Trends in Genetics

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Opinion

Horizontal Gene Transfer in Eukaryotes: Not if, but How Much?

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Horizontal gene transfer (HGT), the movement of genetic material across branches of the tree of life, is well established in prokaryotes and uncontroversial. This is explained in part by relatively compact prokaryote genomes that facilitate assembly and gene prediction, resulting in thousands of complete genomes for analysis. By contrast, their large and often complex genome structure have thwarted HGT studies of eukaryotes. The tide has recently turned with the availability of sufficient high-quality genome data to address quantity and quality of HGT in these taxa. Here, we argue that HGT is a small but significant player in the evolution of microbial eukaryotes and provide examples where HGT has facilitated gain of adaptive functions and in some cases, underpinned major lifestyle transitions.

Horizontal Gene Transfer in Eukaryotes and Prokaryotes

Horizontal gene transfer (HGT) (see Glossary) is the nonlineal movement of genetic material across the web of life that creates reticulate gene phylogenies. This process is of high interest because it can drive functional innovation through the introduction of novel genes and pathways [1]. HGT is a common occurrence across all domains of life; however, most transfers are ephemeral and not transmitted to the next generation. For example, they may be introduced into somatic tissue in a multicellular organism and lost after that generation, or, if integrated into the germline or acquired in a unicellular organism, they do not become fixed within the population (e.g., they are weeded out by natural selection and/or drift) [2]. HGT is well studied in prokaryotes, and extensive or open **pangenomes** have resulted in ambiguous species definitions and problematic phylogenetic reconstruction [3]. Genes are readily exchanged between different bacterial and archaeal cells, either picked up from the environment or transferred by vectors such as viruses, plasmids, and **gene transfer agents (GTAs)**. Due to the unicellular and asexual nature of these cells, once a new gene integrates into the genome, it can be propagated throughout the population. Many studies of prokaryote gene sharing have been published, showing varying degrees of HGT across different taxa [4–7]. In organisms such as *Escherichia coli* that have particularly extensive pangenomes, up to 80% of genes may be the product of horizontal transfer at some point in the organism's evolutionary history [4,5]. By contrast, HGT in eukaryotes is much rarer, and its extent and role in adaptive evolution are the focus of many recent studies, including some that are actively debated [8–12].

HGT in Microbial Eukaryotes

Most microbial eukaryotes (protists and some fungi) are unicellular, predominantly asexual, and represent a taxonomically diverse assortment of organisms and lifestyles whose members comprise the earliest divergences within all major multicellular lineages, such as animals, plants, and seaweeds [13]. Protist and algal clades include a number of other major evolutionary transitions, such as from free-living to pathogenic (e.g., oomycetes [14]), mesophilic to extremophilic (ice algae [15]), and marine to freshwater habitats (and vice versa; green algae [16]) that may have been facilitated by HGT. Many microbial eukaryote genomes have recently become publicly

Highlights

Horizontal gene transfer (HGT) is emerging as a significant contributor to eukaryotic genomes, challenging previous assertions that HGT is restricted to prokaryotes and only relevant to eukaryotes during organellogenesis.

HGTs often confer an adaptive advantage to the 'host' organism, and many of these adaptations significantly enhance metabolic pathways, leading to lifestyle shifts or survival in highly fluctuating environments.

Protists that exhibit a range of lifestyles, including photosynthesis, mixotrophy, polyextremophily, and parasitism, comprise the earliest divergences in major multicellular lineages, making them models for understanding the role of HGT in evolutionary transitions.

HGT represents, on average, about 1% of protist gene inventories, although this 'rule' needs to be tested in the future using more data and a standardized pipeline for HGT quantification.

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available, leading to dozens of studies of HGT and novel gene origin (Box 1) and their role in underpinning novel adaptation in eukaryotes.

Among phytoplankton, HGTs comprise a small but important component of the gene inventory and either serve as replacements for genes with similar functionalities or encode novel functions, some of which have allowed organisms to inhabit new environments, including those considered extreme [17,18]. One such example is in the Cyanidiophyceae, a class within the largely mesophilic red algae (Rhodophyta) that contains various genera, species, and strains of unicellular algae with polyextremophilic lifestyles. These specialized eukaryotes have transitioned to extreme hot spring environments in part due to an array of HGTs that were likely acquired from resident extremophilic prokaryotes [18–20]. A recent study that analyzed 13 genomes proposed that HGTs make up approximately 1% of each cyanidiophycean gene inventory, an observation referred to as the '1% rule' [20]. Another study focused on a more taxonomically diverse suite of mesophilic organisms and reported similar findings of 0.16–1.44% HGT across 23 phytoplankton genomes found within cryptophyte, rhizarian, alveolate, stramenopile, and haptophyte (**CRASH**) lineages [1]. In this opinion article, we discuss the amount and function of HGTs (primarily of prokaryote provenance) that have been characterized in organisms across different lifestyles and niches, what this means for HGT as a fundamental force of eukaryotic evolution, and areas of future research.

