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Review article

Considering the use of the terms strain and adaptation in prion research

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ABSTRACT

Evolutionary biologists and disease biologists use the terms strain and adaptation in Chronic Wasting Disease (CWD) research in different ways. In evolutionary biology, a strain is a nascent genetic lineage that can be described by a genealogy, and a phylogenetic nomenclature constructed to reflect that genealogy. Prion strains are described as showing distinct host range, clinical presentation, disease progression, and neuropathological and PrP biochemical profiles, and lack information that would permit phylogenetic reconstruction of their history. Prion strains are alternative protein conformations, sometimes derived from the same genotype. I suggest referring to prion strains as ecotypes, because the variant phenotypic conformations (“strains”) are a function of the interaction between PRNP amino acid genotype and the host environment. In the case of CWD, a prion ecotype in white-tailed deer would be described by its genotype and the host in which it occurs, such as the H95 + ecotype. However, an evolutionary nomenclature is difficult because not all individuals with the same PRNP genotype show signs of CWD, therefore creating a nomenclature reflecting and one-to-one relationship between PRNP genealogy and CWD presence is difficult. Furthermore, very little information exists on the phylogenetic distribution of CWD ecotypes in wild deer populations. Adaptation has a clear meaning in evolutionary biology, the differential survival and reproduction of individual genotypes. If a new prion ecotype arises in a particular host and kills more hosts or kills at an earlier age, it is the antithesis of the evolutionary definition of adaptation. However, prion strains might be transmitted across generations epigenetically, but whether this represents adaptation depends on the fitness consequences of the strain. Protein phenotypes of PRNP that cause transmissible spongiform encephalopathies (TSEs), and CWD, are maladaptive and would not be propagated genetically or epigenetically via a process consistent with an evolutionary view of adaptation. I suggest terming the process of prion strain origination “phenotypic transformation”, and only adaptation if evidence shows they are not maladaptive and persist over evolutionary time periods (e.g., thousands of generations) and across distinct species boundaries (via inheritance). Thus, prion biologists use strain and adaptation, historically evolutionary terms, in quite different ways.

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1. Introduction

The way in which the terms “strain” and “adaptation” are used in evolutionary biology contrasts with how researchers studying transmissible spongiform encephalopathies (TSEs) use these terms. Below I discuss how evolutionary biologists use these terms and suggest that prion researchers consider redefining their use of strain and adaptation when discussing transmissible spongiform encephalopathies. I focus on studies of Chronic Wasting Disease (CWD) in white-tailed deer (*Odocoileus virginianus*).

2. Use of the term “strain” in evolutionary biology and prion research

In evolutionary biology, a strain is an informal taxonomic rank used below the species and even subspecies level. It is a nascent lineage that might evolve independently, fuse with another lineage or go extinct. Implicit in the definition is the notion that strains are genetically different and a genealogy describes their history at the level of the genetic code. For example, an influenza or COVID-19 strain possesses unique mutations that allow it to survive in or invade novel hosts, and

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reproduce. New strains arise from mutations in previous ones and are connected by a nexus of genealogy. Thus, strains contain information that allows a phylogenetic reconstruction of their evolutionary history, which allows a classification to be constructed that reflects that evolutionary history, such as that for SARS-CoV-2 (<https://nextstrain.org/ncov/global>).

Initially, it was thought that a virus caused TSEs such as CWD, whereas the prion model is now the accepted root cause (Prusiner, 1982; Diringer, 2000; Zabel and Reid, 2015; Brandt et al., 2015; Herbst et al., 2017; see Manuelidis, 2004). It is common today to read statements such as: “These unique infectious agents [prions] exist in a wide variety of “strains”” (Morales, 2017; Espinosa et al., 2020), which has a relatively long history (Dickinson et al., 1968; Caughey et al., 1998). How are strains in prion research defined? Li et al. (2010) defined a prion as “originally characterized by the incubation time and the neuropathology they elicit in a particular host”. Velásquez et al. (2015) state that “Strains are distinguished on the basis of their host range, clinical presentation, disease progression, and neuropathological and PrP biochemical profiles”. For example, some animals (e.g., mice, hamsters) that have the same PrP^c amino acid sequence can be made experimentally to exhibit novel prion protein conformations, or “strains” (Chernoff, 2001). Shorter (2010) referred to this process as “prionogenesis”. Le Dur et al. (2017) wrote that “There is compelling evidence that prion strain diversity reflects differences in PrP^{Sc} conformations, at the level of the tertiary and/or quaternary structure.” Li et al. (2010) state that “Many different strains can be propagated indefinitely in hosts homozygous for the PrP gene (Prnp)”. Morales (2017) asserted that strains are identified by incubation periods, clinical signs, lesion profiles, electrophoretic mobility, glycosylation pattern, and proteolytic resistance. Thus, there is a variety of definitions of strain, most of which refer to a novel protein phenotype and not its underlying genetic basis.

