# Defending the Importance of Lineage-Forming Reproduction in Evolution by Natural Selection

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## Abstract

Charbonneau (2014) and Papale (2021) challenge the necessity of reproduction for evolution by natural selection (ENS) by contending that what really matter for ENS are memory and (re)generation at the population level, rather than lineage-forming reproduction at the local level. In this article, we critically evaluate their reproduction-independent accounts of ENS and defend the importance of lineage-forming reproduction in paradigmatic ENS on both empirical and theoretical grounds. We argue that none of the empirical cases they cite can be used as evidence to demonstrate the existence of paradigmatic ENS without lineage-forming reproduction, and adopting their reproduction-independent views would lead to an account of ENS that is both too liberal and too strict.

**Keywords:** Reproduction; lineage; evolution by natural selection; memory; ecological inheritance; holobionts

## 1. Introduction

The conditions for evolution by natural selection (ENS) are one of the most central and controversial topics among evolutionary biologists and philosophers of biology (Darwin 1859; Lewontin 1970, 1985; Brandon 1990; Ridley 2004; Okasha 2006; Godfrey-Smith 2009; Bouchard 2011; Bourrat 2014; Charbonneau 2014; Papale 2021) . Richard Lewontin (1970, 1985) presents a very influential framework for analyzing ENS, which takes variation, heredity, and differential fitness as necessary and sufficient conditions for a population to undergo ENS.

Although the sufficiency of the above three conditions to produce ENS has been convincingly challenged (Brandon 1990, pp.7-9; Godfrey-Smith 2009, pp.24-26) , they are generally accepted as necessary conditions for ENS to occur. The notion of reproduction, while not explicitly mentioned in Lewontin's three conditions, plays an indispensable role in his account of ENS. For instance, heredity is understood in terms of the resemblance between parents and their offspring, which implies the presence of reproduction; fitness of different variants is compared based on the number of offspring they produce, either actual or expected.

The importance of reproduction in ENS has also been emphasized by many other biologists and philosophers of biology (Dawkins 1976; Hull 1980; Griesemer 2001; Ridley 2004; Godfrey-Smith 2009; Skillings 2016). For example, Mark Ridley (2004), in his textbook *Evolution*, explicitly includes reproduction as a necessary condition for ENS in addition to the three proposed by Lewontin. According to Peter Godfrey-Smith (2009, p.69), reproduction is "at the center of Darwinism," because understanding heritability and fitness requires us to know the parent-offspring relationship between individuals. Richard Dawkins (1976) and David Hull (1980) develop a different framework for analyzing the Darwinian process than Lewontin's, in which ENS involves the interplay of both replicators and interactors. According to Hull (1980, p.318), a replicator is "an entity that passes on its structure directly in replication.” As replication is a special case of reproduction, the concept of reproduction is also essential in the replicator-interactor framework of ENS proposed by Dawkins and Hull.

Although reproduction has traditionally been considered central to the occurrence of ENS, recent scholarship has challenged this assumption from various perspectives (Bouchard 2011; Bourrat 2014, 2015; Charbonneau 2014; Papale 2021; Inkpen and Doolittle 2022). For example, Frédéric Bouchard (2011) argues that many biological systems such as clonal species and multispecies communities are ensembles that can undergo ENS through differential persistence of their parts. Therefore, what is essential for ENS is not the differential reproduction of individuals in a population, but the differential persistence of ensemble parts. Accordingly, the concept of fitness should be defined in terms of the differential persistence of lineages instead of differential reproduction. Pierrick Bourrat (2014) develops a diachronic account of ENS and argues that reproduction and heredity are predictable products, rather than necessary conditions of ENS. He builds a set of individual-based models and demonstrates that paradigmatic ENS, which is defined as ENS that can produce complex cumulative adaptations, is likely to originate from weak ENS that only allows for differential persistence of non-reproducing entities. Mathieu Charbonneau (2014) and François Papale (2021) contend that ENS is a population-level phenomenon, so what really matters for ENS is not reproduction with local-level lineage formation (i.e., reproduction that occurs at the individual level and forms parent-offspring relationships between individuals), but its population-level effects known as memory and (re)generation. Since memory and (re)generation can be realized through mechanisms not involving lineage formation, it is not necessary to consider lineage-forming reproduction as a requirement for ENS.

While all these challenges are intriguing and merit further investigation, in an important sense Charbonneau's and Papale's approaches represent a more radical departure from traditional accounts of ENS than most other recent attempts to challenge the necessity of reproduction for ENS. Scholars who adopt standard approaches to ENS generally do not deny that a population of non-reproducing entities can undergo so-called weak (or marginal) ENS simply through differential persistence (Okasha 2006; Godfrey-Smith 2009). Therefore, the real issue at stake is not whether reproduction is necessary for ENS *in general*, but whether it is necessary for *paradigmatic* ENS, which can produce complex cumulative adaptations such as human eyes. Although Bourrat (2014) argues that reproduction is not necessary for ENS in general, he concedes that some form of reproduction is needed for paradigmatic ENS. Without reproduction, a population cannot maintain its size and will go extinct before complex adaptations can occur. Similarly, Bouchard's (2011) persistence-based account of ENS can explain changes in the distribution of variation in a population, but it needs to include a *reproduction-like* process to explain the origins of novel traits and adaptations (Papale 2021, pp.10435-10436). In contrast, Charbonneau's (2014) and Papale's (2021) accounts claim that even paradigmatic ENS does not require the existence of lineage-forming reproduction. Therefore, their accounts of ENS deserve special attention and require separate treatment.

