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POINT OF VIEW

Use/disuse paradigms are ubiquitous concepts in characterizing the process of inheritance

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ABSTRACT

In recent years, a Lamarckian theme has found its way back into academic discourse on evolution and inheritance. Especially the emerging field of transgenerational small RNAs has provided at least a proof of concept for the inheritance of acquired traits. Yet it remains unclear whether the Lamarckian concept of inheritance will in fact have its rennaisance or whether it will remain the rallying cry for the outlaws, heretics and *enfants terribles* of molecular biology. As unclear as the future of Lamarckian theory is its content and reference. Since the formulation of the *Philosophie Zoologique*, Lamarckian thought has been de- and reconfiguring in and out of the scientific literature and become an umbrella-term for all kinds of unconventional modes of inheritance. This essay will argue that heritable small RNAs might in fact provide a case of genuine Lamarckian inheritance. Moreover, it will be claimed that not only the very broad concept of "inheritance of acquired traits" applies, but also that Lamarck's mechanistic insight into a use/disuse relation might help to explain a specific mode of transgenerational inheritance.

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Introduction

The study of the processes governing inheritance is one of the most fundamental interests in biology. In the middle of the 20th century this preoccupation contributed substantially to the formulation of the "Modern Synthesis" of evolution.[19] Because of its explanatory potential, the *Synthesis* still dominates research paradigms in various branches of biology. Probably a late child of the unity of science movement of Otto Neurath and logical positivist Rudolf Carnap and the *Encylopedia of Unified Science*, it aimed at unifying Darwinian evolutionary theory, Mendelian inheritance, as well as population genetics and paleontology, among other fields. In the light of this context, reaching unity and ruling out instances of Lamarckian inheritance might also have had its political agenda, especially due to the rise of Lysenkoism in Russia and past hurdles much owed to the controversial midwife toads and salamanders of Austrian "toad-whisperer" Paul Kammerer.[20] Despite the paradigm-like status of the *Synthesis*, case studies reporting the inheritance of acquired traits have regained prominence through the field of epigenetics which studies phenotypes that are not mirrored by DNA sequences and their inheritance, respectively. Especially the discovery of heritable small RNAs helped Jean-Baptiste Lamarck's name return to cutting-edge discussions on the trajectories of inheritance.[12,24,25,32] Various scholars have chosen to call this type of inheritance "Lamarckian," "quasi-Lamarckian," or "soft inheritance".[2,6,21,27,31] Despite the polemics surrounding these claims, a thorough assessment of the contents of Lamarckian theory and their correspondence with cutting-edge phenomena is yet missing. However, it has become necessary to assess whether Lamarck's concepts actually describe some unorthodox instances of inheritance. In the following, Lamarckian theory of inheritance will be briefly characterized, and an overview of the mechanisms of small RNA heritability will be provided. This will establish the base for developing the claim that Lamarck's concept of "use" and "disuse" adequately applies to the molecular process of small RNA based inheritance of acquired traits.

Operationalizing Lamarck's theory

As most labels associated with Lamarck's concepts remain vague, this paper will abstain from such terminology. Instead, the term "Lamarckian" will be used exclusively for denoting a correspondence of current scientific results with Lamarck's theory of inheritance, formulated in the *Zoologie Philosophique*. Lamarck's account will be understood as proposing a mechanism of gradual change in response to environmental stimuli, conforming to an inheritance of acquired characteristics that includes three levels of explanation[9]:

(1) the inheritance of acquired traits as a very broad and general concept;
(2) the "use" and "disuse" of "organs" leading to their reduction or augmentation as a mechanistic explanation; and
(3) the "fluides incontennables", i.e. non-discrete, subtle fluids as the mediators of the reduction or augmentation of organs – a concept which clearly stands in opposition to the discreteness of Mendelian agents of inheritance.

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Having established such an operational notion of Lamarckian theory, it shall now be assessed whether the inheritance of small RNAs fulfills one or more of these criteria.