The question is whether prion strains contain information that would allow construction of their genealogy, one that would support a phylogenetic classification. Given the definition of a prion strain by Velásquez et al. (2015) given above, the answer is no because at yet no one has proposed scorable characters with multiple character states that would permit reconstruction of strain phylogeny as has been done for many viruses (Dijkshoorn et al., 2000; Zheng et al., 2006). If novel PRNP phenotypes were inherited across generations and retained phenotypic characteristics that could be scored and used to create a phylogenetic history, such a classification might be possible. Lacking a phylogeny of prion strains, an evolutionary nomenclature is elusive at best. Thus, in my opinion, strains in evolutionary biology and in the prion literature mean different things. I suggest the term “ecotype” for what have been called prion strains, owing to the fact that the protein conformation is an interaction between genotype and host environment. However, in the sense that different strains have the same underlying amino acid sequences, perhaps “ecostrain” would aid in identifying a nongenetic underlying basis.

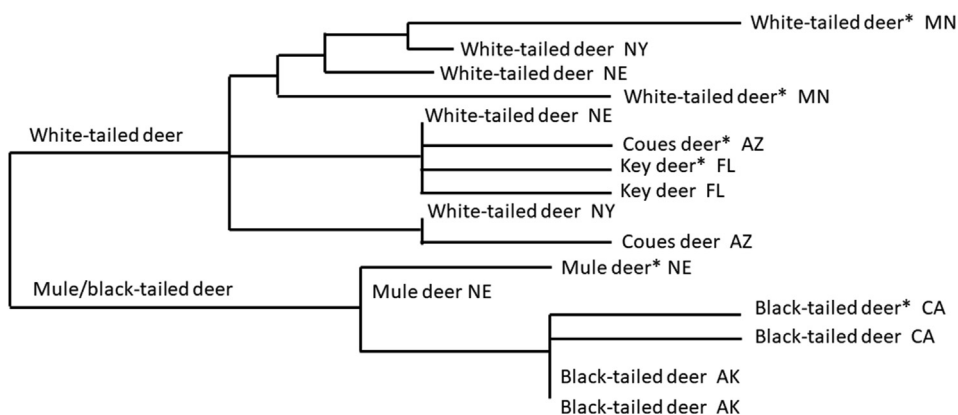


Figure 1. Phylogenetic tree derived from the 771 bases in the PRNP gene of some North American deer, using the maximum likelihood method. Each terminal is an individual allele, not a genotype. MN = Minnesota, AK = Alaska, NY = New York, AZ = Arizona, CA = California, NE = Nebraska, FL = Florida. Individuals lacking an asterisk all have the same amino acid sequence. Each deer except for the two black-tailed deer from Alaska (AK) have different nucleotide sequences. Genbank numbers given in original publication (Vázquez-Miranda and Zink, 2020; Zink et al., 2020).