In this article, we will provide a critical evaluation of Charbonneau's and Papale's reproduction-independent accounts of ENS and defend the importance of lineage-forming reproduction in paradigmatic ENS. While it has been argued that ENS can take place in both biological and cultural systems (Darwin 1981; Richerson and Boyd 2005; Mesoudi 2011), this article focuses solely on ENS in the biological domain. There are two reasons why we would like to make this qualification. Firstly, the framework of evolution by natural selection was initially applied in the biological domain, and subsequently extended to the domain of cultural change. It is therefore possible that the forms of ENS applied in these two domains may exhibit subtle differences due to the different objects being considered. Secondly, and more pragmatically, the alleged examples used by Charbonneau (2014) and Papale (2021) to challenge the necessity of lineage-forming reproduction for ENS are all biological cases. Consequently, the conclusions of our reexamination of these cases are more directly applicable to ENS in the biological domain. We leave it open whether lineage-forming reproduction is a prerequisite for the occurrence of paradigmatic ENS in cultural evolution. But even if it were, this would not automatically discredit our conclusion that reproduction is needed for paradigmatic ENS in the biological domain.

The remainder of the article is organized as follows. Section 2 briefly reviews Charbonneau's and Papale's accounts of ENS without lineage-forming reproduction. Section 3 examines various empirical cases, including cases of prions, holobionts, and ecological inheritance, that have been used by Charbonneau and Papale to challenge the necessity of reproduction for ENS. We argue that none of them can be used as evidence for the existence of paradigmatic ENS without lineage-forming reproduction. In Section 4, we explore the theoretical consequences of adopting Charbonneau's and Papale's reproduction-independent accounts of ENS, arguing that following their approaches would lead to an account of ENS that is both too liberal and too strict. Section 5 concludes by reemphasizing the importance of lineage-forming reproduction in creating evolutionary novelties and complex adaptations through the process of paradigmatic ENS.

## 2. Charbonneau's and Papale's Accounts of ENS without Lineage-Forming Reproduction

This section provides a brief review of Charbonneau's and Papale's accounts of ENS without lineage-forming reproduction.

According to Charbonneau, the notion of reproduction can be divided into two subprocesses: multiplication and inheritance. Multiplication is defined as a process in which "one or many entities or processes produce or participate in the production of a new entity of the relevant kind" (Charbonneau 2014, p.729). Multiplication is a broader concept than reproduction. However, when multiplication is realized within the context of reproduction, the producer and its product are of the same relevant kind, and the producer is the parent of its product, which establishes a *multiplication* (or *productive*) *lineage* between them. However, in Charbonneau's view, if we consider the process of multiplication per se, we will find that it does not require producers and their products to be of the same kind, nor does it imply that producers must be parents of their products. For example, ribosomes and mRNA participate in the multiplication of proteins through the process of translation, but they are not of the same kind of entities as the proteins they produce. Nor do they form parent-offspring lineages with those proteins.

Charbonneau claims that multiplication, which is a local-level process, can have two population-level effects and thus play two roles in evolution: First, it can renew parts of a population, thus preventing the population from fading away as its parts are destroyed and lost. Second, it can serve as a generation marker that distinguishes older and younger generations. These two population-level effects/roles realized by multiplication are called "generation" by Charbonneau.

As for inheritance, Charbonneau adopts the multisystemic view and accepts the existence of inheritance mechanisms that do not rely on genetic transfer. According to this view, inheritance can be understood as any process through which "an entity (a 'parent') affects another entity (its 'offspring') in such a way that the offspring resembles more its parent with regard to the transmitted trait than it does the other constituents of the population" (Charbonneau 2014, p.732) . Such a process can engender an *inheritance lineage* between the "inheritance parent" and its "inheritance offspring."

Charbonneau contends that although inheritance through parent-offspring similarity is usually deemed a necessary condition for ENS, what really matters is its population-level effect called "memory," which refers to the "transgenerational retention of change in the distribution of variation of a population system" (Charbonneau 2014, p.733) . The existence of memory ensures that changes in the distribution of variation due to evolutionary mechanisms (such as natural selection) can be preserved and passed on to the next generation, making cumulative adaptation possible.

Given the above analysis, Charbonneau argues that what is really necessary for ENS to occur is not lineage-forming reproduction at the local level, but its population-level effects, generation and memory. Since these population-level effects can be realized in ways that do not form parent-offspring lineages, lineage-forming reproduction should not be regarded as a necessary condition for ENS to occur.

Papale (2021) follows Charbonneau's (2014) general approach to ENS, but makes several modifications. First, he redefines reproduction as "any causal process of multiplication by which an individual has a privileged causal input on the structure, function or material constitution of one or many individuals in subsequent generations" (Papale 2021, p.10433) . In contrast to Charbonneau, Papale does not emphasize the distinction between multiplication lineage and inheritance lineage. Instead, a parent-offspring lineage can be established between individuals of successive generations as long as certain individuals in the earlier generation make recognizable, privileged causal contributions to the formation of new individuals in the later generation, regardless of whether the contributions are material, functional, or structural.

Second, Papale explicitly recognizes the heterogeneity of ENS and distinguishes between minimal and paradigmatic ENS[1]. According to Papale, ENS is minimally realized when a population features (1) "variation among constitutive individuals that leads to differential fitness" (Papale 2021, p.10444) and (2) memory. ENS is paradigmatically realized when a population satisfies the further requirement of regeneration[2], that is, "the capacity of a population to sustain or increase its size through any process of multiplication of its components" (Papale 2021, p.10447) . While minimal ENS can only explain *changes in the distribution* of traits in a population (distribution explanations), paradigmatic ENS can explain the origin of new traits and adaptations (origin explanations).