Heritable small RNAs

Possible modes and mechanisms of the inheritance of acquired traits as mediated through small RNAs have been extensively reviewed elsewhere.[4,15,18,24] This article will accept it as a fact that information from the exterior can become manifest as a small RNA based response in the germline, which can get transmitted for at least three to five generations in the nematode *C. Elegans*. However, under special conditions this process can be significantly prolonged or maintained *ad indefinitum*.[10,16,22,32] Below, a mechanism based on experimental data will be sketched out to highlight analogies with Lamarckian theory.

The phenomenon of RNAi was discovered by administration of exogenous doublestranded RNA (dsRNA) to *C. Elegans*, hence the response to such stimuli is called exogenous RNAi and is thought to be part of the worm's natural immune response against viruses.[12,33] However, *C. elegans* encodes various species of small RNAs, among them miRNAs, 21U RNAs (the piRNAs equivalents) and endogenous small RNAs (endo-siRNAs).[35] Small RNAs use their own mechanisms of amplification, mediated by RNA dependent RNA Polymerases (RdRPs) which produce "secondary" small RNAs that are characterized by their length of 22 nucleotides and a strong bias for a 5' Guanosine (22G RNAs).[8,13] In a process called nuclear RNAi, endo-siRNAs bound by argonaute proteins can target complementary nascent transcripts, inhibit Polymerase 2 elongation, and lead to the deposition of histone marks culminating in silencing of the respective gene.[4,15] However, it is important to note that endogenous and exogenous RNAi pathways converge upon shared effector proteins such as argonautes and RdRPs, and the administration of exogenous dsRNA can lead to the synthesis of 22G RNAs as well as silencing of endogenous genes as well as transgenes.[4]

In several important studies it has been shown that small RNA mediated gene silencing can be stably inherited for at least three to five generations, most likely due to transmission of various small RNA species to the next generation, including several rounds of amplification.[1,26] This process was shown to be dependent on various effector proteins, among them argonautes, RdRPs, and chromatin modification factors. The sum of these effectors is often called the "RNAi inheritance machinery".[5,7] Yet it remains a tantalizing question to answer why heritable gene silencing – which could in theory be maintained *ad infinitum* by RdRP-dependent rounds of amplification – fades out over time, a problem referred to in the literature as "the bottleneck to RNAi inheritance". Thus it needs to be asked whether RNAi inheritance is a regulated process or an epiphenomenon?

Recently it was shown that the worm indeed actively regulates the duration of this transgenerational epigenetic inheritance. Administration of a "second trigger" of dsRNA in the worm's F1 generation, after exposure to a "first trigger" in the P0, helped to prolong heritable silencing significantly. Importantly, the "second trigger" can be unrelated to the "first trigger", suggesting a mechanism that goes beyond abundances of a specific species of small RNAs.[16] The authors offered an explanation based on a competition model of small RNA species for their respective effector molecules. In the light of this model, two scenarios become imaginable: (1) Without a challenge through exterior stimuli, the endogenous siRNAs occupy the components of the RNAi inheritance machinery and limit their own inheritance by targeting mRNAs of effector proteins of this very machinery. (2) However, when the animal is confronted with an exterior stimulus, manifesting as a dsRNA trigger which in turn activates exogenous small RNAi pathways, occupancy rates of the RNAi inheritance components shifts. A certain amount of effector proteins is now occupied by exo-siRNAs and their derivatives, and the regulation of RNAi inheritance genes by endo-siRNAs is disrupted. Also, less secondary endo-siRNAs are produced, as the relevant effector proteins are also occupied by exo-siRNAs. It is believed that it takes the endo-siRNAs between three to five generations to restore the original equilibrium state, as they are constantly transcribed from the genome and outnumber the exo-siRNAs over time without a further stimulus. This explains the so-called "bottleneck" of heritable gene silencing.[18,17]

It is thought that after the administration of the "second trigger" the inheritance of exo-RNAs gets "boosted" by further rounds of RdRP-dependent amplification of heritable small RNAs deriving from the parental exo-RNAi response at the expense of endo-siRNAs. This hypothesis is supported by small RNA sequencing results after insertion of the respective triggers.[16,22] Thus there exists a critical period in the worm, where exo-RNAi triggers can antagonize the reprogramming of the RNAi inheritance machinery. This further suggests that also other environmental stimuli, such as changes in temperature, diet, or other forms of stress could add to ancesteral RNAi responses, since they were shown to alter the pool of heritable endogenous small RNAs.

