as it should be clear from the following quote: "The IAC mechanism, as distilled in the spirit of Lamarck albeit in modern terms, has two essential aspects: (1) specific, heritable changes in the genome caused by an external factor, (2) specific phenotypic effect of those changes that constitutes adaptation to the causative factor." (Koonin 2018) I used the acronym IAC rather than IAAC solely because of the awkwardness of the latter. In any case, Wideman and

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colleagues submit: "Reinterpreting Lamarck in terms of modern molecular genetics is a questionable endeavor, but surely any suitable reinterpretation must require that "Lamarckian" characteristics (i.e. IAACs) are initially acquired as environmentally-adaptive phenotypes (not genotypes), which come under further selection in subsequent generations."(Wideman et al. 2018) In other words, they believe that only mechanisms that involve assimilation of phenotypic changes into the genotype (that is, the transition from epigenetics to genetics) could possibly be classified as "Lamarckian". I respectfully disagree and think that the fulfilment of the above two criteria is sufficient to justify the classification of an evolutionary process as (quasi) Lamarckian. Wideman and colleagues then indicate that CRISPR-Cas systems mediate directed mutation and thus challenge a key tenet of (neo)Darwinism, namely, that mutation is effectively random (or, at least, mutational biases that might exist have no substantial effect on the outcome of evolution) whereas all non-randomness is introduced into the evolutionary process by the "creative" force of natural selection. I have no objection whatsoever against the characterization of the CRISPR-Cas mechanism as directed mutation. Actually, this is exactly what we point out with Yuri Wolf in our 2009 paper (Koonin and Wolf 2009) on the subject (see Fig. 1 in that paper). Wideman and colleagues, then, place the CRISPR-Cas mechanism into the context of the mutationist vs selectionist debate, where "mutationists" propound non-random mutation as a formative factor of evolution, perhaps, as important as selection if not more (Nei 2013). Again, I have no problem with this perspective at all. What I would like to emphasize, though, is that, in my opinion, the "Lamarckian" view of CRISPR-Cas as an IA(A)C mechanisms and the interpretation in terms of directed mutation are compatible and complementary rather than contradictory.

Woolley and colleagues argue that there is nothing Lamarckian in the CRISPR-Cas mechanism which, to them, is "just" a special case of uptake of exogenous DNA. Actually, I find this characterization to be fair enough but there are two important qualifications to be made. First, not only CRISPR-Cas but also some other mechanisms of exogenous DNA capture, for example, acquisition of antibiotic resistance genes, might qualify as quasi-Lamarckian as I have discussed in some detail in previous publications (Koonin and Wolf 2009; Koonin 2011). Second, it is important to realize just how special CRISPR-Cas actually is. Indeed, this system represents an elaborate, highly evolved engine for genome evolution which direct the incorporation of foreign DNA into s strictly defined sites in the host genome where it can be subsequently utilized for the host's benefit. I would like to emphasize the nature of CRISPR-Cas as a mechanism for a specific form of genome evolution.

Woolley and colleagues introduce a simple classification of potential "Lamarckian" phenomena, from the weakest to the strongest form of "Lamarckism":

L1, any inheritance of acquired characters (horizontal gene transfer is a good case in point)
L2, inheritance of acquired characters that *turn out* to be adaptive
L3, specific inheritance of acquired characters *because* they are adaptive.

In their view, the CRISPR-Cas mechanism fits under L2 but, to them, calling this Lamarckian evolution in unjustified and only leads to confusion. I think the

classification introduced by Woolley and colleagues is sensible and potentially useful. I also agree that L2 hardly qualifies as a Lamarckian process. I would argue, however, that the CRISPR mechanism falls in between L2 and L3, and the classification into one or the other category depends on the efficacy of the discrimination between the parasite (non-self) and host (self) DNA at the adaptation stage: strong discrimination points to L3, weak discrimination to L2. As discussed in this (Koonin 2018) and the previous article (Koonin and Wolf 2016), the level of discrimination seems to differ considerably among different variants of CRISPR-Cas systems, and accordingly, the CRISPR-Cas mechanisms may be considered to span the range from "routine" neo-Darwinian to Lamarckian evolutionary regimes.

Woolley and colleagues, then, express their opinion on a more general subject, namely, that, regardless of the intricacies of the mechanisms involved, the characterization of CRISPR-Cas as a Lamarckian mechanism does no "conceptual work". Predictably, I disagree. I believe that the conceptual advantage of the Lamarckian label is that it emphasizes the special status of the CRISPR-Cas systems as engines of directed evolution. Woolley and colleagues are concerned about the perceived teleological character associated with Lamarckian evolution. I think that it is best not to shun certain terms and the underlying concepts simply because of undesirable connotations (in the case of teleology, it is the specter of intentionality that, certainly, is alien to materialistic science, in general, and evolutionary biology, in particular) but rather, to look into the substance of the matter. Beyond doubt, there is neither intentionality nor an "innate strive for perfection" (as Lamarck believed with respect to evolutionary processes) in the CRISPR-Cas mechanism but I find nothing wrong with the notion that a degree of teleology is injected into the system in the sense that CRISPR-Cas systems mediate an evolutionary process that has a distinct purpose, in the context of survival of a microbial population.