According to Papale's account, reproduction with lineage formation is not a necessary condition for either minimal ENS or paradigmatic ENS. For minimal ENS, individuals in a population need to have differential persisting capacities, but the population does not need processes such as reproduction to regenerate its components. For paradigmatic ENS, maintaining the size of a population requires the regeneration of its parts, but such an effect need not be realized through lineage-forming reproduction.

## 3. Reconsidering the Alleged Cases of ENS without Lineage-Forming Reproduction

Charbonneau (2014) and Papale (2021) not only aim to show the *theoretical possibility* of paradigmatic ENS without parent-offspring lineages, but also draw on various empirical cases to demonstrate the

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[1] Papale's distinction between minimal and paradigmatic ENS echoes Godfrey-Smith's (2009) distinction between marginal and paradigm Darwinian populations, although Godfrey-Smith himself does not use terms such as "minimal ENS" and "paradigmatic ENS" directly.

[2] Papale replaces the term "generation" used by Charbonneau with "regeneration," because he believes that the term "generation" confusingly suggests the importance of establishing a gap between generations (i.e., having discrete generations) for ENS. First, it is widely acknowledged that populations of non-reproducing entities, which do not form different generations at all, can undergo (minimal) ENS through differential persistence. Second, even when populations undergoing ENS do form different generations, they can have overlapping, rather than discrete, generations. It should be noted, however, that both of these criticisms are based on an inaccurate understanding of Charbonneau's view. First, Charbonneau adopts a narrow view of evolution, which refers to *intergenerational* changes of the distribution of variation in a population. For him, populations that do not form different generations cannot be said to be able to evolve at all. Even in Papale's own account, the notion of "regeneration" is only involved in paradigmatic ENS, which does require the formation of new generations. Second, Charbonneau (2014, p.731) explicitly mentions that different generations can overlap and need not be discrete.

*empirical plausibility* of their claims. In this section, we will reexamine three such alleged cases in biology, including cases of prions, holobionts, and ecological inheritance. It turns out that none of them can be used as evidence for the existence of paradigmatic ENS without lineage-forming reproduction.

### 3.1 Prions

Prions are misfolded, infectious proteins that can induce normal proteins with the same amino acid sequence to adopt their abnormal conformation. In general, normal proteins are produced through the process of mRNA translation. When a normal protein is infected by prions, it undergoes a structural change and is induced to fold from a normal cellular form into a pathogenic form (Scheckel and Aguzzi 2018; Sigurdson et al. 2019; Telling 2022).

When Charbonneau (2014, pp.731-732) discusses the case of prions, his first step is to argue that prions are a good example of multiplication without lineage formation. Then he goes further to argue that prions provide a case where a population can undergo ENS without forming parent-offspring lineages at the local level when multiplying its components. His argument can be reconstructed as follows:

(1) Prions come from normal proteins. They have the same material base as normal proteins, but are misfolded in structure.

(2) Normal proteins are products of mRNA translation. Neither preexisting normal proteins nor prions participate in the material production of new proteins.

(3) Thus, no parent-offspring lineages are formed during the multiplication of prions.

(4) Different strains of prions can compete with each other, leading to a process of Darwinian evolution (i.e., evolution by natural selection).

(5) Hence, a population of prions can evolve by natural selection even though no parent-offspring lineages are formed during the multiplication of its components.

The problem with this argument is that Charbonneau conflates “the multiplication of normal proteins” with “the multiplication of prions.” Although normal proteins can be induced to form prions, they do not belong to the same relevant kind of entities, as normal proteins and prions differ significantly in structure, function, and behavior, especially in their infectious properties. Therefore, the multiplication of normal proteins should not be equated with the multiplication of prions. Normal proteins are synthesized through mRNA translation. During this process, the multiplication of normal proteins has been realized, but the multiplication of prions has not. Hence, we cannot derive (3) from (1) and (2), but only that “no parent-offspring lineages are formed during the multiplication of *normal proteins*.”

Prion multiplication is fully realized when normal proteins are converted into the prion conformation by preexisting prions. In this process, preexisting prions play a distinctive causal role in the formation of new prions, i.e., misfolding normal proteins into the prion form and transmitting their conformation to new prions. The upshot is that this causal influence does establish a parent-offspring lineage between preexisting prions and newly produced prions. Godfrey-Smith (2009, pp.79-81) has described cases like this as “formal reproduction.”

Charbonneau is fully aware of the concept of formal reproduction as proposed by Godfrey-Smith. The way he addresses this issue is to deny that the formal reproduction of prions is a case of lineage formation during the process of *multiplication*. Charbonneau seems to suggest that preexisting prions cannot be said to participate in the process of prion multiplication, because they do not materially contribute to the production of new prions. Instead, what preexisting prions really do is to participate in the *inheritance* process and transmit their conformation to new prions. Hence, the parent-offspring lineages found in the formal reproduction of prions, if there are any, are actually established by the inheritance process. He then proceeds to argue that inheritance mechanisms need not rely on local-level parent-offspring lineages in order for a population to evolve by natural selection (Charbonneau 2014, p.732).

This argumentative strategy is faced with two problems. First, it is unclear why material contribution should be considered a necessary condition for an entity to participate in a multiplication process. For example, even Charbonneau (2014, p.730) himself agrees that ribosomes and mRNA, as producers, participate in protein multiplication via the process of translation, although neither of them materially contributes to the production of new proteins. By the same token, if the conversion of normal cellular proteins into prions relies on the causal influence of preexisting prions, it seems perfectly reasonable to think that preexisting prions participate in the multiplication of new prions.

