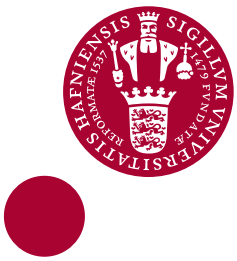

Horizontal transfer of short and degraded DNA has evolutionary implications for microbes and eukaryotic sexual reproduction

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Horizontal transfer of short and degraded DNA has evolutionary implications for microbes and eukaryotic sexual reproduction

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Horizontal gene transfer in the form of long DNA fragments has changed our view of bacterial evolution. Recently, we discovered that such processes may also occur with the massive amounts of short and damaged DNA in the environment, and even with truly ancient DNA. Although it presently remains unclear how often it takes place in nature, horizontal gene transfer of short and damaged DNA opens up the possibility for genetic exchange across distinct species in both time and space. In this essay, we speculate on the potential evolutionary consequences of this phenomenon. We argue that it may challenge basic assumptions in evolutionary theory; that it may have distant origins in life's history; and that horizontal gene transfer should be viewed as an evolutionary strategy not only preceding but causally underpinning the evolution of sexual reproduction.

Keywords:
anachronistic evolution; evolution; horizontal gene transfer; meiosis; natural transformation; sexual reproduction; short and degraded DNA

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Abbreviation:
HGT, horizontal gene transfer.

Introduction

Horizontal gene transfer (HGT) refers to processes in which a cell acquires genetic material from sources besides the genetic material inherited from its parent cell. The concept of HGT – originally termed, "genetic transformation" – dates back to Frederick Griffith's classic mouse experiments with heat-treated Streptococcus bacteria in 1928 [1]. Since then, there has been a steady increase in knowledge on HGT, and today HGT is viewed as common among prokaryotes [2]. As an evolutionary phenomenon, HGT severely complicates our understanding of bacterial evolution and systematics by introducing a reticulate component (linkages between branches after divergence) to simple bifurcating phylogenetic reconstructions [3].

Although HGT often takes place through direct cell-to-cell contact, it is not restricted to that route. Notably, a common form of HGT known as "natural transformation" involves transfer of extracellular DNA [4, 5]. However, so far natural transformation has only been shown to function with kilobase-long DNA fragments released from living or recently deceased cells [6]. Natural transformation is a process that consists of two parts, first DNA uptake and then DNA integration. Canonical integration through homologous recombination relies on sequence homology between donor DNA and the host genome. Therefore, the nature of the process implies that natural transformation mainly takes place between DNA of closely related strains [7, 8].

Kilobase-long DNA fragments do not persist for long in the environment [6, 9, 10]. Therefore, degraded DNA from dead organisms is thought to be simply a source of microbial food rather than the stuff of natural transformation. Intriguingly, such degrading extracellular DNA is found in huge amounts in the environment. There are typically several micrograms of DNA per gram of soil and sediment, and of this, more than half may belong to extracellular fragments in various stages of degradation and fragmentation [11]. Globally, this runs into

gigatons of extracellular DNA [12]. Rivers alone release around 859–14,500 tons of sedimentary DNA yearly [13]. Much of the extracellular DNA ends up as food for bacteria or as single nucleotides as a result of enzymatic fragmentation or spontaneous degradation processes such as hydrolysis and oxidation [14]. Nevertheless, studies have shown that short DNA fragments may persist for tens of thousands of years [15, 16], and over half a million year-old environmental DNA is reported from frozen environments [17, 18]. Additionally, it was recently reported that an ancient horse bone (dated to 735 ± 88 thousand years ago) recovered from permafrost yielded enough short DNA fragments to patch together a full genome, albeit at low coverage [19]. If such ancient DNA still carries enough sequence information to retrieve a genome, it might have a biological effect as well.

In November 2013, we established that small DNA fragments, (down to 20 bp in length), purposely damaged by the introduction of abasic sites, crosslinks, or deamination, can be taken up by natural transformation in the soil bacterium *Acinetobacter baylyi* [13]. Furthermore, we showed that the mechanisms behind such DNA integration is different from that of kilobase-long fragments, and solely depends on the structure of replication forks in DNA replication (Fig. 1). As such, the integration mechanism is spontaneous and very simple when compared to "classical" natural transformation that requires recombination proteins such as RecA. In support of this notion, previous studies with artificial transformation have also reported that short DNA fragments can mutagenize cells in a RecA-independent manner [20–27]. Furthermore, several of these authors have similarly suggested that annealing at replication forks is the mechanism behind the process. However, prior to this, the shortest DNA observed to naturally transform bacteria was 294 bp: consequently, short DNA recombination was considered to be relevant only for genetic engineering [28].