In summary, the inheritance of an acquired gene silencing state through exo-siRNAs is limited by competition with endo-siRNAs for shared effector molecules and can be extended by further disturbing endo-siRNA maintenance equilibria in the F1 generation.

Use/disuse paradigms and the quantitative character of small rna inheritance

The competition model discussed in the above paragraphs suggests that changes in the inheritance patterns and duration of small RNAs can only be explained by looking at changes in *pools* of different small RNA species. The same observation is true for the occupancy of the *pools* of RNAi inheritance effector proteins. Thus we have arrived at an explanatory model of small RNA inheritance that refrains from qualitative explanations but uses quantitative ones. The nature of this quantitative character lies in the fact that small RNAs are not in a strict one-to-one relation with their targets, but instead in a fluid one-to-many relation, i.e., different small RNAs can span different regions of a transcript, what is required for homeostatic fine-tuning and flexible responsiveness to environmental stimuli.

At this point Lamarckian theory enters the stage, and the 3 levels of its depth described above shall be scrutinized for their

adequacy. The first level was characterized as the general claim that an inheritance of acquired traits occurs. For the described mechanism, this is true (at least – under normal conditions – for a few generations). However, such a proposition is only intellectually satisfying if it is accompanied by the designation of an effector mechanism. Here the second level of explanation comes into play, stating that "use" and/or "disuse" of "organs" lead to their respective reduction or augmentation. As a consequence, the question needs to be answered, whether these Lamarckian terms do in fact refer to, and provide an adequate description of, the modes of heritable small RNAs.

If "use" and "disuse" of an organ is regarded as the association of different small RNA species with the RNAi inheritance machinery, an explanation for how "use" and "disuse" can lead to "augmentation" or "reduction" in the next generation is at hand (Fig. 1): "Use" of a certain pool of endo-siRNAs leads to the silencing of the respective target gene and establishes a self-amplificatory loop which mirrors the state of the small RNA pool in subsequent generations. "Disuse" of a certain pool of endo-siRNAs would result for example from an overoccupancy of RNAi inheritance machinery components with exo-siRNAs. In this case, the concentration of endo-siRNAs decreases since they cannot maintain their own self-amplification cycle, and, thus, also less endo-siRNAs are delivered to the next generation. Meanwhile, "use" of exo-siRNAs by the RNAi machinery components leads to an augmentation of exo-siRNA mediated effects and more rounds of amplifications. This use/disuse hypothesis could also account for observed "eri" (enhanced RNA interference) phenotypes[36]: Defects in certain RNAi pathway effectors lead to a hypersensitivity to exterior stimuli in what can be seen as a shift in the use/disuse states of certain pools of small RNAs.

It is necessary to be aware of the fact that the use/disue paradigm is not only a laboratory artifact produced by foreign transgenes and administration of artifical dsRNA triggers. Importantly, it was shown that dsRNAs naturally occur in *C. Elegans* as starvation signals, that affect the constitution of small RNA pools.[14,34] Furthermore, it was suggested that virus derived dsRNA (the naturally occurring exo-siRNA) leads to upregulation of endo-siRNA targets, which is explained by competition between exogenous and endogenous RNAi pathways for shared effector molecules and leads to expression changes in response to viral infection.[28] Heritable effects of these naturally occurring inbalances might in fact be mirrored in the artificial conditions of the laboratory setup. In summary, the "use" and "disuse" equilibrium of a certain pool of small RNAs can result in the augmentation or reduction of a trait (the respective endo/exo RNAi equilibrium state). This specific trait would be "acquired" since it is dependent on the intrusion of exo-siRNAs or the absence of further exterior triggers (Fig. 1). Therefore a mechanism that is proposed by the Lamarckian theory of inheritance might actually be resonant with the processes governing small RNA inheritance. To expand on this claim, two other small RNA dependent systems