Examples of HGT Driving Adaptive Evolution in Microbial Eukaryotes

With significantly more protist, algal, and yeast genomic data and associated studies available, it is possible to investigate robustly supported instances of HGT (Figure 1) to assess if they are adaptive gains associated with environmental shifts and, in some cases, to explore how HGT happens (Box 2). Here, we explore these cases and identify trends in terms of gain of function, recognizing that most HGTs are destined for loss and, in some cases, when neutral or nearly neutral, may later become adaptive [21] (Figure 2).

Extremophiles

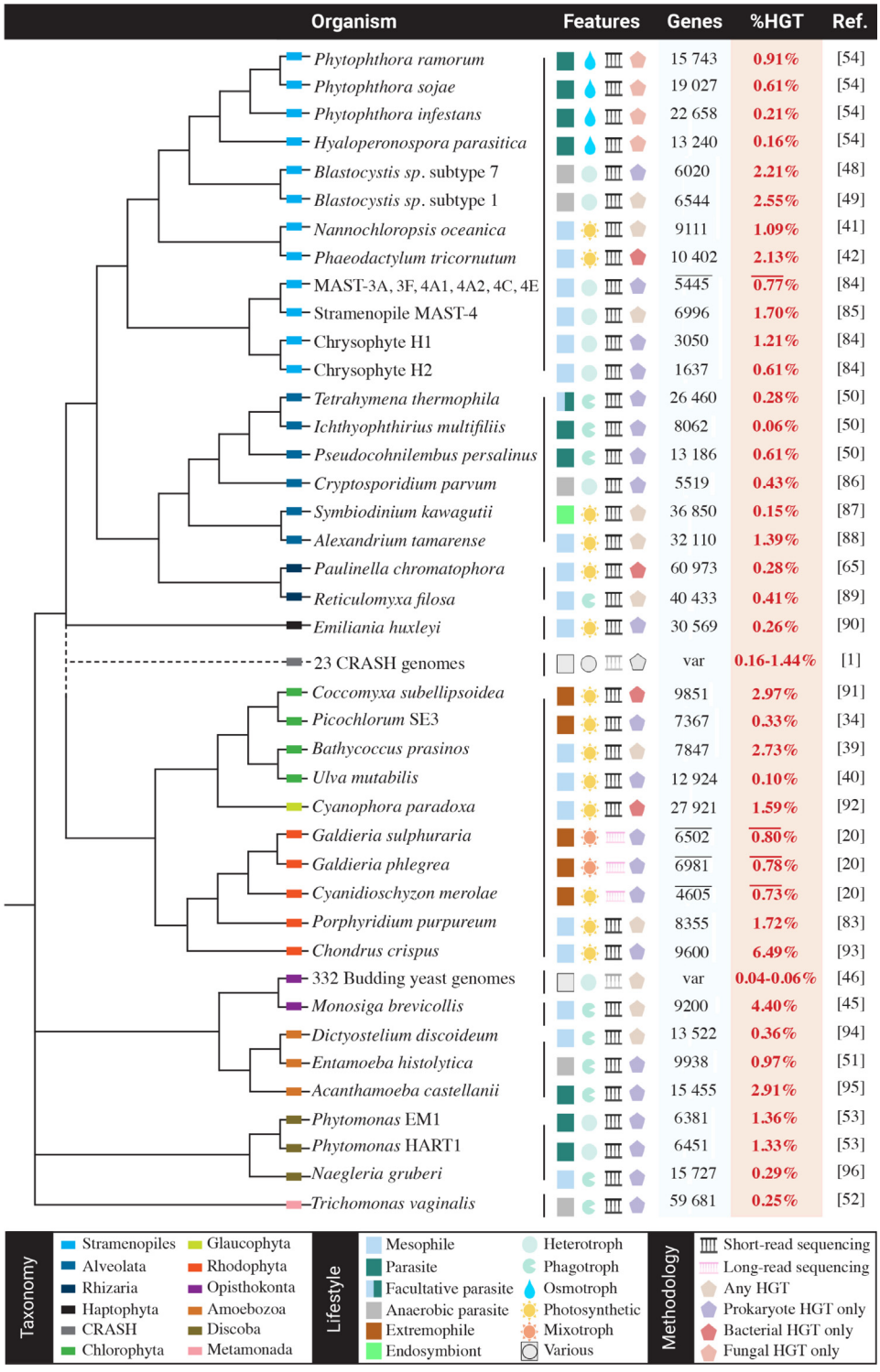
The most striking example of HGTs providing adaptive functions are found in organisms that occupy a new niche, particularly one in which its closest phylogenetic relatives could not survive. In polar climates, ice-binding proteins (IBPs) from prokaryotic donors have been identified and experimentally validated numerous times across disparate protist lineages. In general, IBPs function to modify the external environment to reduce the potential for freezing injury and water loss in cells [22]. Examples include a study of sea ice diatoms, a prymnesiophyte (*Phaeocystis antarctica*), and a prasinophyte (*Pyraminonas gelidicola*) in which the phylogenetic relationships resolved in single-gene trees for IBPs conflict with the expected species relationships based on analysis of 18S rDNA [22]. In addition, IBPs share significant sequence similarity with prokaryotes, including those that exhibit ice-binding activity [22]. Similar findings have been found with *Chlamydomonas* sp. IBPs [23] and links between an ice-specialized bacterial donor (*Psychroflexus torquis*) and other activities that aid in survival in icy environments, such as synthesis of polyunsaturated fatty acids [24]. Although closely related to the *Chlamydomonas* lineage, IBPs of the snow alga *Chloromonas*