3. Exploring PRNP phylogeny and CWD in white-tailed deer

Although relatively conserved, the (diploid) PRNP gene in animals includes many alleles, differing by as few as one base pair up to many. For example, in a sample of 214 white-tailed deer from Nebraska, 10 alleles at the PRNP gene (771 bp) were observed, differing by from 1 to 3 bp (Zink et al., 2020). When these sequences were translated to amino acids ($n = 257$), there were seven variable sites that defined eight distinct amino acid sequences. Some amino acid substitutions delay progression of prion disease in deer (Johnson et al., 2011) and humans (Asante et al., 2015). For example, a white-tailed deer with H95Q S96S G116A might be one of the more resistant genotypes (Haley et al., 2019), with a considerably delayed onset of CWD relative to the wild type genotype. Velásquez et al. (2015) note that the PRNP genotypes that are influential for CWD expression are “Q95 G96 (wild type [wt]), Q95 S96 (referred to as the S96 allele), and H95 G96 (referred to as the H95 allele)”. This nomenclature conflates the allele, genotype and phenotype (protein structure). There are multiple possible genotypes for these two amino acid positions: QQ95GG96, QH95GG96, QQ95SS96, QQ95GS96, HH95GG96, HH95GS96, HH95SS96, although the latter three genotypes (and the 95H allele) are rare (Haley et al., 2019). The nomenclature of Velásquez et al. (2015) pools heterozygotes and homozygotes, for example, QQ95GS96 are QQ95SS96 are considered equivalent, not distinguishing between the occurrence of one or two copies of the allele. Other genotypes in white-tailed deer are considered relevant to CWD resistance, including position 116 (Haley et al., 2019). It is important to consider the entire genotype, not simply amino acids 95 or 96, as they are all linked on the PRNP gene (Cullingham et al., 2020).

A PRNP gene tree for 16 alleles including white-tailed deer, Key deer, mule deer, Coues deer and black-tailed deer (Figure 1) separates white-tailed deer (including Key, Coues) and mule deer (including black-tailed). One might consider the two evolutionary lineages to be different strains, i.e., mule deer strain and white-tailed deer strain. However, the deer without an asterisk behind their labels in Figure 1 have the same amino acid composition because their nucleotide sequences differ only by synonymous substitutions, whereas those with an asterisk have nonsynonymous changes that result in at least one amino acid substitution. Thus, the gene tree based on the nucleotides might not reflect variation in amino acid sequences that are important for the onset and progression of diseases like CWD. One might consider amino acid lineages to be strains. A tree of amino acid differences in 214 deer reveals eight lineages without any phylogenetic structure (Figure 2). However, this tree depicts relationships among alleles, not diploid genotypes, and most of the carriers of the 96S and all of the 95H alleles are in the heterozygous state (Table 1), although being homozygous 96SS does not apparently confer a heightened resistance to CWD. Irrespective of being heterozygous or homozygous for S at 96, there is an even mixture of CWD positive and CWD negative individuals. Thus, the amino acid phylogeny does not reflect the nucleotide tree, and it is unclear how a phylogeny of

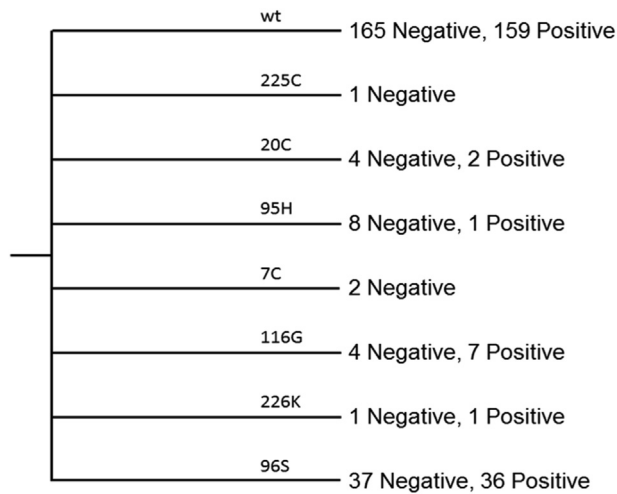


Figure 2. Condensed tree derived from amino acid sequences from 214 white-tailed deer from Nebraska (Zink et al., 2020) at the PRNP locus showing CWD status. Labels on branches are the genotypes at positions 95 or 96; if not shown, then the wild type is present. Genbank numbers given in original publication.

Table 1. Numbers of genotypes at amino acid position 96 and whether individuals were positive or negative for CWD.

	CWD Positive	CWD Negative
SS	5	5
GS	25	26

amino acid alleles could provide an unambiguous classification of strains that cause disease.

