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1 **Lateral gene transfer mechanisms and pan-genomes in eukaryotes**

2

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10

11 **Keywords:** Lateral gene transfer, horizontal gene transfer, eukaryote genomes; pan-genomes

12

13 **Abstract**

14 Lateral gene transfer (LGT) is well known as an important driver of genome evolution in bacte-
15 ria and archaea, but its importance in eukaryote evolution has yet to be fully elucidated. There is
16 now abundant evidence indicating that LGT has played a role in the adaptation of eukaryotes to
17 new environments and conditions, including host-parasite interactions. However, the mecha-
18 nisms and frequency of LGT across the tree of eukaryotes remain poorly understood. Here we
19 review evidence for known and potential mechanisms of LGT into diverse eukaryote lineages
20 with a particular focus on protists, and discuss trends emerging from recently reported examples.
21 We also explore the potential role of LGT in generating ‘pan-genomes’ in diverse eukaryotic
22 species.

23

24 **The role and prevalence of lateral gene transfer in genome evolution**

25 Lateral (or horizontal) gene transfer (LGT or HGT) is an important mechanism by which genetic
26 material is inherited in a non-vertical fashion. In bacteria, DNA is transmitted horizontally by
27 **transduction, transformation** and **conjugation** (see Glossary) (e.g., [1]). Although the frequen-
28 cy of LGT varies greatly amongst taxa, these processes are largely responsible for gene content
29 variation between and within prokaryotic species. Indeed, the total gene content of a given spe-
30 cies – the **pan-genome** – can exceed the number of ‘core’ genes shared between all strains of
31 that species by an order of magnitude or more; those accessory genes are commonly acquired by
32 LGT (e.g., [2] and references therein). It remains unclear what proportion of the genes found in
33 some but not all strains of a bacterial species are adaptive versus neutral or deleterious [3,4], but
34 there is no doubt that LGT has a major role in prokaryote genome evolution.

35 By contrast, the prevalence, mechanisms and roles of LGT in eukaryote evolution are
36 poorly understood. Putative LGTs are increasingly reported as the number of comparative ge-
37 nomic analyses of diverse unicellular and multicellular eukaryotes rapidly grows (Box 1). It is
38 generally accepted that LGT likely occurs less frequently in eukaryotes relative to most prokary-
39 otes (e.g., [5]), but this does not mean that it is an unimportant mechanism in eukaryotic genome
40 evolution [6]. Laterally acquired genes can provide eukaryotic organisms with new functions that
41 impact their biology and evolutionary trajectory (e.g., [7]); LGT has been suggested to play a
42 role in the adaptation of eukaryotes to ‘extreme’ conditions such as high temperature (e.g., [8]),
43 high salt (e.g., [9]), and anaerobiosis (e.g., [10, 11]). LGT also appears to have contributed to the
44 evolution of parasitism (e.g., [12] and references there-in, [13,14]) and in the reversal from a
45 parasitic to a free-living lifestyle (e.g., [15]).

46 How do eukaryotes acquire genes? Numerous potential sources of foreign DNA have
47 been suggested (Figure 1) but the evidence supporting their involvement in LGT varies. Some
48 phenomena, like **endosymbiotic gene transfer** (EGT), are well supported by evidence. Others,
49 such as inter-domain conjugation or natural transformation, have been documented in the lab
50 [16] but their frequencies and relevance in nature is unknown. Regardless of the source, a ‘suc-
51 cessful’ LGT must involve several steps including: (i) the introduction of foreign DNA (or RNA)
52 into the recipient eukaryotic cell; (ii) translocation of the DNA to the nucleus; and (iii) incorpo-
53 ration of the DNA into one or more chromosomes, either accidentally by host DNA repair and
54 recombination pathways, or by an active integration mechanism. For transferred DNA to be
55 faithfully inherited, it must be stably integrated in all sister chromosomes and, for multicellular
56 eukaryotes, must exist in the germline. To persist over longer time periods, newly acquired DNA
57 must be fixed in the population by neutral drift and/or positive natural selection. If the transferred
58 genes are expressed and provide a function that is useful (e.g., a novel adaptive trait, or compen-
59 sation for the loss of a pre-existing gene), they can be maintained by purifying selection. Other-
60 wise, further mutations may erode these genes into pseudogenes (e.g., [17]) and they will even-
61 tually be lost or become unrecognizable intergenic DNA. Clearly the likelihood of any given
62 LGT event being successful is multifactorial, and a number of factors potentially influence the
63 frequency of LGT in any given species (Box 2).

64 Here we review evidence for several means by which foreign DNA is introduced into
65 eukaryotic cells and potential mechanisms that integrate such DNA into eukaryotic genomes. We
66 also discuss aspects of genome biology that may influence the rate of LGT and discuss its im-
67 portance to adaptive evolution in eukaryotes by acting as a ‘catalyst’ for the acquisition of niche-
68 specific traits.

69

70 **Viruses and their hosts exchange genetic material**

71 Virus-mediated transduction has long been recognized as an integral part of the biology
72 of prokaryotic cells. A growing body of literature supports the notion that viruses can act as vec-
73 tors of LGT from prokaryotes to eukaryotes and between eukaryotes (reviewed in [18]). The
74 nature of viral transmission (horizontal), replication (inside the host cell) and survival strategies
75 (e.g., integration into the host genome) together lead to intimate associations with their host's
76 genetic material, providing ample opportunity for gene exchange. At least some viruses can in-
77 fect a wide range of hosts and/or change hosts (e.g., [19]) and some eukaryotes harbour multiple
78 divergent viruses, each of which may enable gene exchange beyond species boundaries. The
79 virus-mediated export of nuclear DNA in plants was recently documented in real time by Catoni
80 et al. [20], where *Beta vulgaris* chromosomal DNA integrated into the viral genome of a single-
81 stranded DNA virus during infection was encapsidated into viral particles and spread throughout
82 the plant population via subsequent viral infections. Notably, the *B. vulgaris* DNA present in the
83 hybrid minicircles was replicated and transcribed in the newly infected host.