Box 1. Novel Gene Origination

Traditionally, gene and genome duplication followed by divergence were considered to be the main drivers of eukaryote evolution. However, recent studies across many different systems have shown that exon shuffling, origin of novel genes from previously noncoding regions, transposon-mediated recombination, gene fusions resulting in promoter capture, and other processes are also important mechanisms of generating genetic novelty [2,98,99]. What is largely missing from these studies is the role of horizontal gene transfer (HGT) in gene origin, particularly in non-model eukaryotes (i.e., excluding classic model systems such as *Drosophila*, many plants, and bacteria). Microbial eukaryotes (i.e., protists and algae), with their thousands of novel or 'dark' genes (e.g., [100]) that are new to science, are ideal models for studying the mechanisms of gene origin, particularly through HGT.

Glossary

CRASH: an informal phylogenetic grouping [1] that includes a diverse collection of photosynthetic and nonphotosynthetic organisms from the following lineages: Cryptophyta, Rhizaria, Alveolata, Stramenopila, and Haptophyta.
Dark genes: genes that are either novel to science, that is, they do not share significant sequence identity with proteins in large databases such as the manually curated UniProt, or are too highly diverged to allow identification of putative homologs. These genes may confer functions that differentiate lineages of eukaryotes and prokaryotes.
Gene transfer agents (GTAs): DNA-containing virus-like particles produced by prokaryotes (bacteria and archaea) that mediate the horizontal gene transfer of approximately 4–5-kbp fragments of DNA between different cells.
Horizontal gene transfer (HGT): also called 'lateral gene transfer' (LGT), is the acquisition of genetic material by the genome of one organism from the genome of another that is not derived from vertical inheritance. HGT can involve the movement of genetic material between similar organisms or across domains of life and can result in reticulate evolution and functional innovation.
Muller's ratchet: the process by which a genome irreversibly accumulates deleterious mutations. This occurs most often in asexual organisms due to the lack of sexual recombination. Muller's ratchet is also a crucial process in organelle evolution whereby the captured endosymbionts no longer freely exchange genes with other organisms and subsequently undergo massive gene loss and genome reduction.
Pangenome: the set of all genes present across all individuals of a particular species; it includes both the 'core' genes found in all members of the species and 'accessory' genes, which are restricted to a subset of species.
Tripartite model: The tripartite model of plastid origin postulates that plastid endosymbiosis in the Archaeplastida ancestor involved three partners – the eukaryotic host cell, the cyanobacterial endosymbiont, and a chlamydial pathogen – that facilitated metabolic integration of the novel photosynthetic organelle.



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Box 2. Horizontal Gene Transfer (HGT) and Experimental Evolution

Due to their small genome size and rapid doubling time, most experimental evolution (EE) studies have been carried out on prokaryotes, with a small subset focusing on HGT [101,102]. In eukaryotes, EE studies dealing with HGT are also sparse, but a few notable examples exist. Grafting experiments in plants have shown that plastids can traverse grafting sites and be incorporated into the cells of a non-interbreeding species [103]. A similar pattern was seen in plant grafting experiments that focus on mitochondria, however parts, and not the whole mitochondrial genome, were transferred [104]. Interestingly, various red algae have plasmids that are capable of transferring foreign DNA into the genomes of plastids, mitochondria, and the nucleus of other eukaryotes [105]. Moreover, it has recently been shown that plasmids from bacteria can replicate in the nucleus of the red algae *Porphyridium purpureum* [106]. These genetic elements can be used as a transformation system in this alga. *P. purpureum* expresses plasmid genes at high levels, but instead of incorporating the plasmid DNA into algal chromosomes, they are maintained episomally in high copy numbers that replicate using the bacterial machinery [106]. It is intriguing that so many plasmids reside in the nucleus of *P. purpureum* and that the nuclear genome of this species contains many horizontally acquired genes [83]. However, direct evidence of plasmids as mediators of HGT in red algae has not yet been reported.

brevispina are more closely related to bacterial sequences than to *Chlamydomonas*, suggesting that both chlorophyte groups acquired IBPs, and thus psychrophilic traits, independently from a prokaryotic source [25]. Comparable findings exist for the Antarctic ciliate *Euplotes focardii* [26] and the dinoflagellate *Polarella glacialis* [27].