Second, even if we agree with Charbonneau that pre-existing prions are involved in the inheritance process rather than the multiplication process, a parent-offspring inheritance lineage is still established here: The preexisting prion that transmits its conformation during the inheritance process works as the parent, and the new prion that receives this conformation works as the offspring. Since Charbonneau conceives the notion of reproduction as a combination of multiplication and inheritance, the prion example is still a case of reproduction with lineage formation.

Charbonneau may agree with the second point we have made. However, if he does, his discussion of the prion case would fail to support his main thesis—that a population can undergo paradigmatic ENS without forming parent-offspring lineages. To substantiate this thesis, it is not enough to show that paradigmatic ENS can occur without multiplication lineages; Charbonneau must demonstrate that there can be paradigmatic cases of ENS in which both multiplication and inheritance lineages are absent.

In order to eliminate inheritance lineages in the case of prions, Charbonneau (2014, pp.734-738) proposes a hypothetical scenario in which the formation of new prions does not require the direct inducing action of preexisting prions on normal proteins. Proteins need to fold into correct three-dimensional conformations to function, and they usually need the assistance of chaperones, which themselves are a class of proteins, to fold correctly. In Charbonneau's hypothetical case, preexisting prions can incapacitate the normal functioning of chaperones, which increases the probability of an unfolded protein misfolding into the prion conformation. He argues that in this case prion multiplication occurs, but there is no parent-offspring inheritance lineage established between preexisting and newly formed prions, because preexisting prions do not serve as a template to directly induce the misfolding of newly formed unfolded proteins. If the requirement of memory is also fulfilled under suitable conditions, this hypothetical case could potentially provide an example of paradigmatic ENS without lineage-forming reproduction.

However, Charbonneau's argument based on the hypothetical case is flawed, because he assumes without justification that "a newly formed unfolded protein's probability to misfold into the prion conformation proportionally increases" with the decrease of the density of functional chaperones (Charbonneau 2014, pp.737–738). While all prions are misfolded proteins, the converse is not true. Prions have *specific conformations* that give them their unique infectivity. Only those misfolded proteins that exhibit these specific conformations are prions. Although it is not logically impossible for a normal protein to automatically take the conformation of prions, this possibility is very small. In the context of prion evolution, a mechanism should be in place to ensure that the appearance of new prions in the next generation is not a mere coincidence (Sigurdson et al. 2019; Hoyt et al. 2022; Manka et al.

2022; Ribes et al. 2023). In actual scenarios, this mechanism is realized by preexisting prions serving as templates to convert normal proteins. In Charbonneau's hypothetical case, however, such a mechanism is lacking. Although preexisting prions may increase the likelihood of protein misfolding by impairing the normal functioning of chaperones, without a mechanism in place it is unclear whether and how this can also proportionally increase the probability of a normal protein misfolding into the prion conformation. Therefore, contrary to what Charbonneau thinks, the requirement of memory is not obviously satisfied. This analysis also reveals a limitation of using hypothetical examples to challenge the necessity of reproduction for paradigmatic ENS: Challengers can construct hypothetical populations with features that support their conclusion, but their depictions often lack important details. This leaves it unclear how those relevant features can be realized in non-mysterious, biologically plausible ways.

Some may contend that our criticism is directed at the specific hypothetical example presented by Charbonneau and it is possible for him to modify certain details of his example in a way so as to address our criticism. We agree. Nevertheless, merely asserting the possibility of something without demonstrating *how* it might be achieved offers little in the way of support for a thesis. We cannot predict how Charbonneau would modify his example. However, at least now his hypothetical example of prions, given our best current knowledge, does not provide a biologically plausible mechanism of how a population of prions might be able to regenerate its parts and ensure memory without forming lineages.

## 3.2 Holobionts

Papale (2021) believes that holobionts provide a better case for multiplication without lineage-forming reproduction. A holobiont is a functional entity consisting of a host and all its symbiotic microbes (Zilber-Rosenberg and Rosenberg 2008; Gilbert et al. 2012; Ebert 2013; Doolittle and Booth 2017). The host in a holobiont can acquire its symbiotic microbes in two main ways: vertical transmission and horizontal transmission. In vertical transmission, the host in a newly formed holobiont acquires its symbiotic microbes directly from its parent. In horizontal transmission, however, the host acquires its symbiotic microbes from a microbial source pool in the environment, which consists of microbes coming from many preexisting holobionts. The existence of vertical transmission of microbes across different holobiont generations does not raise particular challenges for standard accounts of ENS, because in such cases the host and its symbiotic microbes reproduce as a whole and parent-offspring lineages are formed among holobionts of successive generations. Papale therefore focuses on the case of horizontal transmission.

In constructing his argument, Papale appeals to a mathematical model of holobiont evolution with horizontal microbial transmission constructed by biologist Joan Roughgarden and her collaborators (Roughgarden et al. 2018; Roughgarden 2020). The minimal model of horizontal transmission consists of three sequential processes. The first step is microbial transmission, in which microbes in a microbial source pool are distributed to juvenile hosts in a host source pool according to a Poisson sampling process. This process produces a new generation of holobionts. The second step is microbial proliferation, in which the number of microbes within each holobiont increases according to an ecological growth model. The third step is holobiont selection, in which each holobiont reproduces as a whole, and holobionts with more beneficial microbes can leave more holobiont offspring. Then all the microbes and hosts from the newly reproduced holobionts enter a microbial source pool and a host source pool respectively, and a new cycle begins.