84 It is well established that host genes can be integrated into a viral genome and persist,
85 particularly in double stranded DNA (dsDNA) viruses that infect a variety of eukaryotes. In the
86 case of nucleocytoplasmic large DNA viruses, host DNA can make up a substantial portion of
87 the virus' genome (e.g., [21]). A recent study of the giant DNA virus of the green alga
88 *Tetraselmis*, TetV, identified 110 protein coding genes (17% of its protein coding genes) that
89 appeared to be acquired from eukaryotes, including many specifically of Viridiplantae origin
90 [21]. At least four of the eukaryotic homologs identified in TetV were novel viral genes that sug-
91 gested recent incorporation of genes from both its host (*Tetraselmis*) and other unicellular eukar-

92 yotes that likely allow manipulation of the host fermentation pathways during anaerobic condi-
93 tions. Giant virus genomes are highly mosaic – not only do they acquire genes from their eukar-
94 yotic host(s), they also pick up genes from bacteria and other viruses present within the host cell
95 (e.g., [22]).

96 The fact that genes of host origin exist in viral genomes implies that host DNA is regular-
97 ly introduced into viral genomes with varying consequences on fitness; while some genes are
98 fixed, others presumably persist temporarily over a limited number of replication cycles. Short-
99 lived insertions, however, could still allow the foreign genes to be transferred to a new host. This
100 was shown experimentally by Gilbert et al. [23], who investigated the frequency of gene transfer
101 in 21 baculovirus (AcMNPV) populations infecting two moth species. Around 5% of AcMNPVs
102 were found to harbor at least one moth-derived DNA sequence at a given time, all of which per-
103 sisted only temporarily. There is nevertheless evidence for a continuous flow of genetic material
104 from the host to AcMNPV and for repeated transfers of the short-lived insertions from the viral
105 genome into the one of the various insects they infect. Host DNA was predominately inserted
106 into viral genomes via transposition. However, there is also evidence for a variety of
107 microhomology-mediated and homology-independent recombination events at double stranded
108 breaks. Whether these recombination events were mediated by host or viral factors, or some
109 combination of the two is currently unknown, but Gilbert et al. [23] speculate that as long as
110 there is a double stranded break, viral and host DNA have the potential to be joined.

111 A large number of viral genomes and genome fragments from a wide range of both retro-
112 viruses and other viruses have been found in eukaryote genomes, including those of multicellular
113 organisms (e.g., [24-26]). In order to replicate, retroviruses become endogenized, i.e., integrated
114 in their hosts genome via an autonomous mechanism using enzymatic machinery they encode.

115 Beyond retroviruses, it has been suggested that viral DNA is inserted via accidental chromoso-
116 mal integration at double stranded DNA (dsDNA) breaks through homology mediated recomb-
117 nation, non-homologous end joining repair, or a hybrid mechanism that utilizes the enzymatic
118 machinery of retrotransposons [24]. For example, inactive remnants of endogenous
119 florendoviruses (non-retroviral viruses that lack their own integrases) found across a broad diver-
120 sity of plant genomes are frequently inserted in transposable element (TE)-rich regions, specifi-
121 cally at TA dinucleotide simple repeats [27]. These repeats are thought to give rise to fragile
122 chromosome regions, making them more prone to dsDNA breaks – breaks that create potential
123 integration sites for foreign DNA.

124 Further clear evidence for the flux of genetic material from virus to host and *vice versa*
125 has been reported in various eukaryotes. Substantial gene exchange is particularly evident be-
126 tween the amoebozoan *Acanthamoeba castellanii* and its large DNA viruses [28]. 267 genes of
127 probable viral origin were identified in the genome of *A. castellanii*, many of which were tran-
128 scriptionally active and clearly integrated in the nuclear genome. Phylogenetic analyses also de-
129 tected a kinase gene that appears to have been transferred on multiple occasions – at least once
130 from eukaryotes to viruses and at least twice subsequently from viruses to amoebae [28]. Recent-
131 ly, experimental evidence suggested that two densovirus genes transferred to the nuclear genome
132 of pea aphids from a densovirus that infects rosy apple aphids are responsible for their ability to
133 produce winged offspring during population crowding, a phenotype with clear adaptive benefit
134 [29]. Similarly, endogenous chromosomally-transmitted bracoviruses in parasitic wasps produce
135 dsDNA circles encoding virulence genes that are injected into host caterpillars, integrate into
136 their genomes and are expressed suppressing the immune response allowing the wasp larvae to
137 survive in the lepidopteran body. These endogenous viruses appear to have been vectors for

138 transfer of C-type lectin genes from the wasps to lepidopterans that have been domesticated by
139 the latter and allow them to resist baculovirus infection [30].

140

141 **Transposable elements—agents of intra- and inter-genomic mobility**

142 Transposable elements (TEs) have an intrinsic ability to move around within a genome,
143 which may make it easier for them to be transferred to other genomes. Numerous examples of
144 laterally transferred TEs have been reported in animals, plants, insects and fungi (e.g., [31–34]).

145 A large-scale survey of TEs in 195 diverse insect genomes identified 2,248 lateral transfers of
146 TEs, constituting 2-24% of a given insect genome [33]. Reiss et al. [34] recently extended this
147 survey to screen 460 insect genomes for patterns of TE transfer. They concluded that DNA
148 transposons were the most frequently transferred TE class in arthropods, followed closely by
149 Long Tandem Repeats (LTRs), and that there was a clear excess of TE transfer in species hosting
150 baculoviruses.

151 With the exception of the studies mentioned above, large-sale surveys of TEs in eukary-
152 otes are generally lacking, especially in protists, making it difficult to make general conclusions
153 about the extent of inter-genomic TE mobility and its global impact on eukaryotic genome evolu-
154 tion. Regardless, studies looking at the rate of TE transfer have found that geographic proximity
155 as well as genetic distance between the host and recipient lineage impact the rate of LGT (e.g.,
156 [33]). Some of the most abundant retrotransposon families in eukaryotes were suggested to have
157 been transferred multiple times between aquatic organisms (L1) and between animals (BovB) via
158 various parasite vectors [35]. Some viral genomes contain TEs acquired from eukaryotes (e.g.
159 [23,36]), including the widespread invasion of miniature inverted-repeat TEs found in high copy
160 numbers in viruses that use host machinery to transpose themselves [37]. Several TEs have been

161 shown to produce extrachromosomal circular forms during transposition [38], which if
162 encapsidated in a viral particle may be more likely to undergo virus-mediated LGT than linear
163 DNA [18].