On the other end of extremes is high temperature, for which HGTs have been implicated in the evolution of thermophily [18,20]. The most prominent example is the Cyanidiophyceae, including the genera *Galdieria* and *Cyanidioschyzon* that split about 1 billion years ago and have maintained a thermophilic lifestyle [28]. The common ancestor of all Rhodophyta may also have been an extremophile because of its highly reduced predicted gene inventory and the presence of bacterium-derived HGTs with functions related to withstanding salinity and other stresses [28]. Fossil evidence suggests that mesophilic red algae are at least 1.2 billion years old [29,30]; therefore, the ancient split of Cyanidiophyceae and other red algae, supported by many studies [20,31,32], predates this time point. HGTs associated with the transition to high temperature stress in Cyanidiophyceae include an *Hsp20* homolog and thermostable enzymes such as α-xylosidase that is present in all sequenced *Galdieria* strains and species, as well as proteins that mitigate free radicals that result from the higher metabolic rates due to high temperature [20]. Other examples include thioredoxin oxidoreductase and a putative glutathione-specific γ-glutamylcyclotransferase 2 found in both genera, multiple peroxidase-related enzymes in *Galdieria*, and a cytosolic and/or extracellular peroxiredoxin-6 in *Cyanidioschyzon* [20]. Species in the genus *Galdieria* are far more versatile in terms of habitats than *Cyanidioschyzon*, with *Galdieria sulphuraria* (nine diverged strains sequenced) and *Galdieria phlegrea* (two closely related strains sequenced) able to thrive in conditions ranging from hot springs to acid mining sites characterized by pH approaching 0, high-salt environments, and waters or sediments rich in arsenic and mercury [33]. *G. phlegrea* is adapted to dry habitats near fumaroles such as fissures between rocks or cryptoendolithic environments [19]. Analysis of *Galdieria* genomes identified HGTs related to all of these traits that function in metal and xenobiotic resistance/detoxification, carbon metabolism, amino acid metabolism, one-carbon metabolism and methylation, urea uptake and utilization, and osmotic resistance and salt tolerance [19,20]. *Galdieria* species are facultative heterotrophs, able to discontinue photosynthesis and feed on a multitude of

Figure 1. A Schematic Phylogenetic Tree Showing Traits Associated with Horizontal Gene Transfer (HGT) in Protists and Algae. This tree includes data from species with completed nuclear genomes that explicitly quantified HGT. The tree topology is based on [82], and within-clade branching order is based on the National Center for Biotechnology Information taxonomy. Under 'methodology,' viruses were excluded because some papers specifically searched for HGTs from viruses and some did not, but many did not specify. Note that the branch that contains 23 cryptophyte, rhizarian, alveolate, stramenopile, and haptophyte (CRASH) genomes in the tree represents polyphyletic taxa that individually would appear at different places within this phylogeny. Because these data are from one large study that groups them together, we included them as a single, independent branch solely for the sake of simplicity. 'Features' icons were created with BioRender.com. See [1,20,34,39–42,45,46,48–54,65,83–96].