CWD presence transcends phylogenetic patterns in gene and amino acid trees and suggests causes other than DNA or amino acid sequences (Mysterud and Edmunds, 2019; Zink, 2020; Seabury et al., 2020). For example, in the 214 white-tailed deer from Nebraska, six genotypes were observed for positions 95, 96, and 116, each with two alleles (Table 2). Apart from the small sample of 95H, these multi-site genotypes had equivalent frequencies of prion disease, although it is unknown whether they all represent the same or different prion protein conformations (i.e., ecotypes). The obvious point is that similar-aged individuals with the same multilocus genotypes might or might not acquire CWD (Table 1). Whether this is a function of the deer's age, insufficient time since infection, or lack of exposure to prions in its biotic or abiotic environment, is unknown. Possibly, deer possessing a susceptible genotype, but without CWD, have a prion phenotype that is more resistant to misfolding that does not reflect its genotype. That is, such a deer might present an ecostrain that resists misfolding but differs from that in other deer with the same genotype. What is lacking is a large-scale survey of prion phenotypes in wild populations of deer, and

Table 2. Distribution of CWD positive and negative deer by multilocus amino acid genotype at positions 95, 96, and 116 in white-tailed deer from Nebraska. No individuals were 95HH in this sample.

Genotype 95-96-116	CWD Negative	CWD Positive
QQGGAA	71	66
QQSSAA	4	5
QQGSAA	24	24
QQGGAG	3	6
HQGGAA	8	1
QQGSAG	1	1

their CWD status, as to date most studies explore prionogenesis in transgenic strains of mice.

The lack of correspondence of PRNP nucleic acid and amino acid gene trees and CWD presence, suggests a classification system should at least incorporate both genotypes and phenotypes. For example, Velásquez et al. (2015) concluded from inoculation of transgenic mice with homogenized brain tissue from a H95-PrP deer that "Transmission of the deer H95/wt and H95/S96 CWD allotypes resulted in the emergence of a distinct CWD strain (H95⁺)." Note that the genotype H95 is not new, rather a novel prion protein conformation (phenotype) was documented experimentally in a particular strain of mouse. Instead, the novel phenotypic response should be linked to the multi-site PRNP protein genotype, which would minimally include 95HQ. That is, it would be logical to refer to this novel phenotype as an "H95⁺ ecotype," or "H95 + ecostrain" because the host environment elicited the response from the PRNP genotype in either the current or a previous generation. Whether this ecostrain occurs in natural populations of deer and its relationship to CWD susceptibility are unknown.

Are different prion ecotypes deleterious? In elk (*Cervus elaphus nelsoni*) variation at position 132 involves LL132, ML132 and MM132, with incubation periods ranging from longest to shortest in this order (Moore et al., 2020). They concluded that prions from genotypes LL132 and MM132 elk produced different phenotypes when inoculated into transgenic mice, which they referred to as strains. I entered the two PRNP amino acid sequences differing at position 132 into the program Provean (Choi et al., 2012), which tests whether substitutions are likely to be neutral or deleterious. The analysis returned a score of -0.564, which is predicted to be a neutral substitution. The two protein phenotypes derived from genetic variation at position 132 in elk should be considered ecotypes labeled LL132 and MM132, indicating they differ genetically and phenotypically.

4. Darwinian vs epigenetic evolution of prions, and the concept of adaptation

Traditionally, genetic variation is thought to provide the raw material for adaptive change. Adaptation results when new genotypes experience higher fitness in a new environment, or existing genotypes move into a novel environment and the organisms that possess these adaptations leave more offspring than those bearing alternative genotypes. Influenza strains adapt because they are in an evolutionary arms race with a host immune system – to survive the virus must present new mutations that allow it to counter the host's evolved responses, with a concomitant increase in, or maintenance of, viral fitness. A phylogenetic tree based on the nested pattern of mutations reveals the evolutionary history of influenza adaptations. What about the process of prionogenesis? Is it driven by adaptation, as often claimed for ecotypes of CWD (Espinosa et al., 2020)? Velásquez et al. (2015) stated that "Serial passage in tg60 mice resulted in adaptation of a novel CWD strain (H95) with distinct biological properties." This conflicts with evolutionary usage because the new "strain" is a phenotypic response by a genotype to a novel host environment rather than an adaptive process driven by mutation and natural selection at the gene level. There is no reference to enhanced fitness of the H95 ecotype. Thus, you might consider that the ecotype adapted to a novel host environment, but this is not the same as adaptation (differential fitness) in an evolutionary sense. Although one can speculate about the fitness of a new ecotype, it ultimately depends on how this ecotype affects fitness of the individual virus and its host. Natural selection can focus on the PRNP locus to favor genotypes more resistant to misfolding (such as the 95H in white-tailed deer) and hence increase the fitness of individual deer (Haley et al., 2019). Because CWD is always fatal in cervids, a hypothesis of adaptation seems inappropriate for these prions (but not others, see Shorter, 2010). I suggest that "phenotypic transformation" is a better term than adaptation for classically defined CWD ecotypes.