164 Under stress conditions, LTR retrotransposons are activated and generate double-stranded
165 breaks in DNA (e.g., [39]); this could potentially facilitate integration of foreign DNA at these
166 breakpoints via normal DNA repair pathways. Furthermore, imperfect excision of a TE from a
167 chromosome, as has been observed in bacteria [40], can result in large adjacent portions of the
168 genome accidentally being carried along with a TE during transposition or horizontal transfer.
169 Due to the ability of TEs to capture and transduce sequences at a high frequency, it has been
170 suggested that horizontal TE transfer could be responsible for the direct movement of genes
171 among eukaryotes as well [41]. Consistent with this hypothesis, transferred genes are often asso-
172 ciated with TE-rich regions in metazoan genomes [14]. The transfer of a wheat virulence protein
173 (*ToxA*) between three fungal wheat pathogens in two independent events was likely facilitated by
174 a type II DNA transposon [42]. Long-read sequencing based genome assemblies for several of
175 these fungi showed that the genomic context of the two *ToxA* LGTs included 14 kbp of both cod-
176 ing and non-coding DNA, including conserved terminal inverted repeats bearing strong resem-
177 blance to features of type II DNA transposons, suggesting LGT and lateral TE transfer can occur
178 simultaneously [43]. Altogether, this strongly suggests that TEs are not only transferred laterally,
179 but that they also can facilitate the movement of other genes between species.

180

181 **Endosymbiosis and gene transfer**

182 A large variety of eukaryotes harbour prokaryotic and/or eukaryotic **endosymbionts** ex-
183 hibiting varying degrees of cellular and genomic integration. In a number of cases (e.g., mito-

184 chondria and plastids), the endosymbionts have co-evolved with the host to the point where they
185 have become permanent, semi-autonomous organelles. In any case, the close proximity of the
186 host nucleus and endosymbionts or endosymbiont-derived organelles provides an opportunity for
187 the movement of genetic material [43,44]. Endosymbiont-to-host nucleus gene transfers played
188 an important role in the evolution of mitochondria and plastids, a process specifically referred to
189 as endosymbiotic gene transfer (EGT) [45,46]. In these instances, if the transfer of genes from
190 the endosymbiont-derived organelle to host is followed by the retargeting of the gene product to
191 the organelle, the copy of the gene in the organellar genome can be lost. In the fullness of time,
192 the result of EGT is the existence of complementary gene inventories and coordinated metabo-
193 lisms in host and endosymbiont-derived compartments [10,44]. The substrate for EGT is thought
194 to be organelle lysis; the frequency of EGT over short evolutionary timescales appears to be pos-
195 itively correlated with the number of organelles per cell (see [16,47] and references therein).

196 The extent to which bacteria other than the endosymbiotic progenitors of mitochondria
197 and plastids contributed to organelle establishment remains controversial (e.g., [10, 48–50]). In
198 the case of plastids, phylogenetic evidence suggests that LGTs from diverse bacteria – not just
199 cyanobacteria – may have facilitate organelle evolution (e.g., [51]). Beyond canonical plastids,
200 the contribution of LGT to the recently evolved ‘chromatophores’ in the rhizarian amoeba
201 *Paulinella chromatophora* is clear. Sequencing of the *P. chromatophora* genome revealed 228
202 nuclear genes of various bacterial affinities, only ~25% of which appear to come from the
203 cyanobacterium-derived chromatophore [52]. The suite of EGTs and LGTs, along with native
204 host nuclear genes, contribute to many essential biosynthetic pathways partially encoded by the
205 substantially reduced chromatophore genome, and encode transporters facilitating host-
206 chromatophore metabolite exchange [52,53]. The phagotrophic nature of the ancestors of *P.*

207 *chromatophora* likely played an important role in sourcing these various bacterial genes, driving
208 LGT and, ultimately, chromatophore integration.

209 Various other examples of endosymbiosis-associated gene transfers have also been doc-
210 umented. Some of these transfers are directly from endosymbionts to host, while others seem to
211 be LGTs from ‘third party’ organisms that allow endosymbionts to lose the corresponding genes.
212 The latter is exemplified by the mealybug *Planococcus citri*, in which the loss of enzymes for
213 synthesizing eight amino acids in its endosymbiont *Tremblaya* were made possible by >20 LGTs
214 from various bacteria to the *P. citri* nuclear genome [54]; a related mealybug harbors a less
215 genomically streamlined *Tremblaya* endosymbiont that still encodes all the enzymes necessary
216 for the synthesis of these amino acids. This endosymbiosis actually involves a tripartite relation-
217 ship between *P. citri* and two bacteria – a betaproteobacterium (*Tremblaya*) and its
218 gammaproteobacterial endosymbiont *Moranella* – which exhibit complementary gene losses and
219 are themselves metabolically intertwined [54]. Peptidoglycan biosynthesis is just one pathway
220 that appears to be a mosaic of *P. citri* encoded LGTs and endosymbiont encoded genes. Using a
221 variety of techniques including Nanoscale Secondary Ion Mass Spectrometry (NanoSIMS),
222 Bublitz et al. [55] recently showed that the laterally acquired genes for peptidoglycan synthesis
223 are translated in the cytoplasm of *P. citri* and the resulting proteins are transported across multi-
224 ple membranes to the *Moranella* endosymbiont where peptidoglycan is assembled. These results
225 clearly show that LGT can give rise to stable, complex, mosaic biochemical pathways in
226 endosymbioses outside the bounds of mitochondria and plastids.