Papale argues that in this model, the regeneration of the holobiont population is realized by the random assembly of hosts and microbes from two source pools. However, this process is too diffuse to invoke lineage formation at the holobiont level, because the microbes in each new holobiont may come from numerous holobionts in the previous generation, making it almost impossible to identify which of the preexisting holobionts has a privileged causal role in the formation of a new holobiont.

We agree that this is a case of regeneration without lineage formation. However, what is really relevant here is whether there is paradigmatic ENS at the holobiont level. Or, put another way, do holobionts that rely largely on horizontal transmission count as paradigmatic units of selection (Bordenstein and Theis 2015; Moran and Sloan 2015; Douglas and Werren 2016; Skillings 2016; Lloyd and Wade 2019)?

The criteria for units of selection have been extensively discussed and remain controversial in the literature. Nevertheless, some minimum criteria seem to be widely accepted. One such criterion is called fitness alignment. Bourrat and Griffiths (2018) formulate this criterion as follows:

> Considering both a spatial and temporal scale at which an ensemble can be divided into units, the higher the probability that manipulating properties of one unit reliably leads to effects of the same magnitude and direction on the realized fitness of all the partners and only the partners constituting that unit at the temporal scale considered, the more highly each unit scores on individuality (Bourrat and Griffiths 2018, p.16).

Roughly speaking, the fitness alignment criterion tells us that when considering whether a unit consisting of multiple components counts as an evolutionary individual or a unit of selection, we can perform a test by manipulating one character of the unit and see whether that manipulation would affect the fitness of all the components in that unit by the same magnitude and in the same direction, i.e., whether the fitness of different components is kept aligned. A unit with a higher level of fitness alignment among its components will score higher on evolutionary individuality.

Let us apply this criterion to the case of holobionts with horizontal transmission. Consider a scenario where a lethal mutation occurs in the host. This mutation will significantly affect the fitness of the host, reducing it to zero. The fitness of its symbiotic microbes will also be temporarily reduced, but to a much lesser extent. On an evolutionary timescale, those microbes are ultimately not "in the same boat" as the host, and they can maintain their fitness at a fairly good level by living freely in the environment or by colonizing other hosts. In other words, there is a very low level of fitness alignment between the host and the microbes within a holobiont that rely largely on horizontal transmission. Therefore, such holobionts do not count as paradigmatic evolutionary individuals or units of selection.

Another closely related but somewhat different criterion is partner fidelity, which refers to "a stable association of host and symbionts across multiple host generations" (Skillings 2016, p.884). For the holobiont to qualify as a unit of selection, its components must co-occur over evolutionary time and evolve as a whole. Such a criterion is consistent with the fitness alignment criterion introduced above, because partner fidelity helps bind the different parts of a unit to a common fate and promotes the alignment of their fitness (Skillings 2016, p.884). Another important, but less appreciated, role of partner fidelity is to facilitate the evolution of complex cumulative adaptations. A biological entity must be able to exhibit complex adaptations at the level of that entity in order to qualify as a paradigmatic unit of selection, and the appearance of complex adaptations requires the interaction of multiple genes acting in an orchestrated manner. If partner fidelity is low for the components of a biological entity, it will be difficult to maintain, let alone improve, the orchestration among the genes contained in its different components.

In Roughgarden's (2020) simple model of holobiont evolution with horizontal microbial transmission, the partner fidelity criterion is superficially satisfied, because the model assumes only one host taxon, one microbial taxon (either all pathogenic or all mutualistic), and no genetic variation among hosts or microbes. Different holobionts differ only in the number of microbes they contain. However, a closer look reveals that such a case lacks the features that the criterion of partner fidelity is supposed to ensure. On the one hand, as already argued above, the components of such holobionts have a very low level of fitness alignment. On the other hand, for complex cumulative adaptations to evolve at the holobiont level, appropriate mutations must occur in both hosts and microbes. Under horizontal microbial transmission, partner fidelity among particular variants of hosts and microbes is expected to be low, making it difficult to achieve and maintain the orchestration among different genes required for the emergence of complex adaptations.

The above analysis shows that holobionts with extensive horizontal transmission, as discussed by Papale, do not qualify as paradigmatic units of selection. If this is correct, then the fact that a population of holobionts can be regenerated without lineage formation cannot be used as a counterexample to challenge the necessity of lineage-forming reproduction for paradigmatic ENS.

### 3.3 Ecological Inheritance

We have shown that the cases of prions and holobionts cannot be used to support the empirical plausibility of paradigmatic ENS involving (re)generation at the population level without the formation of parent-offspring lineages at the local level. This section reexamines the case of ecological inheritance, which Charbonneau has invoked to argue for the empirical plausibility of memory in the absence of parent-offspring lineage formation.

Charbonneau (2014, pp.734-736) begins with a thought experiment in which a researcher artificially synthesizes a population of bacteria that cannot reproduce themselves. Suppose the researcher adds a toxic chemical compound to the bacterial population. Different bacterial variants in the population show different levels of resistance to this compound and thus have different survival rates. After a period of time, the researcher counts the number of surviving bacteria of each variant and records information about the corresponding distribution of variation in a spreadsheet. Using this information, the researcher performs another round of synthesis to create a new population of bacteria that has the same distribution of variation as the earlier generation after being exposed to the chemical compound, and places it in the same toxic environment as the previous population. If the researcher repeats the above procedure, the bacterial variant that is most resistant to the chemical compound will eventually dominate the population after enough generations.