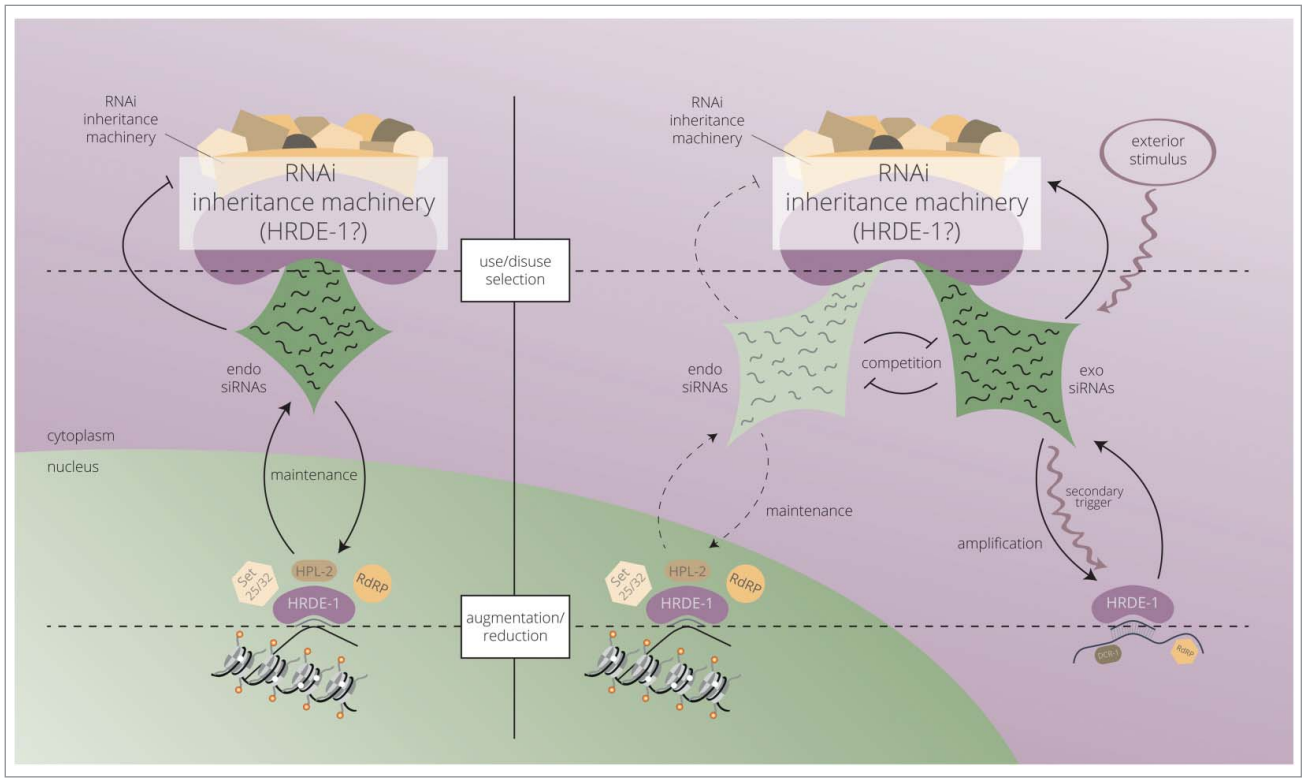

Figure 1. Use and Disuse of different small RNA species leads to their respective augmentation or reduction. If the organism is unchallenged by the exterior, endo-siRNAs maintain a steady-state between rounds of nuclear RNAi and self-amplification in the nucleus and the duration of RNAi inheritance (left panel). If the organism is challenged by an artificial dsRNA trigger or an environmental condition that translates into an RNA state (e.g., a virus) endo-siRNAs have to compete with exo-siRNAs for components of the RNAi inheritance machinery. If they can use less of their effector proteins, they cannot maintain their impact on the duration of RNAi inheritance. Through binding to effector proteins, intruding exo-siRNAs can themselves use a self-amplificatory loop, that can get boosted through further triggers in the subsequent generation (right panel). Thus, through use or disuse of endo- or exo-siRNA species by their effector proteins leads to their respective augmentation through self-amplificatory loops or reduction, if those loops cannot be maintained.

will be introduced, where the "use"-"disuse" dichotomy might also apply.

The RNAa (licensing)/RNAe (silencing) model claims that the association of 22G RNAs with different species of nuclear argonautes in the germline helps cells decide which genes to silence and which genes to express.[25] HRDE-1 associated small RNAs complementary to certain genomic regions can cotranscriptionally silence the coding genes, which is also correlated with the deposition of repressive histone marks.[29] This process is thought to be antagonized by CSR-1 bound 22G RNAs complementary to actively transcribed genes.[3,5,23,29] However, direct effects are yet to be shown. Thus the licensing capacities of CSR-1 are currently under debate. Yet another theory suggests that uridylation of CSR-1 bound small RNAs leads to their destruction, keeping them from becoming associated with the silencing HRDE-1 argonaute.[30] Importantly, both CSR-1 and HRDE-1 have been associated with transport of small RNAs into the next generation.[3,5,23,29] In a scenario where CSR-1 and HRDE-1 compete for the 22Gs loaded into them, CSR-1 bound 22G RNAs would execute a "use" signal by preventing the deposition of repressive histone marks on their target gene and thus promote increased expression (= augmentation). On the other hand, HRDE-1 bound 22G RNAs would execute a "disuse" signal by promoting heritable silencing of a complementary region, thus reducing expression (= reduction).