227 Several species of single-celled Foraminifera harbor intracellular bacteria capable of
228 denitrification – a process widespread in prokaryotes but not in eukaryotes, which allows for the
229 storage and use of nitrate in energy metabolism [56]. Recently, a novel denitrification pathway

230 was identified in the genome of the foraminiferan *Globulimina* where two of the key enzymes
231 are clearly of prokaryotic origin and likely originated from the denitrifying endosymbiotic bacte-
232 ria [57]. In the ctenophore *Mnemopsis leidyi*, various LGTs have been suggested to be a result of
233 the association between these enigmatic invertebrates with various bacteria and protists such as
234 amoebae [58]. Numerous studies have shown a variety of LGTs from *Wolbachia* endosymbionts
235 into various insect and nematode hosts ([59] and references therein), ranging from smaller inser-
236 tions to genome-sized fragments. These are just a few of many examples illustrating that
237 endosymbionts contribute DNA to their hosts and that gene transfers from various sources during
238 and/or prior to the establishment of a permanent endosymbiont can influence host-endosymbiont
239 integration. The fact that EGTs have occurred in diverse lineages across the eukaryotic tree
240 shows that there are few – if any – barriers to the uptake and integration of foreign DNA into
241 eukaryote nuclear genomes.

242

243 **“You are what you eat”**

244 Many protists feed on other organisms via phagocytosis. Doolittle’s ‘you are what you
245 eat’ hypothesis [60] posits that both prokaryotic and eukaryotic prey could act as sources of new
246 genes simply as a by-product of digestive vacuole rupture and release of nucleic acids. In this
247 respect, the overall mechanism of gene transfer from phagocytosed prey is similar to gene acqui-
248 sition from endosymbionts. Even if food vacuole or symbiont rupture is a very rare occurrence,
249 both generate free DNA that could enter the nucleus and be incorporated into the nuclear genome
250 at a low frequency. Such sequences will accumulate mutations and may be deleted. But if the
251 gene within the inserted DNA happens to be expressed, it could be fixed in the population by
252 drift or, if some advantage is conferred, positive selection. Because of their overall similarity in

253 mechanism and outcome, it is difficult if not impossible to determine if gene transfer events orig-
254 inated from transient ancestral endosymbionts or phagocytosed food. A number of potential ex-
255 amples of the latter have nevertheless been suggested.

256 Adaptive gene transfers of bacterial origin in the bacterivorous protist *Halocafeteria*
257 *seosinensis* allow it to produce an osmoprotectant that relieves significant osmotic stress found in
258 its hypersaline habitat. This, in combination with other LGTs from bacteria, likely facilitated the
259 adaptation of *H. seosinensis* to a high salt environment [9]. In the anaerobic oxymonad
260 *Monocercomonoides*, a laterally acquired SUF-system from a bacterial donor (with genes veri-
261 fied to be in the nucleus by fluorescent in situ hybridization) is likely linked to the ability of
262 *Monocercomonoides* to ingest and digest bacterial prey [61]. This laterally acquired pathway
263 allows for iron-sulfur cluster assembly to take place in the cytosol rather than *via* the typical ISC
264 pathway in mitochondrion, ultimately making the last essential function of *Monocercomonoides*'
265 mitochondria-related-organelle redundant and dispensable [61]. Eukaryovores are relatively rare
266 compared to bacterivorous protists, which may contribute to apparent differences in the frequen-
267 cy of gene transfer between eukaryotes and between eukaryotes and prokaryotes [62]. However,
268 these differences may also reflect the difficulties associated with confidently identifying eukary-
269 ote-to-eukaryote gene transfer events (Box 3).

270

271 **Parasites as LGT recipients and vectors**

272 LGT has played an important role in the adaptation of many successful parasite lineages
273 to their host and interactions with other host-associated microbes. Cases of adaptive LGT from
274 prokaryotes to a variety of unicellular and multicellular parasitic eukaryotes have been well doc-
275 umented (e.g., [7,12]). For example, LGTs have been reported in diplomonad protozoa (e.g.,

276 *Spiroucleus* and *Giardia intestinalis* (e.g., [63]), *Plasmodium*, *Toxoplasma*, *Leishmania*,
277 *Trichomonas* and *Entamoeba* [12,64] as well as parasitic nematodes (e.g., [14, 65]).

278 Several trends in the patterns of laterally transferred genes into parasitic lineages have
279 been noted. For example, there is an excess of genes received from organisms that live in the
280 same ‘host’ environment as the parasites (e.g., [12, 64]). Furthermore, the functions of the ac-
281 quired genes tend to reflect the lifestyles of the different parasite types. For example, the muco-
282 sal parasite *Trichomonas vaginalis* has acquired genes related to harvesting glycans from host
283 epithelial tissues and secretions (Box 4). A recent analysis of the anaerobic gut mucosal parasite
284 *Blastocystis* sp. is consistent with these observations. 2.5% of the protein-coding genes in this
285 organism were acquired laterally from a wide range of both prokaryotes and eukaryotes, allow-
286 ing for successful host infection, evasion of host immune systems, modulation of other gut mi-
287 crobes (e.g., via production of indole and polyketides) and metabolic adaptation to the gut envi-
288 ronment [13]. Several of the LGTs in *Blastocystis* appear to have come directly from animal
289 hosts. Others appear to have been transferred multiple times between this organism and other
290 mucosal protists (e.g., *Trichomonas*, *Giardia* and *Entamoeba*), suggesting the potential existence
291 of a pool of adaptive genes shared between anaerobic mucosal parasites. Numerous other strong
292 examples of LGT facilitating parasite adaptation have been reported across the eukaryotic tree.
293 For example, Dean et al. [66] used a combination of ancestral gene reconstruction and functional
294 assays to show that the acquisition of a single nucleotide transporter from bacteria to the com-
295 mon ancestor of *Microsporidia* species allowed them to become energy parasites that steal host
296 ATP, GTP and NAD⁺ and subsequently lose the capacity to synthesize nucleotides on their own.

297 In phytoparasitic nematodes, functionally important genes encoding enzymes involved in
298 plant cell-wall degradation and related metabolism were identified as LGTs from bacteria that

299 are plant pathogens of the same hosts, as well as from a variety of eukaryotes (including the plant
300 pathogen *Phytophthora infestans*) [14]. Interestingly, LGTs in these nematodes are often found
301 in TE regions of the genome, and the bacterial homologs are found on plasmids, suggesting a
302 mechanistic role for mobile genetic elements in LGT. Another example of host-parasite mediated
303 LGT is the acquisition of mammalian TE families by the blood-sucking insect *Rhodnius prolixus*
304 and the pond snail *Lymnaea stagnalis*. These TEs had extremely high sequence identities to TEs
305 in the genomes of host mammals of the insect and trematode parasites for which the snail acts as
306 an intermediate host [67], implicating host-parasite interactions in horizontal transmission of the
307 TEs. Based on the foregoing examples, it seems likely that parasites that infect a wide range of
308 hosts or species that exist in general symbiotic associations could be particularly potent vectors
309 for LGT.