Charbonneau argues that in this case, the requirement of memory is satisfied without the formation of local-level inheritance lineages. On the one hand, the change in variation distribution due to natural selection in the earlier generation has been encoded and redistributed to the later generation. On the other hand, no parent-offspring lineages are formed between the individual bacteria in successive generations, because “the individual contributions of the constituents of the earlier generation are fused in the computation of the relative frequencies of each variant type,” which “makes any single individual’s contribution indiscernible from another’s” (Charbonneau 2014, p.735).

Despite the interesting setting of this thought experiment, it fails to present a convincing case of paradigmatic ENS. First of all, in Charbonneau’s imaginary case, there is no evolutionary relationship between successive generations of bacteria at all. It needs to be clarified that the problem is not simply that it is an imaginary case or that it involves some artificial settings or interventions. What really matters is *what kind* of artificial settings or interventions is involved. There is no doubt that populations of various species can evolve under artificial conditions, such as domestic pigeons discussed by Darwin and *E. coli* studied by evolutionary biologists in the lab. Despite the artificial settings or interventions in these cases, new organisms (pigeons or *E. coli*) in the later generation are reproduced by preexisting organisms in the earlier generation, establishing an evolutionary relationship between successive generations. This allows us to make the reasonable claim that the later population of pigeons or *E. coli* evolved from an earlier population. In Charbonneau's imaginary case of bacteria, however, each new generation of bacteria is completely artificially synthesized, which cuts off the biological link between different generations. As a consequence, these artificially synthesized bacteria cannot be said to have *evolved* from an earlier population. Secondly, the realization of generation and memory in this case is almost entirely due to the intervention of the researcher, without which the initial laboratory population of bacteria is doomed to dwindle away. Given this, it is unreasonable to think that such a population system itself has the capacity to produce complex adaptations through the accumulation of small changes. To better understand this point, it might be helpful to consider an analogous case where we want to evaluate whether someone is a good swimmer. Suppose that the testee's performance in the water satisfies all the criteria of a good swimmer, except that her every movement is controlled and finished by some other people underwater. In this case, it would be unreasonable to call the testee a good swimmer. Similarly, in Charbonneau's imaginary case of bacteria, although the requirement of generation and memory is superficially satisfied, it has nothing to do with the capacity of the bacterial population itself to evolve by natural selection at all. In fact, the setting that bacteria in each new generation are artificially synthesized rather than reproduced is deliberately designed by Charbonneau, with the aim to eliminate parent-offspring lineages between successive generations. But as we have argued, this setting also undermines its alleged status as a case of paradigmatic ENS. Thus, Charbonneau's imaginary example, even if realized in the laboratory, cannot serve as a counterexample to challenge the necessity of lineage-forming reproduction for paradigmatic ENS.

To address the limitations of the hypothetical example, Charbonneau (2014, pp.736-737) appeals to a group of naturally occurring phenomena known as "diffused ecological inheritance": Information concerning the state of a population (such as its distribution of variation after selection) is recorded in the state of an external environment. This environmental state can be passed on to the next generation and influence its development, resulting in a similar distribution of variation as the previous generation. For example, males and females of many fish species can release different kinds of steroids (a class of organic compounds) into their surrounding water. The level of steroids in the water can be transmitted through ecological inheritance to the next generation, ultimately determining the sex ratio of the fish population.

In this scenario, the sex of each fish in the subsequent generation is determined by the overall steroid level in the water, which is in turn influenced by all the fish in the previous generation. As a result, no specific inheritance lineages can be identified between individuals in successive generations. This is intended by Charbonneau to be a case where memory is realized on the population level without forming inheritance lineages on the local level. However, this interpretation of the case faces several problems.

First, strictly speaking, the case of sex determination in fish does not fulfill the requirement of memory. As Charbonneau himself acknowledges, the sex ratios in successive generations are negatively, rather than positively, correlated. While this mechanism allows the population to adjust its sex ratio through negative feedback, it cannot guarantee that the subsequent generation will have the same or similar sex ratio as the previous one. As a response, Charbonneau (2014, p.737) argues that "one can easily imagine cases where a positive correlation would obtain, showing the empirical plausibility of diffused ecological inheritance."

It is simply not true that the fact of our being able to imagine something shows its empirical plausibility. But even if we grant the actual existence of cases where a positive correlation obtains between the sex ratios of successive fish generations, parent-offspring lineages would still persist. This is due to the fact that each individual fish in the later generation was born from its parents in the earlier generation, which forms multiplication lineages between parents and offspring. Additionally, the realization of memory of sex ratio between successive generations is dependent on the existence of inheritance lineages at the local level. Each fish inherits from their parents the capacity to develop a certain type of sex based on the steroid level in the water, rather than a specific sex. This capacity itself may be a complex adaptation that relies on specific genetic mechanisms and can evolve by natural selection. The inheritance of this capacity establishes local-level inheritance lineages between parent fish and their offspring. The above analysis shows that not only do both multiplication and inheritance lineages exist in these cases, but they also overlap with each other. Therefore, standard accounts of ENS involving lineage-forming reproduction are entirely compatible with Charbonneau's alleged counterexample.

Compared to Charbonneau's reproduction-independent account of ENS, standard accounts also provide a better perspective for understanding why there can be correlations of sex ratios between successive generations. Ecological inheritance may pass on information about the sex ratio in the earlier generation to the next one, but merely focusing on ecological inheritance leaves unexplained why the steroid level in the water can determine the sex ratio of the next generation. From the perspective of traditional accounts, however, there is no mystery here: The correlation of sex ratios between successive generations, no matter positive or negative, is the result of each fish expressing its inherited capacity to develop a certain type of sex based on the steroid concentrations in the water. Notice that traditional accounts of ENS do not deny the role of ecological inheritance in explaining such evolutionary phenomena. The point here is that the presence of ecological inheritance in Charbonneau's alleged counterexample does not actually undermine the necessity of lineage-forming reproduction for paradigmatic ENS.