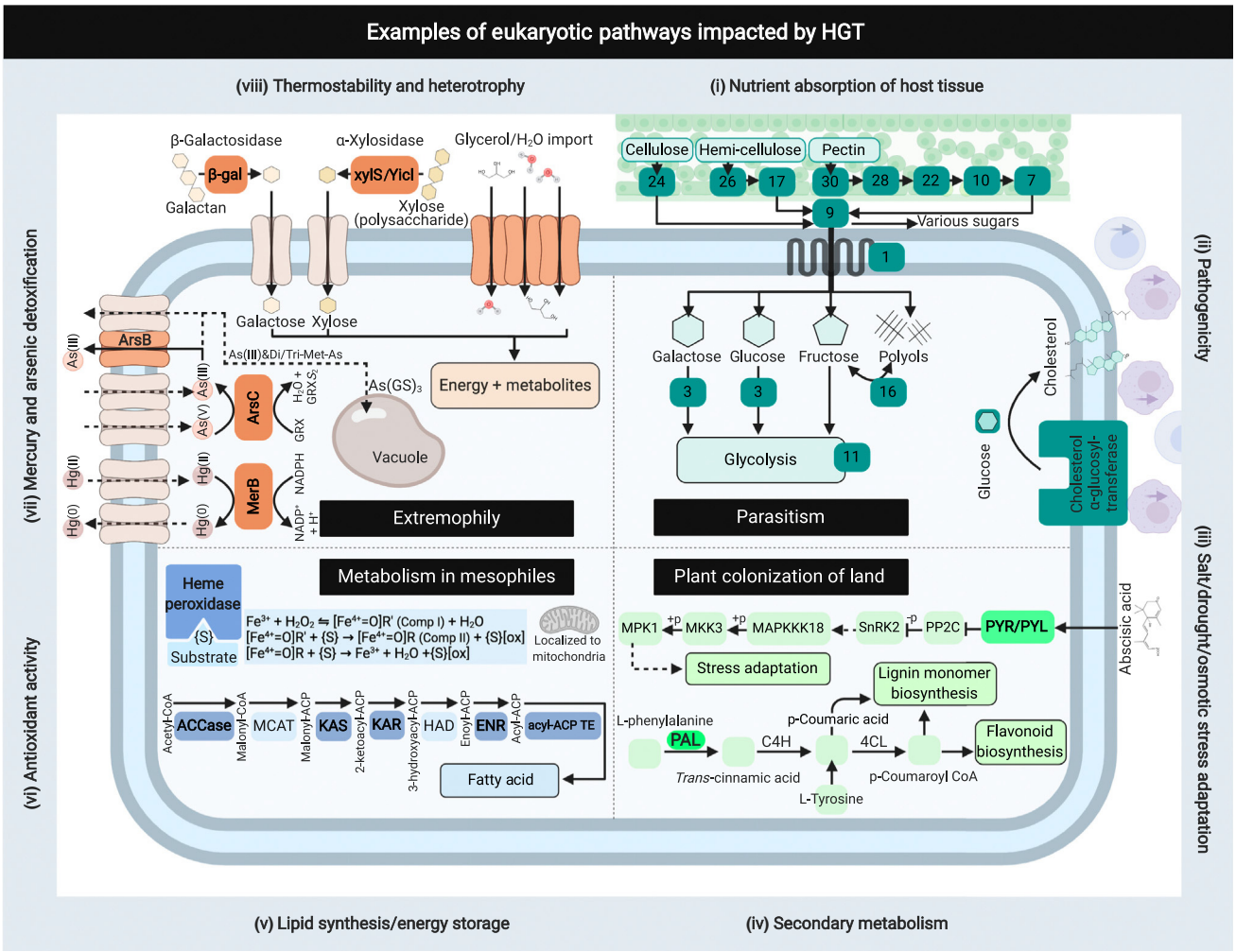

Figure 2. Functions of Horizontal Gene Transfer (HGT)-Derived Genes in Different Algae and Protists Shown in a Single Idealized Cell Image. Schematic image highlighting some major eukaryotic cell pathways impacted by HGT. These gene acquisitions play key roles in protist and algal transitions to different environments. All dark colors indicate putative HGTs, whereas muted colors indicate native pathway components. The figure was created with BioRender.com. Top right quadrant: Parasitism. (i) Nutrient absorption of host tissue: this pathway is adapted from [54] and shows how oomycetes are able to use both extracellular and intracellular enzymes derived via HGT to break down plant polysaccharides into molecules compatible with their own energy-generating mechanisms. The numbers in the protein boxes correspond to numbered HGTs from [54]. (ii) Pathogenicity. this pathway is adapted from [49] and shows the α-glucosylation of cholesterol, which leads to host immune response evasion by *Blastocystis* sp. Bottom right quadrant: plant colonization of land. This region shows two metabolic pathways with HGT-derived enzymes that enabled plants to colonize land. (iii) Salt/drought/osmotic stress adaptation. This pathway is adapted from data in [67] implicating the PYR/PYL protein (a putative HGT) as a possible contributor to plant terrestrialization and the corresponding KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway in *Arabidopsis thaliana*, which places this protein in a pathway related to stress adaptation. (iv) Secondary metabolism. This pathway is adapted from [97], where PAL is implicated as an HGT-derived enzyme that was acquired in the land plant ancestor and plays a role in phenylpropanoid metabolism. Bottom left quadrant: metabolism in mesophiles. This region shows two metabolic pathways with HGT-derived adaptations in mesophiles. (v) Lipid synthesis/energy storage. This pathway is adapted from [41] and shows the fatty acid synthesis pathway in *Nannochloropsis oceanica* IMET1. (vi) Antioxidant activity. Adapted from [40], this pathway shows the function of heme peroxidase. This HGT-derived gene in the *Ulva mutabilis* genome has undergone repeated duplications. Top left quadrant: extremophily. This region shows multiple metabolic pathways related to survival in extreme environments. (vii) Mercury and arsenic detoxification. These pathways are from [20] and show HGT-derived enzymes in the *Galdieria* lineage for the detoxification of Hg and As. (viii) Thermostability and heterotrophy. Also taken from [20], from left to right shows β-galactosidase activity, thermostable α-xylosidase activity, and a glycerol transporter, all pathways related to carbon metabolism. Abbreviations: ACCase, acetyl-CoA carboxylase; Acyl-ACP TE, Acyl-acyl-carrier-protein thioesterase; ENR, Enoyl-acyl-carrier-protein reductase; HAD, Hydroxyacyl-acyl-carrier-protein dehydrase; KAR, Ketoacyl-acyl-carrier-protein reductase; KAS, Beta-ketoacyl-acyl-carrier-protein synthase; MAPKKK18, mitogen-activated protein kinase kinase kinase 17/18; MCAT, malonyl-CoA-acyl carrier protein transacylase; MKK3, mitogen-activated protein kinase kinase 3; MPK1, mitogen-activated protein kinase 1; PP2C protein phosphatase 2C; SnRK2, serine/threonine-protein kinase SRK2.