310

311 **Conjugation and natural transformation in eukaryotes**

312 A wide variety of eukaryotes have been transformed in the lab. While artificial in nature,
313 such experiments indicate that there is no fundamental obstruction to LGT when barriers to
314 transfer are intentionally removed. Established eukaryotic transformation systems are frequently
315 exploited for commercial applications such as the production of genetically modified crops. For
316 example, *Agrobacterium tumefaciens*, which uses a type IV secretion system to inject a tumor
317 inducing (Ti) plasmid carrying specific genes into plant cells, has been used to modify plant spe-
318 cies to confer pest resistance [68]. Once in the host cell, the Ti plasmid utilizes the plants ma-
319 chinery to enter the nucleus and integrate into the genome, using eukaryotic promoters to express
320 its genes. Importantly, there is evidence that this transformation occurs naturally in plants as
321 well. Genes from *Agrobacterium* have been found to reside and be expressed in the germ line of

322 various plant species that have not been artificially transformed (e.g., [69,70]), suggesting that
323 these gene transfers are maintained and functional.

324 Recently a 100% efficient transformation system was developed in the red alga
325 *Porphyridium purpueum* where the transformation vectors are maintained episomally as
326 extrachromosomal high-copy number plasmids [71]. This shows that different bacterial origins
327 of replication can function in a eukaryote system and result in stable, autonomous replication
328 within the nucleus. Naturally occurring extrachromosomal plasmids of unknown origin have
329 been found in some red algae; these plasmids contain both bacterial and viral ORFs (e.g., [72]).
330 The apparent genetic compatibility between plasmid replication systems of bacteria and compo-
331 nents of DNA replication machinery in the red algal nucleus [73] raises intriguing questions
332 about algal genome evolution. The extended presence and replication of a nuclear episomal ele-
333 ment could provide opportunities for gene transfer to the nuclear genome and may have facilitat-
334 ed the contribution of bacterial genes to red algae (estimated to be between 1% and 9.3% of the
335 total gene content, depending on the lineage [73,74]).

336 Interestingly, a conjugation-based transformation system capable of delivering episomal
337 vectors from *E. coli* has been developed for the diatoms *Phaeodactylum tricornutum* and
338 *Thalassiosira pseudonana* [75]. Although direct conjugation has not been observed in nature,
339 this system suggests that bacterial conjugation may be one mechanism that could explain the
340 high percentage of putative bacterial-derived genes (5-10% of genes) in diatom genomes [76].
341 The transfer of plasmids via bacterial conjugation has been observed in yeast [77], as has the
342 natural transformation of exogenous DNA under non-artificial conditions [78].

343

344 **Difficulties in detecting LGT in eukaryotes**

345 Eukaryotes typically have relatively large and complex genomes compared to prokary-
346 otes, making genome sequencing, assembly and analysis difficult. The paucity of high-quality
347 genome data from diverse eukaryotes, especially from closely related species and multiple
348 strains of the same species, is a present barrier to the study of LGT [79]. In regions of the eukar-
349 yotic tree in which there is denser genomic sampling of lineages, the extent of adaptive LGT is
350 becoming clearer. For example, Ropars et al. [80] analyzed 10 high-quality genomes from
351 *Penicillium* in the context of genome data from 416 strains of 65 fungal species. The authors con-
352 fidently identified 104 LGTs between *Penicillium* species, including some large recently trans-
353 ferred genomic regions spanning more than 10 kb, some of which show signatures of TE mediat-
354 ed insertion [80]. Gene expression analysis and fitness experiments confirmed that at least some
355 of the LGTs encode functional genes that provide metabolic adaptations to a cheese environment
356 (e.g. lactose metabolism) or antifungal proteins used to out-compete other fungal species [80].
357 Until a similar density of high-quality genomic sampling is available for other branches of the
358 eukaryote tree— particularly for microbial eukaryote lineages – it is impossible to know whether
359 the dynamic nature of fungal genomes is the exception or the rule.

360 Distinguishing true LGTs from contamination (see 81) can be difficult and mitigated by
361 careful inspection of candidate genes (Box 3). Many (but by no means all) published studies
362 have used appropriately stringent phylogenetic criteria for reporting LGT. Initial bioinformatic
363 steps generally lean towards the exclusion of bacterial/symbiont sequences from assemblies,
364 which means that the impact of LGT to a given genome can be underestimated. Additional diffi-
365 culties arise when trying to determine the scale of how far back in evolutionary time one has to
366 go to consider a gene as having been vertically or laterally transferred. More ancient LGTs are
367 harder to detect as the sequence similarity of a transferred gene to the gene of the donor lineage

368 decays over time. At the same time, confidently detecting eukaryote-eukaryote LGT is generally
369 more difficult than prokaryote-eukaryote LGT due to the more recent common ancestry shared
370 between donor and recipient, resulting in less obvious phylogenetic incongruence. A ‘patchy’
371 gene distribution across the eukaryote tree is often interpreted as evidence of LGT between eu-
372 karyotic lineages. However, secondary loss of ancestral genes is well known to have occurred in
373 parallel in many eukaryotic lineages (e.g. genes in mitochondrial genomes, reviewed in [10]) and
374 is sometimes an equally plausible alternative explanation for the gene distribution pattern.
375 Therefore, strongly supported single gene trees that contradict the expected species tree relation-
376 ships are needed to support suspected LGT cases. This standard is sometimes challenging to
377 achieve due to the limited phylogenetic signal within many single gene alignments. Identifying
378 gene transfers in eukaryotes also requires a sufficient level of taxonomic sampling of the given
379 gene of interest, which is often lacking. Strategic sequencing of diverse eukaryote lineages will
380 make LGT detection easier in the future.