### 3.4 Who Has the Burden of Proof?

The preceding analysis suggests that, at least in the realm of biological evolution, Charbonneau and Papale have yet to provide convincing empirical cases of paradigmatic ENS without lineage-forming reproduction. While some cases involve paradigmatic ENS, they still depend on the existence of parent-offspring lineages; other cases lack parent-offspring lineages at the relevant level but fail to meet the criteria for paradigmatic ENS.

One possible response to this critique is that the lack of convincing empirical cases at the moment does not necessarily mean that such cases do not exist or cannot be found in the future, since we have not examined all relevant scenarios or proved the impossibility of finding such cases. For example, Papale (2021, p.10499) contends that "the proposed reformulation of Lewontin's three conditions has shifted the burden of proof on those claiming a priori that populations of non-reproducers cannot sustain ENS."

But is this conclusion really justified? While it cannot be proven a priori that populations of non-reproducers cannot undergo paradigmatic ENS, this at best shows that paradigmatic ENS without lineage-forming reproduction is logically possible, that is, the proposition that "there are cases of paradigmatic ENS without lineage-forming reproduction" is consistent with the laws of logic (Mallozzi, Vaidya, and Wallner 2023). However, mere logical possibility provides very little justification for advocating a reproduction-independent account of ENS. To make a conceptual change in science, one needs to develop a case for the *necessity* of making such a change. As widely acknowledged in the literature, one central purpose of providing an account of ENS is to identify the necessary features of empirical phenomena that are considered to be cases of ENS (Godfrey-Smith 2009; Papale 2021). If no convincing empirical cases of paradigmatic ENS without lineage-forming reproduction are identified in nature, there will be no compelling reason to replace traditional accounts of ENS with a reproduction-independent one. In their discussions, Charbonneau and Papale have also appealed to hypothetical examples to show the empirical plausibility of paradigmatic ENS without reproduction. But as we have pointed out, a closer look at the details of these hypothetical examples reveals that none of them can serve the intended purpose. Therefore, it is those who attempt to challenge and revamp traditional accounts of ENS that have the burden of proof to demonstrate the necessity of making such a conceptual change.

There is another and perhaps more substantial reason for resisting this change: If we do adopt Charbonneau's and Papale's reproduction-independent accounts of ENS, we will confront a series of theoretical problems with respect to ENS. This will be our focus in the next section.

## 4. Problems with Charbonneau's and Papale's Reproduction-Independent Accounts of ENS

One issue with Charbonneau's and Papale's reproduction-independent accounts of ENS is that they may incorrectly include some unqualified cases as paradigmatic ENS. Recall the thought experiment described in section 3.3, where a researcher artificially synthesizes a population of bacteria that cannot reproduce themselves. This population possesses all the features that Charbonneau and Papale consider to be characteristic of paradigmatic ENS:

(1) Variation and differential fitness: Different bacterial variants exhibit varying degrees of resistance to the toxic chemical compound, resulting in different survival rates.

(2) Memory: The change in the distribution of variation is retained between successive generations.

(3) (Re)generation: The bacterial population is resynthesized for each new generation.

However, as we have argued in section 3.3, this population cannot undergo paradigmatic ENS, because there is no evolutionary relationship between successive generations, and without heavy artificial interventions, this population system cannot produce cumulative complex adaptations.

The second issue with Charbonneau's and Papale's accounts is that they may exclude at least some genuine cases of paradigmatic ENS. For an example, consider a population that contains two kinds of phenotypic variants (such as Black and White in Fig. 1) and forms discrete generations. The distribution of variation in Generation 1 is 50% of Black and 50% of White. Suppose that individuals carrying different variants have the same viability, but differ significantly in their fecundity: Each black individual has three black offspring, while each white individual only has one offspring. As a consequence, the distribution of variation in Generation 2 will become 75% of Black and 25% of White. According to standard accounts of ENS, such a population can undoubtedly undergo paradigmatic ENS. However, according to Charbonneau's and Papale's accounts, we would get a different answer.

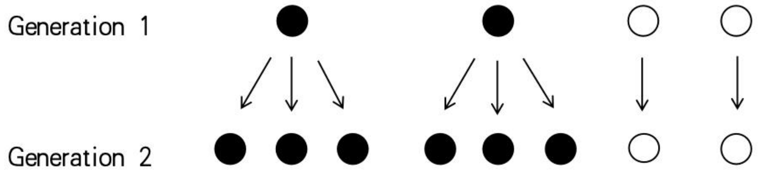
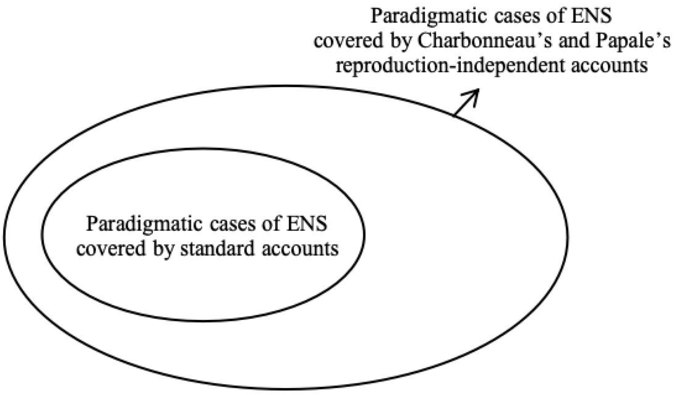