The universality of the mechanism behind the uptake of short and damaged DNA implies that its integration can in principle occur in any cell. Thus, it is not unreasonable to imagine that each time a cell encounters short and degraded DNA there is a probability, albeit small, that the cell is transformed. This may even happen with truly ancient DNA, as we showed with the natural transformation of a bacterium even with DNA from a 43,000-year-old woolly mammoth. Importantly, we would like to emphasize that at this time it remains unknown how often natural transformation of short and degraded DNA takes place in natural settings. As such its evolutionary implications – the topic of this essay – are highly speculative, but nevertheless interesting, considerations.

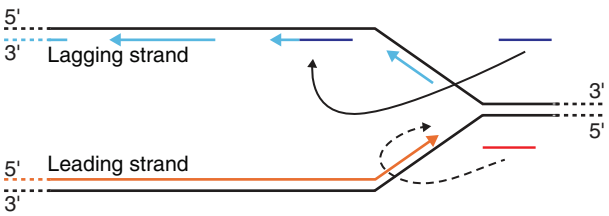

Figure 1. Short DNA integration at replication forks. During DNA uptake both gram-negative and gram-positive bacteria degrade one strand of the DNA helix and release single-stranded DNA into the cell. Subsequently, during genome replication, single-stranded DNA fragments can bind the open chromosome near the replication fork and function as primers of new DNA. In this way DNA can be incorporated in the genome of one of the daughter cells. Because of more open, accessible, chromosomal DNA in the lagging strand, DNA fragments have a better chance of binding there rather than at the leading strand.

Short extracellular DNA recycles and causes anachronistic evolution

Nucleic acids are an important source of phosphate, which is often a growth-limiting substrate for plants and animals. In addition, DNA represents an energy source supplementing that derived from carbohydrates, lipids, and proteins [12, 29]. Thus, it is not surprising that many microbes transport DNA chains into their cells.

When DNA is deposited outside the cell it gradually disintegrates into short fragments – often less than 100 bp in length – and accumulates damage such as abasic sites, crosslinks, and miscoding lesions [10, 30, 31]. Thus, degradation produces genetic diversity in extracellular DNA. If integrated into bacterial genomes, such damaged DNA may further produce new genetic variation during genome replication because of the increased "risk" of replication errors. In other words, two direct evolutionary implications of natural transformation of short and degraded DNA in natural settings are: (i) that DNA of dead cells is a direct contributor to the genetic diversity on which evolution works in living cells and (ii) that higher genetic diversity in dead cells will speed up the evolutionary processes in living cells (Fig. 2). These principles distinguish themselves fundamentally from classical evolutionary theory where dead organisms have no direct genetic impact on the evolution of the living. The recognition of HGT as an evolutionary driver in microorganisms has pushed back this boundary, but only to recently dead organisms, because gene-length DNA does not persist beyond a few contemporary generations [6]. Nonetheless, there is an additional possibility of genetic recycling after many generations – in principle as long as short DNA fragments persist in the environment. We call this phenomenon anachronistic evolution [13]. We now know that fragmented and damaged DNA down to at least 20 bp can in principle be incorporated into bacterial genomes. Thus, even DNA from the last ice age retains the potential to change bacterial genomes. In other words, DNA that has remained "dormant" in the environment for many generations can be re-acquired by microbes and result in genetic recycling.

For the long-term evolutionary impact of genome changes, the frequency of introduction of fragmented foreign DNA is not the key factor. Selective advantage will determine if a genome change will become established in a population or not. Rate of transfer in the population merely determines the speed with which the establishment occurs (how many generations). It does not matter if a thousand (or a million) transformation events are disadvantageous; it is the one transformation event that results in a selective advantage that matters to the following generations [2]. What is more, the one-hit-kinetics that we determined experimentally in the