381

382 **LGT and pan-genomes in eukaryotes**

383 In prokaryotes, within-species variation in gene content is such that the core genome (i.e.,
384 gene families found across all or most strains) represents 3-84% of the total gene families found
385 in all sequenced strains of a given species (reviewed by [3]). The accessory genome includes
386 genes found in a single or subset of strains (Figure 2). This pan-genome pattern is due to the
387 combined effects of LGT and differential gene loss.

388 The extent to which a pan-genome-like pattern is observed in various eukaryote lineages
389 – and, in particular, protists – is less clear, as inferences are limited by the paucity of genomes
390 that cover the breadth of diversity for a given species outside of animals, plants and fungi [79].

391 Another problem is the difficulty in obtaining accurate and comprehensive gene predictions in
392 eukaryotes, as gene prediction is much more difficult than in prokaryotes because of the pres-
393 ence of spliceosomal introns and alternative splicing. As a result, potential pan-genome patterns
394 must be cross-checked to be certain they are not simply artefacts of failure to predict existing
395 genes. Nevertheless, across various model fungal species where a dozen or more genomes have
396 been sequenced, pan-genomes have been observed with core genomes ranging from 80-90% of
397 the species coding capacity [e.g., 82 and references therein]. Some of the accessory genes are
398 inferred to be a result of LGT; others appear to be a consequence of gene duplication,
399 pseudogenization and outright gene loss. In plants, the accessory genome component of the spe-
400 cies pan-genome seems to be more extensive (up to ~47% of the pan-genome). For example,
401 across >3,000 genomes of *Oryza sativa* L., 46.5% of the pan-genome was found to be accessory
402 genes, most of which are related to immune and defense responses [83].

403 In the world of protists and algae, fascinating insight into the eukaryotic pan-genome has
404 come from investigation of the coccolithophorid *Emiliania huxleyi* [84]. In analyzing the ge-
405 nomes of 14 highly-similar strains of *E. huxleyi* (>98% 18S ribosomal DNA (rDNA) identity),
406 Read et al. [84] found a core genome (experimentally confirmed via microarrays) comprised of
407 only 69.5% of the total gene set. While Read et al. were unable to confidently assign LGTs from
408 bacteria or viruses as the main source of the accessory genes, they did find that *E. huxleyi* ge-
409 nomes contain a lot of repetitive elements, which may foster accessory gene formation and serve
410 as preferential target sites for LGT.

411 As with prokaryotes, there are serious problems in defining the ‘pan-genome’ because it
412 is confounded with how species are defined and delimited. For example, Worden et al. [85]
413 found significant gene content differences between two isolates of *Micromonas* that were origi-

414 nally considered the same species on the basis of morphology and 18S rDNA similarity. The fact
415 that these two isolates were found to share only 90% of their genes was interpreted to mean that
416 they were different species. Yet, an alternative interpretation is that these two isolates are mem-
417 bers of the same pan-genome-containing species. In the absence of evidence for sexual recomb-
418 ination between individuals of a putative species – information that is lacking for the majority of
419 protists – defining a species is difficult. Without a clear species definition for most microbial
420 eukaryotes, it is difficult to distinguish between these two scenarios.

421

422 **Concluding Remarks**

423 The past few years have seen a rapid increase in the sequencing of complete genomes
424 from diverse eukaryotes and, concordantly, abundant evidence for LGT into eukaryotic genomes.
425 Many questions nevertheless remain unanswered (see Outstanding Questions). Indeed, for most
426 documented cases that are not obviously EGTs, the means by which the transferred DNA was
427 introduced into cell and incorporated into the chromosomes remain unclear. Many of the mecha-
428 nisms that could have been involved, and their relative importance, are ‘known unknowns’. For
429 example, endosymbiont- or ‘you-are-what-you-eat’ mediated transfers are probably much more
430 common in eukaryotes that commonly harbor endosymbionts and phagocytose prey, respective-
431 ly. On the other hand, virus-mediated transfer will depend on the number and types of viruses
432 that infect particular lineages and the degree to which they can integrate into host genomes and
433 mobilize host DNA. To address these questions the following data are needed. First, a greater
434 density of sampling of eukaryotic genomes from each of the major eukaryotic groups should
435 allow us to ‘catch’ more very recent transfer events that may still bear the hallmarks of their ori-
436 gins (e.g., flanking TEs, viral genes, breakpoints of recombination events etc.). This should also

437 allow us to assess the extent to which pan-genomes are a common feature of diverse eukaryote
438 species, assuming we can get reliable information about how such species are delimited (i.e.,
439 evidence for recombination within species). Our understanding of eukaryote LGT will also be
440 greatly enhanced by the development of new model systems and long-term laboratory evolution
441 experiments that allow the process of LGT to be studied in real time. Ideally, organisms from
442 different branches of the eukaryotic tree should be developed as model systems and the various
443 insights they yield considered in isolation and together. Long-term progress in our understanding
444 of the frequency and significance of LGT in eukaryotes will rely on the synthesis of information
445 gleaned from both experimental and bioinformatic approaches.

446

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770

771 **Glossary**

772 **Transduction**

773 Virus-mediated transfer of genetic material. In bacteria, generalized transduction involves the

774 integration and transfer of random pieces of bacterial DNA into the viral genome, while in spe-

775 cialized transduction only defined regions of the donor genome can be packaged into the virus

776 and transferred.

777

778 **Transformation**

779 Uptake of exogenous DNA by a cell. This can occur naturally or in an experimental context.

780 Both prokaryotic and eukaryotic cells are capable of being transformed.

781

782 **Conjugation**

783 Transfer of genetic material mediated by cell-cell contact; single-stranded DNA moves through a
784 thin connection between the donor and recipient cells. Although best known to occur between
785 bacteria, artificial conjugation between bacteria and eukaryotes such as diatoms has been carried
786 out.

787

788 **Pan-genome**

789 The term refers to the entire set of genes in the genomes of members of a given species. The
790 word was invented to underscore the fact that individuals or strains of a given species can vary in
791 their gene content. In bacterial and archaeal species genome content variability can be immense
792 such that the set of genes found in all members of a given species (the 'core') can represent a
793 small fraction of the total pan-genome. This variability in gene content has a number of causes
794 including gene duplications, gene losses, *de novo* gene formation and the acquisition of new
795 genes by lateral gene transfer (LGT).