carbon sources, an ability that can be linked to many HGTs found to function in carbon metabolism [20,33]. Similarly, analysis of the green alga *Picochlorum* SE3 identified HGTs with potential roles in tolerance to fluctuating salinity and light conditions in its estuarine habitat, including enhancement of carbohydrate metabolism and cell surface modification [34]. HGTs have also been associated with increased salt tolerance in heterotrophic protists, such as the stramenopile *Halocafeteria seosinensis*, in which horizontally acquired peroxidase and NADPH-dependent alcohol dehydrogenase–encoding genes have been identified that both show higher expression under salt stress [35].

Mesophiles

HGTs with potential adaptive functions are easier to discern in extremophiles that often contain small genomes with reduced and specialized gene inventories. However, most microbial eukaryotes are not extremophiles but must nonetheless deal with stressful and changing environments. Can HGTs be attributed to adaptive functions in these latter taxa? Past work suggests that genes encoding metabolic enzymes are good candidates for HGT because they may provide novelty by enhancing metabolic networks already present in recipient organisms [36]. In addition, adding an enzyme to an existing pathway likely increases the chance of successful gene integration into a new genome [37]. If the HGT increases fitness (even marginally), then this gene may undergo duplication to exploit the benefit [38]. Recent work on eukaryotic HGTs supports this idea. Some examples include the green alga *Bathycoccus prasinos*, where about 50% of all putative HGTs encode metabolic functions [39]. In another chlorophyte, the sea lettuce *Ulva mutabilis*, one-half of the HGTs are involved in gene family expansion, with one candidate encoding a heme peroxidase from a single HGT event, giving rise to 36 copies in the genome [40]. By scavenging H_2O_2, this enzyme confers a clear adaptive advantage in dealing with stressors in the intertidal habitat occupied by *U. mutabilis*, including high light, salinity fluctuations, and dehydration [40]. In *Nannochloropsis oceanica*, a marine single-celled stramenopile, most of the HGTs function in metabolic processes, and HGT-derived genes involved in lipid biosynthesis are implicated in oleaginousness and thus energy storage, making this organism a promising candidate for biofuel production [41]. In other stramenopiles such as the marine diatom *Phaeodactylum tricornutum*, HGTs encode novel metabolic functions such as organic carbon and nitrogen utilization, enzymes of the diatom urea cycle, and enzymes involved in cell wall silification, all of which putatively enhance fitness [42]. Other diatoms such as *Pseudo-nitzschia* and *Fragilariopsis* species are able to sequester iron with ferritin, whose encoding gene was horizontally acquired from bacteria, allowing them to bloom in iron-limited waters, a trait absent in most other diatoms [43]. The trend of HGTs encoding metabolic enzymes is also apparent in phagotrophs such as *Dictyostelium discoideum*, an amoeboid slime mold [44], and in the choanoflagellate *Monosiga brevicollis* [45]. In *M. brevicollis*, over one-half of the identified HGTs lack annotations (i.e., are **dark genes**; Box 1); however, those with known functions often encode enzymes involved in carbohydrate metabolism, which likely enhances the ability of the organism to digest diverse food sources [45]. Last, in yeasts, non-protistan microbial eukaryotes, HGT has been studied across 332 genomes that together represent a level of diversity consistent with the plant and metazoan clades [46]. Within these data, 878 putative HGTs were identified in 186 genomes that can be traced back to 365 discrete acquisitions, leading to a quantification estimate of 0.04–0.06% across this dataset, of which a majority of the HGTs are associated with metabolism-related Gene Ontology terms [46].

Parasites

Pathogens and parasites live in close association with their hosts, resulting in reciprocal gene sharing [47]. Many diverse protists are parasitic, some obligate and some facultative, with hosts ranging from plants to fish to humans. These species have relied on HGT from various sources to better adapt to the parasitic lifestyle. *Blastocystis* sp. is an anaerobic parasite that infects humans. When analyzed for putative HGTs, one isolate (subtype 7) encoded genes most closely related to bacterial donors but