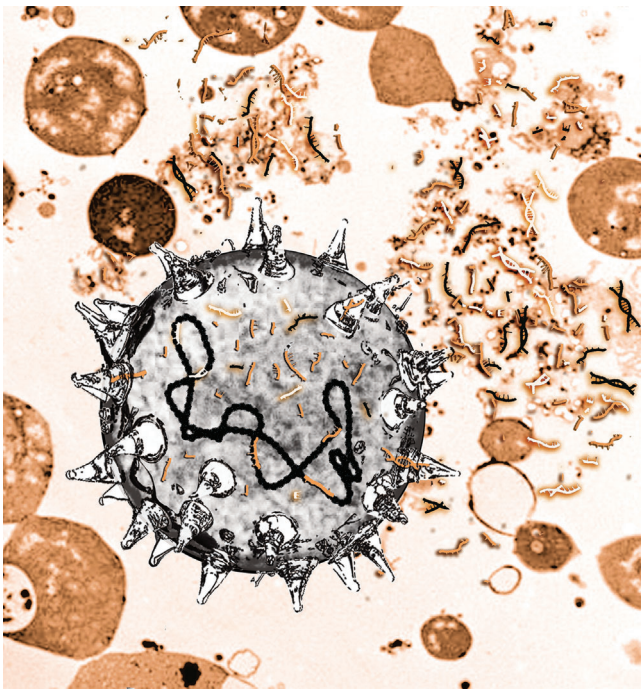

Figure 2. Microbe salvaging DNA garbage: Extracellular DNA that a microbe encounters can be taken up via surface pili structures. Although most of the extracellular DNA will be re-metabolized, some short and damaged DNA may diffuse into contact with the cell's genome. When this happens there is a probability that the incoming DNA binds during replication and causes genome changes in one of the daughter cells. Depending on the DNA sequence, new diversity may be generated or old genotypes may be reintroduced.

PNAS paper [13] shows that individual DNA molecules have an equal probability of transforming the bacteria regardless of high or low DNA concentration. Of course, if there is never an introduction event of short DNA into bacteria, then no genome changes are induced and no selection will operate. However, because of the simplicity of the short DNA natural transformation process, we find that this process is likely to occur once in a while in the microbial world.

Environmental conditions change through time and may result in specifically adapted gene variants being outcompeted by others. Later, the environment may change again and become similar to earlier conditions. In such circumstances, incidental reactivation of old dormant DNA sequences already adapted to prior conditions may take place. Because of the short length of old DNA, entire genes are unlikely to transfer across time. However, short sequence variants of functional importance may change parts of extant cellular genes. Through this process evolution may loop back on itself and restore a previous genotype. This concept expands the idea of microbes having access to a gene bank in the environment. Importantly, however, genetic recycling and anachronistic evolution is not a microbial "Jurassic Park". A microbe will not be turned into a completely different microbe. By definition, short DNA natural transformation occurs with fragments of DNA that hardly ever include full genes. Therefore, the evolutionary result of such recombinations will often be difficult to distinguish from mutational processes. Actually, it may be that a significant fraction of DNA changes that we identify as random mutations are in fact the result of recombination with damaged environmental DNA.

Did early life experience horizontal gene transfer through short DNA natural transformation?

In recent years HGT has emerged as being a pervasive, fundamental and important evolutionary process across many microorganisms. It is even argued that HGT has been highly active and important since the beginning of cellular life and that the complexity of life, as we know it, may not have evolved without HGT [32, 33]. Some scientists have argued that even the genetic code has been, and probably still is, under evolutionary optimization [34]. They propose that the code probably reached the current state early in life's history, but they also suggest that the code is maintained in its current form as a result of the long-term advantages of having access to a large pool of genetic innovation. Furthermore, it has been argued that the amino acids arginine and tryptophan were added to the universal genetic code only after the divergence of the three domains, indicating that the universal genetic code is not a frozen accident, but under constant evolutionary adaptation including HGT [35–37]. Given the simplicity of natural transformation by short and degraded DNA, it is tempting to speculate that such transformation may, functionally speaking, represent one of the earliest forms of cellular HGT as a simple by-product of utilizing nucleotides as food sources.