796

797 **Protists**

798 Protists are any eukaryotic organisms that are not animals, plants or fungi. Protists are an ances-
799 tral 'grade' of eukaryotic organization and encompass the bulk of eukaryotic diversity. Although

800 most protist species are unicellular microbes, charismatic multicellular forms are well known
801 (e.g., macroalgae like giant kelp).

802

803 **Endosymbiont**

804 An organism that thrives within another organism, typically without killing its host. Endosym-
805 biosis of bacteria, archaea and microbial eukaryotes (e.g., algae) is relatively common in free-
806 living protists, but more rare in parasites. Organelles such as mitochondria and chloroplasts
807 evolved from ancient bacterial endosymbionts. Readers should keep in mind that (en-
808 do)symbiosis encompasses a continuum from mutualism to parasitism, and determining where a
809 given symbiotic system lies along this continuum can be very difficult.

810

811 **Endosymbiotic gene transfer (EGT)**

812 The transfer of genetic material from endosymbionts and their organellar derivatives to the host
813 cell nuclear genome. This process involves endosymbiont / organelle lysis, migration of released
814 DNA into the nucleus, and integration of random pieces of foreign DNA into nuclear chromo-
815 somes by DNA repair enzymes.

816

817 **Box 1: Robust examples of LGT in eukaryotes**

818 This review focuses on a select few of the numerous reports of LGT in eukaryotes. A
819 recent example of adaptive LGT concerns the acquisition of the gene *rquA*, which is involved in
820 rhodoquinone (RQ) biosynthesis, in various anaerobic protistan lineages [11]. The RQ produced
821 by RquA allows for fumarate to be used as an alternative terminal electron acceptor during mito-
822 chondrial ATP production under anaerobic conditions, allowing these protists to adapt to low
823 oxygen conditions. Phylogenetic analyses suggest that *rquA* was transferred on at least two sepa-
824 rate occasions from bacteria to eukaryotes, followed by multiple eukaryote-to-eukaryote trans-
825 fers.

826 Single gene-encoded transporters may be especially amenable to LGT as they could pro-
827 vide a direct selective advantage to the recipient by allowing for the uptake and utilization of a
828 particular metabolite. Numerous examples of LGTs involving transporter genes have been re-
829 ported (e.g., [68,86-88 and references therein]). Milner et al. [88] identified seven cases of trans-
830 porter-gene LGT between fungal phyla and demonstrated that they conferred a competitive ad-
831 vantage in certain environments. They proposed a “transporter gene acquisition ratchet” where
832 the range of transporters encoded in a given genome are continually being altered by various
833 mechanisms, including LGT.

834 Another notable report of LGT is the acquisition of an entire operon involved in
835 siderophore biosynthesis from Enterobacteriaceae into a group of closely related yeasts [89]. The
836 co-linearly arranged genes were found to be expressed and functional (via transcriptomics, meta-
837 bolic assays, and mass spectrometry), allowing for the sequestering and uptake of iron from the
838 environment – a likely beneficial feature as the yeast reside in the iron-limited insect gut envi-
839 ronment co-inhabited with numerous Enterobacteriaceae. After the initial LGT, the operon struc-

840 ture appears to have undergone a ‘eukaryotification’ process that included the addition of
841 polyadenylation sites, various structural rearrangements, addition of some eukaryotic genes, and
842 loss of some genes encoded by the operon in closely related yeast lineages. In principle, acquir-
843 ing an entire operon in a single LGT would allow for complex multi-gene pathways to spread
844 rapidly and effectively from donor to recipient.

845 Analyses of chromosome-level genomes for the grass *Alloteropsis semialata* also re-
846 vealed recent acquisitions of chunks of foreign DNA encoding multiple genes, including genes
847 related to disease resistance, abiotic stress tolerance and C4 photosynthesis [90]. Phylogenetic
848 analyses including genomes from >140 other grass species led to the discovery of 59 unambigu-
849 ous, recent LGTs into *A. semialata* from at least nine distantly related donor grasses. These genes
850 were found to be mostly expressed and clustered in 23 transferred fragments up to 170 kbp long,
851 indicating that large stretches of DNA containing multiple functional genes as well as non-
852 coding regions were transferred in a single event. In some cases, TEs were also encoded on the
853 transferred region.

854

855 **Box 2: Factors influencing the frequency of lateral gene transfer**

856 The frequency of gene transfer into a given species may hinge on a number of genomic
857 and environmental factors. For example, non-homologous end joining repair could be a major
858 mechanism of incorporation of foreign DNA [44] and thus impact the frequency of LGT in a
859 given genome. It is generally thought that successful LGTs can occur more frequently between
860 closely related organisms due to similarities between their genomes (codon usage, cis-acting
861 regulatory elements, etc.); high levels of sequence identity also foster homologous recombina-
862 tion, which can lead to the transfer of alleles from one species to another [1]. Genetic exchange,

863 however, is not completely limited to very similar sequences. As long as there are conserved
864 flanking regions, divergent alleles can recombine by homologous recombination [1] and the
865 presence of small short repeats may allow for micro-homology mediated recombination as well
866 [91].

867 Dynamic and repetitive regions of the genome that are more prone to duplication are like-
868 ly to have increased rates of gene integration. LGTs have been observed in regions enriched in
869 DNA transposons and retrotransposons and in gene-poor regions such as telomeres or in/near
870 TEs (discussed in [43]). Additionally, selfish genetic elements (e.g., self-splicing introns, inteins
871 etc.) are common in multicellular eukaryotes and could promote LGT and genome rearrange-
872 ments [1]. ‘Fragile’ genomic regions that are prone to double stranded breaks or environmentally
873 induced DNA damage and repair could also create opportunities for foreign gene integration.
874 LGTs may occur when viruses or parasites manipulate DNA damage/repair pathways. Certain
875 life/developmental stages may be more likely entry points for foreign genes into the nuclei of
876 both unicellular eukaryotes (particularly those who are not phagotrophic), and in particular, the
877 germline of multicellular eukaryotes (see the ‘weak-link’ model [92]).