Li et al. (2010) claimed that “prions show the hallmarks of Darwinian evolution: They are subject to mutations evidenced by heritable changes of their phenotypic properties, and to selective amplification, as documented by the emergence of distinct populations in different environments”. That is, prions that take on novel conformational changes result in variant phenotypes irrespective of underlying genotype, they can be transmitted across generations, and natural selection might influence the fate of these new phenotypes. This view suggests an alternative to classical Darwinian evolution presented above, where information flows unidirectionally from gene to protein to phenotype (Bussard, 2005). Manjrekar (2017) suggests that prions might be an example of transgenerational epigenetic inheritance, which “raises obvious questions about a possible evolutionary role for epigenetic ‘Lamarckian’ mechanisms in evolution, particularly when epigenetic modifications are induced by environmental cues.” It is possible for a female deer to pass her mutant prion conformations to a fawn in utero (Selariu et al., 2015), and deer sperm can contain prions (Kramm et al., 2019). The question is whether ecotypes propagated epigenetically represent evolutionary adaptations?

Epigenetic variation can be heritable, at least in the short term, and the source of new adaptations (Klironomos et al., 2013; Donohue, 2014; Heard and Martienssen, 2014; Chakravarty and Jarosz, 2018). One example is the transgenerational epigenetic inheritance of methylation (Angers et al., 2010), in which a methyl group (CH₃) can be attached between a cytosine and guanine (“CpG”). Skinner et al. (2014) claimed that epigenetic mutations were a source adaptive variation in Darwin’s finches, and that phylogenetic and epigenetic distances were correlated; however, their study relied on a phylogenetic hypothesis now known to be incorrect (Zink and Vázquez-Miranda, 2018). McNew et al. (2017) concluded that in two species of Darwin’s finches, “epigenetic changes accumulate over macroevolutionary time and further suggest that epigenetic changes may contribute to the evolution of adaptive phenotypes.” These studies raise the possibility of a role for epigenetic evolution of prions, although here the question is whether this applies to the prions that cause TSEs.

Skinner et al. (2014) suggested that epigenetic changes that “persist over thousands of generations” could contribute to adaptation and increased fitness. Chakravarty and Jarosz (2018) wrote that “Although the first prion discovered—mammalian PrP—is the causative agent of debilitating neuropathies, many additional prions have now been identified that are not obviously detrimental and can even be adaptive.” Shorter (2010) noted that “prions are units of selection. Thus, natural selection inescapably enriches or depletes various prion strains from populations depending on their conformational fitness (ability to self-replicate) in the prevailing environment. The most successful prions confer advantages to their host”. As with genetic inheritance, whether epigenetic changes are adaptive depends on their relationship to organismal fitness (Shorter 2010). In the case of TSEs, and CWD in particular, the classically defined ecotypes are maladaptive to their hosts and would not lead to their integration into the evolutionary lineage, although they might persist for short periods. In the case of CWD, if new mutations or host-induced modifications yield novel protein phenotypes that are more prone to cause misfolding, or cause it at a faster rate, the consequence is killing off their hosts at an earlier point in their reproductive lifespans; i.e., the antithesis of an adaptive response by a prudent virus. Shorter (2010) argued that prionogenesis led to fitness advantages in yeast owing to heightened ability to respond to environmental stress. In the case of CWD, new ecotypes would have to inhibit misfolding to be adaptive, but there is no evidence of this to date, although the lack of some deer with susceptible genotypes raises the possibility (see above). This argues against a role for epigenetic evolution of prions the cause TSEs and prionogenesis being a process akin to adaptation.