Fig.1 The change of the distribution of variation between two successive generations

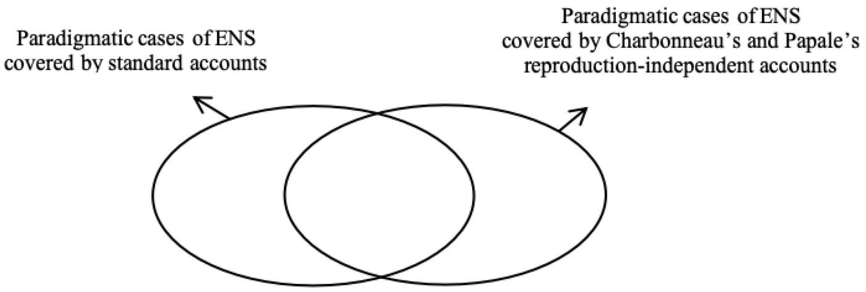
To see why, recall that Charbonneau (2014, p.733, my italics) defines memory as “the process of transgenerational retention of change in the *distribution of variation* of a population system, with inheritance systems being those local-level mechanisms the agglomeration of which effects the retention,” and Papale (2021, p.10447, my italics) defines memory as “the correlation between successive states of a population relatively to the *distribution of variation* (the more similar states are, the stronger memory is).” There is some difference between their definitions of memory: Charbonneau’s definition only concerns different generations, while Papale’s definition also applies to successive states of a population that does not form different generations. However, given the context of the above example in Fig. 1, where the population does form different generations, their definitions of memory are essentially the same—both Charbonneau and Papale define memory in terms of the resemblance of the distributions of variation between two generations. In biology, the distribution of variation simply refers to the frequencies of different types of variants in a population, which is also how Charbonneau and Papale understand it when they use this notion to define the concept of memory. However, if we follow their definition of memory, the population in Fig. 1 would exhibit a very low level of memory and hence cannot undergo paradigmatic ENS, because the distribution of variation at the beginning of Generation 2 (75% of Black and 25% of White) differs significantly from that at the end of Generation 1 (50% of Black and 50% of White). This finding yields an unpleasant result for Charbonneau’s and Papale’s accounts: One central motivation for them to reformulate the conditions for ENS is to provide a more general account that can cover cases of ENS not included in standard accounts. However, their revamped accounts mistakenly exclude some genuine cases of paradigmatic ENS already covered by standard accounts. Indeed, the fact that some acknowledged cases of paradigmatic ENS do not satisfy the requirement of memory indicates that it should not be regarded as a necessary condition for ENS.

One may wonder how it is possible for Charbonneau's and Papale's accounts to exclude cases of paradigmatic ENS, given that both of them recognize that reproduction with lineage formation is a means to realize memory. To address this concern, the key is to realize that "A is one of many possible ways to realize B" does not mean that "the presence of A always realizes B." Although both Papale and Charbonneau emphasize that classical lineage-forming reproduction is merely one of many possible ways to realize memory, it does not follow that a population with lineage-forming reproduction always satisfies the requirement of memory. Our counterexample shows that there are cases where a population with lineage-forming reproduction can undoubtedly undergo paradigmatic ENS, but does not satisfy the requirement of memory. Although this does not mean that Papale's and Charbonneau's accounts of ENS generally exclude *all* paradigmatic cases of ENS, it does show that the requirement of memory in their accounts is sometimes too strict so that *at least some* paradigmatic cases of ENS will be excluded.

One central aim of Charbonneau's and Papale's reproduction-independent accounts of ENS is to provide a more general account such that paradigmatic cases of ENS featuring lineage-forming reproduction as delimited by standard accounts are only a subset of a broader class of paradigmatic ENS (see Fig. 2(a)). However, the above analyses show that their accounts are sometimes too loose so that they may erroneously include some unqualified cases as paradigmatic cases of ENS, while sometimes too strict so that they may exclude some genuine cases of paradigmatic ENS (see Fig. 2(b)). This provides another reason to be skeptical about the need to replace traditional accounts of ENS with a reproduction-independent one.



(a) Charbonneau and Papale's proposal about the relationship between standard accounts and their reproduction-independent accounts of ENS



(b) The actual relationship between standard accounts and Charbonneau's and Papale's reproduction-independent accounts of ENS

Fig. 2 The envisaged (a) and actual (b) relationships between two types of accounts of ENS

## 5. Conclusion

This article provides a critical evaluation of Charbonneau's and Papale's reproduction-independent accounts of ENS and defends the importance of lineage-forming reproduction in paradigmatic ENS on both empirical and theoretical grounds. Our empirical analysis shows that none of the cases cited by

Charbonneau and Papale can be used as evidence to demonstrate the existence of paradigmatic ENS without lineage-forming reproduction. Our theoretical analysis indicates that focusing solely on memory and (re)generation at the population level when formulating ENS would lead to an account of ENS that is both too liberal and too strict. Therefore, there is no compelling reason to replace traditional accounts of ENS with a reproduction-independent one.

Charbonneau's and Papale's attempts to reject reproduction as a necessary condition for paradigmatic ENS reflect a longstanding tradition of generalizing the theory of ENS by abstraction since Darwin (Griesemer 2005). While generalizing Darwinian theory is not inherently problematic, there are limits on the extent to which such generalization-by-abstraction can be carried out. The analysis presented in this article suggests that, at least in the realm of biological evolution, reproduction remains a necessary condition for a population to undergo paradigmatic evolution by natural selection.

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