878 In the testate amoeba *Paulinella micropora*, Matsuo et al. [93] identified a common ~30
879 bp sequence added post-transcriptionally by spliced leader (SL) trans-splicing to a diverse set of
880 mRNAs, including those derived by LGT and EGT. Based on these observations, the authors
881 suggested that SL trans-splicing could mitigate the potential detrimental effects of LGT in eu-
882 karyotes by ensuring translation of native nuclear genes disrupted by the insertion of foreign
883 genes in their 5’UTRs. This could in principle apply not only to *Paulinella* species but to other
884 trans-splicing organisms including euglenids, dinoflagellates and some metazoans.

885

886 **Box 3: Detecting LGT with confidence**

887 Genomic features that differ between eukaryotic and prokaryotic genes can be used to
888 differentiate between bona fide LGT and prokaryotic contaminants. One useful distinction is the
889 presence of introns in eukaryotic genes and lack thereof in prokaryotes; if a candidate LGT of
890 bacterial origin contains an intron it most likely resides within a eukaryote genome. Other char-
891 acteristics such as nucleotide composition, amino acid usage and the presence of a poly-A tail on
892 transcripts can help identify genuine LGTs. Further support can be found by examining the ge-
893 nomes of closely related organisms; unless the transfer is very recent, the suspected LGT will
894 likely be found in multiple genomes. For example, most of the LGTs identified in the gut para-
895 site *Blastocystis* sp. subtype 1 were also found in genomes of seven of the other subtypes [13].

896 Phylogenetic analysis is essential for differentiating candidate LGTs from gene duplica-
897 tions and differential loss. A patchy gene distribution, combined with a phylogenetic relationship
898 that contradicts the species relationship, is suggestive of an LGT. If it is assumed that all patchily
899 distributed eukaryotic genes are due to differential gene loss rather than lateral acquisitions, then
900 there becomes a genome of Eden problem [94] – the unlikely scenario that the last eukaryotic
901 common ancestor (LECA) had almost every gene seen in modern eukaryotes and an enormous
902 range of metabolic capabilities. Phylogenetic reconstruction of LGT is not without its limitations,
903 with certain transfers being more difficult to detect and interpret (such as sequential transfers,
904 which results in more complex gene distributions [62]). The lack of resolution associated with
905 single gene trees can make it tough to infer the validity and origin of candidate LGTs. Phyloge-
906 netic artifacts, leading to falsely resolved relationships that may mislead interpretations of a
907 gene's evolutionary history, are also of concern.

908 Some of the other most conclusive bioinformatic evidence for LGT can come from inves-
909 tigations of genomic context. A candidate LGT contained within a larger genomic scaffold clear-
910 ly of host origin is likely to be real (e.g., viral LGTs in *Acanthamoeba* that are surrounded by
911 amoebal host genes [28]). This can be difficult to assess if a reference genome is poorly assem-
912 bled and highly fragmented. Fortunately, long read sequencing technologies make it possible to
913 produce more highly contiguous genome assemblies, allowing for greater insight into genomic
914 context, particularly in repetitive regions and near telomeric repeats that typically do not assem-
915 ble well (e.g., [95]). Classical molecular biology techniques can also be used to verify genomic
916 integration of candidate LGTs into host chromosomes (e.g., fluorescence in situ hybridization
917 (FISH) has been used to confirm insertions of *Wolbachia* DNA on multiple chromosomes in the
918 grasshopper *Chorthippus paralleulus* and Tsetse flies (*Glossina* spp.) [96, 97].

919

920 **Box 4: *Trichomonas* and gene transfer in the microbiome**

921 *Trichomonas vaginalis* is a sexually transmitted parasite that colonizes an environment
922 occupied by other parasites and bacteria. 149 LGTs of both prokaryotic and eukaryotic origin
923 were identified in the *T. vaginalis* genome, including genes likely facilitating pathogenicity and
924 to take advantage of the rich metabolic opportunities in its environment (e.g., glycan degrada-
925 tion) [64]. Further study on LGTs in *T. vaginalis* identified and confirmed the functionality of
926 NIPC/P60 peptidoglycan hydrolases that were acquired laterally from bacterial co-members of
927 the microbiota [98]. Pinheiro et al. [98] showed that the bacterial NIPC/P60 hydrolases in the
928 genome of *T. vaginalis* were capable of degrading bacterial cell envelopes and that their expres-
929 sion was significantly upregulated in the presence of bacteria. The transfer of NIPC/P60 hydro-
930 lases in *T. vaginalis* appears to be critical in controlling bacterial abundance in its environment

931 and the establishment of a successful infection. This example shows that co-inhabitants of a giv-
932 en microbiome can act as a source of genes for one another, particularly in cases of phagocytic
933 species like *T. vaginalis* [99].

934 The human microbiome has been referred to as “a hotspot of microbial horizontal gene
935 transfer” [100]. While studies on LGT in the human microbiome has almost entirely focused on
936 the extensive prokaryote-prokaryote transfers (e.g., 13,514 high confidence LGTs between 308
937 human microbiome bacteria [100]), diverse eukaryotes with lifestyles ranging from parasitic to
938 beneficial also reside in and are important components of the human microbiome [101]. The
939 abundance of a variety of species and phages in close proximity presumably creates a favorable
940 environment for LGT for microbial eukaryotes; by increasing the frequency of donor DNA in the
941 environment you increase the exposure of a species to a given DNA source [13,44,64].

942 **Figure 1. Possible sources of foreign DNA contributing to LGT in eukaryotes.** Eukaryotic
943 cells are exposed to many potential sources of exogenous DNA including from: i) lysed
944 endosymbiont-derived organelles (mitochondria and chloroplasts), prokaryotic or eukaryotic
945 endosymbionts, ii) viral infection, iii) bacterial conjugation (e.g., *Agrobacterium tumefaciens*)
946 and iv) lysed phagosomes containing food organisms.

947

948 **Figure 2. The pan-genome concept.** The pan-genome consists of all genes found in all strains
949 of a given species and is depicted at the top of the figure as the union of all ellipses (each ellipse
950 is the set of genes present in that strain) in the Venn diagram. The pan-genome can be further
951 broken down into the ‘core’ genome (genes found in all strains shown in dark teal) and the ‘ac-
952 cessory’ genome (shown colours ranging from green to blue) which are genes found in a single
953 strain (i.e. strain specific) or a subset of strains (i.e. strain select).