Herron and Freeman (2014) stated “The impermanence of most epigenetic marks precludes a substantial contribution by epigenetic variation to long-term evolution (Slatkin 2009).” An interesting question involves the evidence for this statement. That is, what are the

phylogenetic footprints of epigenetic evolution and how would we discover them? In the case of methylation, there appears to be some evidence of long-term maintenance of epigenetic changes. If epigenetic propagation of prion strains are important in long-term evolution, one might not find evidence at the DNA level. Instead, one might discover a phylogenetic hierarchy of prion ecotypes that exhibit an explicit evolutionary history, which might or might not reflect the history encoded in genes. That is, sister taxa would share the same ecotypes or ones more closely related to each other than to those in their sister taxon (or taxa). At the very least, ecotypes should be found across well-defined species boundaries. As noted above, this would require the scoring of characters from novel protein confirmations to assess their phylogenetic distribution and relationships. To my knowledge, no one has documented a phylogeny of prion ecotypes, based on characters derived from the ecotypes themselves, that would be expected if epigenetic propagation the mode of evolution. Thus, the possibility exists that epigenetic transmission of ecotypes could explain at least in part the evolution of prion ecotypes that had a positive effect on fitness (Shorter 2010), which likely excludes the PRNP locus and its protein derivatives that induce TSEs.

If multiple prion phenotypes originating from the same genotype were each adaptive, it might stabilize nucleotide sequences and reduce variability. Although the PRNP gene exhibits relatively low sequence variation, genetic variation does exist (cf. above). Buchanan and Zink (submitted; see Zink, 2020) found that for 102 mammal species (representing 20 orders, 58 families, and 85 genera) that the PRNP gene and amino acid tree, and a tree based on 20 independent loci, were mostly congruent. If epigenetic changes influenced PRNP gene evolution, one might find that species with TSEs share particular motifs or amino acid sequences irrespective of phylogenetic relationships, which was not the case, suggesting that epigenetics did not produce clusters of particular prion phenotypes that were susceptible or resistant to neurodegenerative disease. The phylogenetic predictions of epigenetic inheritance of prions should be investigated.

5. Conclusion

Alternative protein configurations derived from the identical amino acid sequences at the PRNP gene can exhibit different physiological properties, have been called strains, and might undergo what some consider short-term Darwinian evolution via natural selection. If a prion strain is distinguished based on host range, clinical presentation, disease progression, and neuropathological and PrP biochemical profiles (Velásquez et al., 2015), it conflicts with the evolutionary usage of the term, which implies an underlying discoverable genealogy, described by a nested hierarchy of mutations. As defined, no characteristics that could be used to reconstruct an evolutionary history of strains have been presented. In my opinion “strain” has been misappropriated in prion research, and what have been termed strains should be relabeled as ecotypes or ecostrains to reflect their origin as a genotype × environment interaction. The idiosyncratic relationship between PRNP phylogeny and presence of CWD in cervids precludes classification of strains at the level of the gene, and to construct a nomenclature of strains requires specifying the PRNP genotype, the resulting phenotype, and the host in which it was transformed. It is likely that genetic factors other than mutations at PRNP influence susceptibility to CWD (Seabury et al., 2020). Furthermore, there has not been an extensive survey of CWD prion phenotypes throughout wild populations of cervids, and most of the information about ecotypes comes from in vitro experiments with genetically transformed mice. Whether prion strains undergo adaptation depends on the fitness consequences of new ecotypes. Whereas some prion ecotypes might enhance the fitness of their carriers, others such as CWD are fatal and a new strain that induces earlier or more rapid prion propagation would not be considered adaptive. If ecotypes are propagated epigenetically, one might not expect a phylogenetic signature at the level of the PRNP gene. However, the criteria for documenting evolutionary transmission of prion ecotypes via epigenesis have not been met at this time.

Thus, in my opinion, prionogenesis of prions causing TSEs is not a result of adaptation *per se*. In any case, judicious use of “adaptation” is warranted when referring to prions.

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Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

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