To give a last example, it has been proposed that stress induced tRNA fragments could interfere with RISC pathways components. Especially, it has been hypothesized that such tRNA fragments could compete with endo-siRNAs for binding their respective argonautes and dicers.[11] In this case an exterior stimulus would again lead to a shift in small RNA pools by interfering with their biosynthesis and effector mechanisms. Similar phenomena have also been associated with stravation induced long non coding RNAs.[14]

Conclusions

Above, several mechanisms were proposed that could be governed by a use/disuse interaction and lead to augmentation or reduction of traits in future generations as a response to shifting environmental conditions. It needs to be stressed that all aforementioned mechanisms do interact with each other as they all converge upon effects on the equilibrium states of small RNA pools. Thus the predicate "Lamarckian" is not only a very broad term to coin odd instances of inheritance, but it can be narrowed down to a certain mode of inheritance.

For now, it only remains to be shown what to do with the third level of Lamarckian inheritance that was proposed in the beginning. In principle, it is clear that there is no point in claiming that "fluides incontennables" do exist. However, granting Lamarck interpretative charity, one might suggest an alternative for handling this third level: Lamarck's "fluides" do not refer to actual molecules but may be taken to point to the "fluid" character of the epigenetic state of a cell as opposed to the discreteness of its genetic state. This suggests that it might be time to pay greater attention to quantitative explanations, since there might exist various instances, not only in inheritance, that cannot be fully accounted for in a qualitative manner. Preoccupation with mechanistic and qualitative explanations might keep us from discovering processes that are guided by quantitative properties, such as the use/disuse paradigm. The nature of an explanation of inheritance intrinsically corresponds to the nature of its effector molecules. Hence, it should only be natural that different explanatory frameworks are required for different modes of inheritance. The primacy of DNA in all biochemical processes within one generation must not be confused with a much more doubtable primacy in all processes of inheritance. Admitting to different modes of hereditary transmission and different types of explanations might enhance our understanding of the variety of processes governing inheritance and – in the long run – also evolution.

Declarations

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