In some environments, especially the oceans, nucleotides can diffuse far from their source and still carry retrievable information. During early life, this might have promoted a shared evolution of microbes living across a large physical area, where cells integrate similar, albeit partly degraded, sequences and maintain "genetic coherency" despite lacking close proximity. For example, microbial life in deep-sea vents, which by some are believed to be the original habitat of life [38], would imply that cells would not easily be able to change genetic information across vents unless they can exchange and take up degraded molecules. In this manner, early life in ecological niches physically separated from each other may still have shared a common evolution due to diffusion of nucleotides through seawater. Furthermore, short DNA recombinations are a consequence of strand annealing, which is simply the chemical and physical behaviour of polynucleotides, both RNA and DNA strands. Therefore, similar recombinations are to be expected for RNA/DNA strands at pre-cellular evolutionary stages, which are hypothesized to precede the establishment of fully fledged cells with tight membranes.

Eukaryotic meiosis is a sophisticated type of horizontal gene transfer

Random recombination occurs in all cells. Assisted recombination appears to occur in most cells in the form of

homologous recombination. Homologous recombination is important in asexual single-celled microorganisms because it counters a build up of mutational load of slightly deleterious mutations: this is termed "Muller's Ratchet" [39, 40]. Since all cell lines experience mutations, and because most mutations are detrimental, a clonal population with no HGT will deteriorate over the long term. The reason is that a clonal population selects for the least deleterious – though still disadvantageous – mutations, rather than expelling such mutations through homologous recombination. In eukaryotes, sexual recombination is thought to prevent the "Muller's Ratchet" [41]. In bacteria and archaea it is HGT that works against the Mullerian accumulation of mutational load. Therefore, homologous recombination must be an ancient biological process that predates the establishment of sexual reproduction/meiosis in eukaryotes.

Recombination with random extracellular DNA must often cause deleterious effects. For a single-celled species this may not be a significant problem because an unlucky cell simply dies, while other colonial cells continue uninterrupted. However, for multicellular species such deleterious mutations may have severe consequences for the remaining cells. In combination with protective measures (physical and enzymatic) against entirely random recombination, sexual recombination allows stable evolution of complexity in cell lines that can support division of labour and, by extension, proper cellular differentiation.

All eukaryotes seem once to have had sexual reproduction. Meiosis is thought to have evolved at the establishment of the eukaryotic domain, and sexual reproduction is therefore basal to eukaryotes [42, 43]. Many, especially single-celled, eukaryotes can reproduce clonally, but still they sometimes undergo sexual reproduction; a traditional example is that of mating types in yeasts. Thus, although for example the invertebrate group of Bdelloid rotifers lacks sexual reproduction today, it is likely a secondary loss [44]. Simple eukaryotes may be able to thrive asexually; however it is striking that Bdelloid rotifers are amongst the few eukaryotes known to carry out "traditional" HGT by natural transformation [45, 46]. Perhaps Bdelloid rotifers reverted to the strategy of unspecific HGT to compensate for the loss of sexual recombination. The unavoidable genetic interference from unspecific recombination is disruptive to cellular differentiation, especially in multicellular organisms. Cellular differentiation depends on precise regulation of specific functions. Both regulation and function are therefore highly vulnerable to mutations and random recombination. As eukaryotes – via meiosis – only recombine with homologous sequences of high similarity (i.e. species members), gene function is maintained, while Muller's Ratchet is avoided.

Based on the above observations and considerations, it is tempting to view the homologous recombination of classical natural transformation with long DNA fragments as a mechanism that evolved because it improves on random short DNA recombination; the improvement being that genetic exchanges are biased toward longer homologous events with fewer deleterious results. By logical inference, short DNA natural transformation occurred first because it only requires DNA uptake (for nutrient-salvaging for example). Classical natural transformation requires the coupling of DNA uptake and homologous recombination: as a result, homologous recombination must have evolved before classical natural transformation – a merger of DNA uptake and recombination – could evolve. Successful repair of DNA damage could very well be the direct underlying selection pressure that has driven such evolution, which then subsequently found use as a mechanism of genome evolution.

Similarly, sexual reproduction may be considered to be a later refinement of evolutionary processes, as genetic exchange is biased toward even longer homologous exchanges with fewer deleterious results. As such, sexual reproduction is similar to classical natural transformation. It ensures homologous recombination of useful genes and reduces interfering random recombination across the genome. In that respect, we suggest that there has been a progression in evolutionary strategies (but not necessarily through protein homologs) from random short DNA recombination over long DNA natural transformation to tightly regulated homologous recombination in the form of meiosis. Sexual reproduction is traditionally considered to be in opposition to and fundamentally different from HGT, because – more or less by definition – sexual reproduction occurs within species and HGT happens between species. However, the uncovering of short DNA natural transformation has led us to see meiosis as a refined gene-exchange mechanism that *defines* species borders and not a process that is *limited* by species borders. Seen in that light, sexual reproduction is a sophisticated type of horizontal gene transfer.