homologous to other anaerobic eukaryotic parasites, suggesting that these genes are transferred horizontally between eukaryotes [48]. This species also contains HGTs that encode major facilitator superfamily (MFS) proteins that aid in nutrient absorption from host tissues and others for fermentation that allow it to thrive in anaerobic environments [48,49]. In subtype 1, the patterns of HGT copy number throughout the *Blastocystis* lineage may have an effect on virulence [49]. HGT has also been implicated in conferring virulence in a comparative study of ciliate (alveolate) pathogens, in which the facultative parasite *Pseudocohnilembus persalinus* and the free-living *Tetrahymena thermophila* were compared with the obligate parasite *Ichthyophthirius multifiliis* with respect to quantity and quality of HGTs [50]. The amoebozoan *Entamoeba histolytica* is another parasite with HGTs that appear to play a role in its obligately anaerobic lifestyle, as well as enzymes that allow the cell to metabolize a diversity of amino acids [51]. HGTs in the *Trichomonas vaginalis* genome encode enzymes such as cysteine peptidase that may affect virulence [52]. In the plant pathogen *Phytomonas* sp., HGTs function in carbohydrate metabolism, including a bacteria-type α,α-trehalose phosphorylase that allows this organism to use trehalose, a plant disaccharide, as an energy source that likely supports the parasitic relationship [53]. In trypanosomes, HGTs from bacteria that encode central metabolic functions are so integral to the machinery of the organism that they may serve as effective drug therapy targets [36]. In the fungus-like stramenopiles, the oomycetes, numerous fungus-derived HGTs have been found in four species in two genera, suggesting that fungal plant pathogens likely donated genes that allowed oomycetes to become successful pathogens of plants [14,54]. These HGTs encode enzymes that break down plant cell walls and acquire nutrients from the environment, as well as proteins that aid in resisting plant defenses and attacking plant cell walls [54]. In the microsporidia, fungal obligate intracellular parasites with highly reduced genomes, HGTs have been implicated in facilitating parasitism [55]. Examples include genes encoding ATP transporter and H^+ symporter functions that are present in all sequenced microsporidia, as well as genes that provide bacteriocin resistance, reactive oxygen species (ROS) scavenging, UV protection, and various metabolic functions, many of which likely derive from co-occurring intracellular bacterial donors [55]. Although not exhaustive, the studies highlighted here show a clear trend toward HGT-driven metabolic enhancement and novel functions leading to increased fitness and adaptation to a variety of lifestyles across many protist lineages.

HGTs and Major Evolutionary Transitions

Not only is HGT a ubiquitous process implicated in novel adaptations throughout the web of protist life, but HGT-driven adaptations are also an integral part of major evolutionary transitions. Two of the most noteworthy in this respect are the origin of primary plastids and the colonization of land by plants. The primary endosymbiosis of a cyanobacterium by a phagotrophic protist resulted in the canonical plastid in the ancestor of Archaeplastida [56–58]. This endosymbiotic event is widely hypothesized to have been aided by the contemporaneous acquisition of dozens of Chlamydiae-derived genes, many of which encode proteins that are plastid-targeted [59–61]. These proteins that complement the function of the plastid likely assisted in the transition of the captured cyanobacterium to an organelle and carried out various other vital functions [59–61]. It is postulated that the chlamydial cell(s) implicated in this relationship were either endoparasites or endosymbionts in the Archaeplastida host, and their close association made possible these HGTs (for details, see [60]), although it is important to note that this hypothesis (**tripartite model**) is still an area of active investigation and debate [62–64]. Similar types of compensatory functions of HGTs are also associated with the only other known endosymbiotic event that led to the advent of a primary plastid. In *Paulinella chromatophora*, a rhizarian amoeba with a relatively recently (~100 mya) acquired photosynthetic organelle ('chromatophore'), HGTs compensate for endosymbiont genome reduction due to **Muller's ratchet**. HGTs to the nucleus of *P. chromatophora* fill metabolic gaps resulting from the reduced organellar gene inventory, and many of these bacterium-derived genes have undergone gene family expansion [65,66].

With regard to plant origin, the Zygnematophyceae, streptophyte algae that are sister to embryophytes (land plants), contain HGTs that predate the split of these lineages and played a major role in terrestrialization [67]. As more streptophyte genomes have become available, it is clear that the molecular toolkit for terrestrial adaptation existed in algae prior to embryophyte origin [67,68]. These genes, including microbial terpene-synthase-like genes, *PAL*, *YUC* genes, and possibly *GRAS* and *PYL*/*PYR*/*RCAR* genes, were likely donated by soil bacteria associated with ancestral streptophytes. Following diversifying selection, they underwent gene- and genome-level duplication events and neofunctionalization to result in many of the terrestrial adaptations evident in extant embryophytes [67]. Similar links between HGT and major evolutionary transitions have been suggested in the fungi, animal, and plant kingdoms [69–71], all of which are rooted within protist assemblages.