Along a different line of reasoning, Woolley and colleagues fault my article over a perceived confusion between the Lamarckian character of the CRISPR mechanism and Lamarckian evolution of CRISPR-Cas systems themselves. I plead not guilty. The idea that CRISPR-Cas systems could evolve via some kind of Lamarckian mechanism makes no sense, and I do not see how anything like this could be gleaned from my article. Moreover, I strongly emphasize the role of chance in the origin and evolution of the CRISPR-Cas systems, embodied in the contribution of random insertion of mobile genetic elements, both the casposons and the ancestors of Class 2 effectors.

All of the above is legitimate, at times, I think, important conceptual debate that I welcome. But then, at the end of their Commentary, Woolley and colleagues introduce an idea from which I wish to distance myself. They submit that a true "Lamarckian" route of evolution mediated by CRISPR would involve goal-oriented modification of the human germline. I am afraid I find this suggestion to be both disingenuous and bordering on irresponsible. Disingenuous, because such artificial genetic manipulation would not equal to a true evolutionary process. Irresponsible, for obvious reasons: at this stage, at least, with the safety of the CRISPR-mediated genome modification being far from fully established, any discussion of the implications of such procedures is premature, even apart from the host of more fundamental ethical concerns (Caplan et al. 2015).

Turning now to the two "pro-Lamarckian" commentaries, I have, probably, predictably, much less to discuss. Veigl raises a genuinely interesting point, by drawing a connection between CRISPR-Cas and Lamarck's use/disuse paradigm through the recently discovered phenomenon of interference-driven spacer acquisition (IDSA) (Staals et al. 2016). In the case of IDSA, acquisition of spacers by subtype I-F CRISPR-Cas is enhanced by interference, resulting in reinforcement of protection against frequently encountered foreign DNA through additional spacers. I agree that this is a notable Lamarckian connection that has escaped my own attention.

I would like to conclude this discussion of the Lamarckian theme by referring to the Commentary of Jablonka who emphasizes that CRISPR-Cas is a highly evolved molecular engine that promotes evolvability. I completely agree that this is a crucial, arguably, the most important biological feature of CRISPR-Cas and other (quasi) Lamarckian systems, for example, mechanisms of DNA uptake that facilitate horizontal gene transfer and those repair mechanisms that cause stress-induced mutagenesis. Actually, emphasizing the status of CRISPR-Cas as a dedicated engine for directed genome evolution was the primary incentive for introducing the Lamarckian connection in the first place. As originally emphasized, there was no intent to closely follow Lamarck's actual reasoning (Koonin and Wolf 2009). Thus, in the historical perspective, perhaps, connecting CRISPR-Cas to Lamarck is not entirely accurate. I nevertheless think that "Lamarckian" is a good label for processes of directed evolution, and there is nothing wrong in honoring the great early evolutionary biologist, even if much of his thinking inevitably got outdated.

I now briefly turn to the two Commentaries on subjects distinct from Lamarckian evolution. The key point of Baxter is that the CRISPR-Cas technology combines the naturally evolved mechanisms for genome editing with innovations introduced through human ingenuity and that, this being the case, the distinction between fundamental and applied research in the CRISPR field 9 and, conceivably, in many other fields as well is not particularly sharp. I completely agree, the natural and the engineered blend in the CRISPR research. I think an excellent case in point is "dead" Cas9 (dCas9), that is, Cas9 protein in which the nuclease domains have been artificially inactivated and that is employed for gene expression regulation rather than genome editing (Xu and Qi 2018). To the best of my knowledge, no dCas9 has ever been discovered in bacteria: as judged by the conservation of the catalytic sites, all Cas9 variants are active nucleases. However, "dead" CRISPR-Cas effectors do seem to exist, namely, the V-U5 variant in which the catalytic site of a type V effector appears to be disrupted (Shmakov et al. 2017). Nevertheless, the V-U5 systems are broadly conserved among Cyanobacteria and, in all likelihood, are functional. The nature of this function remains to be elucidated and could well involve gene regulation. So once again, human inventiveness recapitulates biological evolution.

Finally, Pradeu and Moreau emphasize in their insightful Commentary that the functionality of CRISPR-Cas systems is not limited to self vs non-self discrimination or to defense, in general. More specifically, they note that self-targeting by CRISPR-Cas systems might be not only deleterious but also functionally relevant in various contexts and that non-defense functions, such as participation in repair processes, quorum regulation and more. I quite agree. A recent comprehensive comparative-genomic analysis of CRISPR-cas loci reveals multiple connections of CRISPR-Cs systems to signal transduction networks some of which, at least, do not appear to be directly involved

in defense (Shmakov et al. 2018). These non-defense functions and their evolution are discussed in detail in a forthcoming article (Faure et al. 2018). Furthermore, there seems to be a distinct possibility that the CRISPR effector modules originate from a distinct signal transduction system that might be involved in stress-induced cell dormancy induction in bacteria (Koonin and Makarova 2018). Nevertheless, and all this functional diversity of the CRISPR-Cas systems notwithstanding, I wish to stress that the immune function, with varying degrees of self vs non-self discrimination, is by far the most common, dominant CRISPR-Cas functionality from which all the others were derived.

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