Gene transfers in eukaryotes maintain genetic links to bacteria and archaea

The many characteristic features of eukaryotes did not arise in the blinking of an eye. However, today we only see the successful end product of that development. It is interesting to speculate that early eukaryotes passed through multiple bottlenecks at high evolutionary speed. The establishment of mitochondria is plausibly the founding event for eukaryotes [47]. Introns, the nuclear envelope, the endoplasmic reticulum and other cell compartments likely developed as a consequence of the endosymbiotic establishment of mitochondria [48]. Endosymbiotic relationships allow (unidirectional) HGT from (usually bacterial) endosymbionts to the host cell (often a eukaryote): this is termed "endosymbiotic gene transfer". Several examples are known. Mitochondria and chloroplasts are the most obvious, but there are also several other types of plastids of endosymbiotic origin, for example the apicoplast of the malaria-causing parasite *Plasmodium falciparum*. Furthermore, in lichen a symbiotic relationship exists between fungus and photosynthetic algae or cyanobacteria; a further example of endosymbiosis is *Wolbachia spp*., a parasitic intra-cellular bacterium that infects many insects and other arthropods [49]. Endosymbiotic gene transfer is in contrast to sexual reproduction. We see sexual reproduction as a process that helps organisms to maintain complex biological systems, which are sensitive to disturbances, by selectively recombining only with other organisms that are very similar, thereby defining species. In

case of endosymbiotic gene transfer the eukaryotic barriers to HGT are not very efficient. These kinds of transfers are predominantly evolutionary one-way transfers, where parasites or symbionts are donors of genes to host cells. Transfers of DNA can occur in the opposite direction, but these rarely acquire any function and therefore they are normally not maintained evolutionarily. There seems to be a fundamental drive towards collecting cellular genes in one genetic system [50]. The reason behind this is not resolved yet, but the observed reduction of organellar genomes apply to all types of endosymbiotic cell compartments that have, or have had, separate DNA. Because endosymbiotic gene transfer does not happen with random environmental sources of extracellular DNA it differs from HGT, which is typically an arbitrary phenomenon. The physical and enzymatic barriers that eukaryotes have raised against HGT work poorly against endosymbiotic gene transfer, because the DNA is already inside the cell. Consequently, the relatively low eukaryotic barriers against endosymbiotic gene transfer compared to random HGT represents a continuous genetic link to archaea and bacteria that is probably still evolutionary active today. This could be assisting in maintaining a more or less universal genetic code across the three domains of life.

Concluding remarks

Horizontal gene transfer of long DNA fragments from one extant cell to the next is already known to influence the rate and means of bacterial evolution. Now, we need to consider that the massive amounts of highly degraded DNA in the environment may also be subject to HGT, implying that the diversity of dead cells in the environment influences the speed of evolution of living cells. Currently, we do not know to what extent this process takes place under natural settings; however, the immeasurable amounts of DNA and microbes in the biosphere supply ample opportunity for short DNA natural transformations to occur. DNA is found in most environments on Earth, and, particularly in sediments, DNA can survive tens of thousands of years. This provides an unrecognised potential for genetic transfers across timescales of many generations, and a cause of anachronistic evolution. Furthermore, the simplicity of the DNA integration process suggests it was occurring far back in time, maybe even in early life on Earth.

Sexual reproduction has a longstanding position as the supreme type of reproduction in evolution – one that gave rise to complex multicellular plants and animals. Sexual reproduction is viewed as opposite to HGT, a paradigmatic divide that is often presented as vertical descent versus horizontal/lateral exchanges. However, this opposition is an artificial construct in our view. Rather, sexual reproduction represents a type of controlled selective HGT that optimizes the evolutionary advantage of homologous genetic exchange by maintaining relatively high barriers to random interfering transfers. Furthermore, in our opinion sexual reproduction was a prerequisite for the evolution of multicellularity due to this shielding from "genetic interference" from random HGT that allowed for a stable cooperation of cell consortia, eventually resulting in true multicellularity.

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