Extent of HGT in Protist Genomes Based on Available Data

Although generally underrepresented in the literature, a majority of eukaryote diversity is microbial. Protists comprise a polyphyletic assemblage of many kingdom-level groupings of organisms, and due to their largely unicellular and asexual nature, they form the bridge between prokaryotes and more complex eukaryotes, maintaining genomic features and traits of each domain. Thus, focusing on them with respect to HGT is the logical next step to building on the many prokaryotic HGT studies and informing future wide-scale studies in morphologically more complex eukaryotes. With the expansion of whole-genome sequencing projects and increased use of long-read data, the detection of HGTs has become more reliable [72,73]. Long reads (e.g., 50–100 kbp in length) physically link HGT and native genes in a single molecule of DNA, thereby avoiding potential issues with incorporation of contaminant DNA in assemblies derived from short-read data [20]. Figure 1 shows a schematic phylogeny of protist genomes that have been sequenced in which the associated manuscript explicitly quantified HGT. These studies were carried out between 2004 and 2019 and thus used different pipelines, programs, and criteria to identify HGTs. Therefore, the data need to be interpreted with caution because the quantification of HGT was not done in a unified fashion. As denoted in the figure, some studies included all forms of HGT, whereas others focused on prokaryotic donors only, or in the case of the oomycetes, only on fungus-derived HGTs. In addition, differences in filtering parameters such as level of bootstrap support (e.g., >70%) to identify an HGT event or taxon sampling could lead to over- or underestimates of this number. Finally, only the most recent studies represented in this figure used long-read sequencing platforms. Due to this uncertainty, it is difficult to reach final conclusions about the frequency of HGTs in eukaryote nuclear genomes.

Reports of HGTs that are distributed throughout the vast eukaryotic tree of life provide an incomplete picture of the extent and impacts of this process. These data provide important but limited insights rather than a comprehensive understanding of how HGT may have shaped eukaryote evolution. Thus, genome-based studies targeting novel or poorly studied lineages (e.g., *Rhodelphis*, Hemimastigophora [74,75]), or so-called orphan lineages (e.g., Telonemia, 'CRuMs' [76,77]) are valuable. These data complement existing HGT studies and confirm the presence and importance of HGT across many distantly related organisms, thereby disproving the previously held view that HGT in eukaryotes is rare and tied to endosymbiosis [12,78].

Concluding Remarks

Although hotly disputed when it first rose to prominence, the hypothesis of HGT in eukaryotes, in particular protists, has been substantiated by multiple high-quality analyses of genomes across the tree of life. The time is ripe to move beyond the debate 'if HGT' to focusing on its adaptive and evolutionary implications and filling 'genome gaps' in the eukaryotic tree of life (see Outstanding Questions). Based on published analyses of HGT, the incidence of these gene transfers varies

Outstanding Questions

How can we more accurately characterize the extent of HGT in eukaryotes? What types of data do we need to generate to have confidence in the results, and what level of taxonomic sampling will allow us to distinguish between HGT and differential loss scenarios?

How does effective population size impact HGT, and does a smaller population size allow more HGTs to be fixed (i.e., not weeded out by selection)? Can we use protist models to study HGT in the laboratory by applying the correct selection pressures? What sorts of vectors are responsible for HGT in eukaryotes? Is phagocytosis a major driver of novel gene origin?

Is it possible to establish and implement a standardized pipeline for HGT quantification in eukaryotic genomes? What challenges remain to harness genomic data in a fashion that is consistent across different journals and international borders to create a robust framework for quantifying HGT in eukaryotic genomes, and do we need to require evidence based on long-read sequencing to accept HGT candidates?

Can we encourage or require future genome studies to include a putative HGT determination in their analysis? Can we push this field forward by creating a eukaryotic HGT toolkit that is freely available and, similar to BUSCO (Benchmarking Universal Single-Copy Orthologs) for determining genome completeness, can be used to screen all novel genomes for cases of foreign gene acquisition?

With more genome data available and more standard methodology, will HGT quantification begin to converge on a more specific number? Will these trends reflect the gain or loss of key lifestyle features, or will HGTs be found at some background level, regardless of lifestyle transitions?

from 0.04 to 6.49% among microbial eukaryotes (including yeasts). This estimate is impacted by methodological biases but also reflects the fact that percentage HGT is based on the gene inventory size that grows and shrinks as lifestyles evolve. In general, HGT is much less prevalent [perhaps by 80-fold (e.g., 1% vs. 80%)] in eukaryotes than in prokaryotes. Going forward, we suggest that genome sequencing and analysis methods should be standardized to allow a meaningful comparison of HGT across all eukaryotes. Ideally, long-read sequencing data (e.g., [20]) and taxon-rich datasets should be used for phylogenetic analyses, and an explicit definition of HGT should be applied, such as whether candidates are nested within well-supported clades (strong evidence) or are sister to them (weaker evidence). Moreover, phylogeny-independent methods such as protein network analysis [79,80] or alignment-free methods for increasingly large datasets [81] should be used to corroborate HGT candidates. We strongly encourage all studies in which a new genome is sequenced and the results are reported in a genome paper to include a list of putative HGTs so that this often-overlooked fundamental force of eukaryotic evolution can become part of the mainstream scientific discourse. As it stands, understanding HGT in the broader context of eukaryotic evolution is a work in progress but a worthy endeavor, considering all of the evolutionary insights it has generated thus far.

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