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Opinion

Bacterial cooperation through horizontal gene transfer

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Cooperation exists across all scales of biological organization, from genetic elements to complex human societies. Bacteria cooperate by secreting molecules that benefit all individuals in the population (i.e., public goods). Genes associated with cooperation can spread among strains through horizontal gene transfer (HGT). We discuss recent findings on how HGT mediated by mobile genetic elements promotes bacterial cooperation, how cooperation in turn can facilitate more frequent HGT, and how the act of HGT itself may be considered as a form of cooperation. We propose that HGT is an important enforcement mechanism in bacterial populations, thus creating a positive feedback loop that further maintains cooperation. To enforce cooperation, HGT serves as a homogenizing force by transferring the cooperative trait, effectively eliminating cheaters.

Highlights

Cooperation is ubiquitous in bacterial populations. Bacteria produce and share public goods, providing indiscriminate benefits to their neighbors at cost to themselves.

Bacteria often engage in horizontal gene transfer (HGT) to rapidly disseminate traits in a population. HGT provides an important mechanism for cooperation to spread, effectively acting as an enforcement mechanism.

Public goods can also promote HGT, potentially resulting in positive feedback loops between the two.

HGT could itself be considered a public good due to the costly benefits it provides a population.

Evolution of cooperation

Cooperation has evolved many times across the Tree of Life and at all levels of biological organization, from genes forming genomes, to cells forming multicellular organisms, to eusocial insects forming superorganisms, and even to different species forming communities [1–3]. However, social adaptations such as cooperation are not always locally advantageous, making their prevalence puzzling to researchers for decades [4–6]. Several explanations for this social evolutionary paradox have since been developed; however, the same underlying concept holds: prosocial behavior benefits the collective, whereas selfishness provides a local advantage to individuals within collectives [5–7]. Charles Darwin described this notion in his observation that morality gives little to no advantage to an individual over another of the same tribe, yet will give an immense advantage to one tribe over another [4]. This multilevel selection framework requires that the benefits of cooperation go mostly towards other cooperators than to their selfish counterparts [5]. This can occur through sorting, kinship, conformance, or enforcement strategies such as punishment, which all increase the likelihood that a cooperator is interacting with, and thus benefiting, other cooperators [5,7]. We propose that the transfer of genes in bacteria is unique in that it serves as driver, product, and form of cooperation.

In bacteria, cooperation is often in the form of public goods whereby their production benefits all individuals [8,9]. These public goods are vulnerable to exploitation by individuals that benefit from their production but do not share in the costs. Nonetheless, it is not clear under which conditions bacterial cooperation is favored and whether it is an evolutionary stable strategy (i.e., a strategy that when adopted by most individuals in a population cannot be invaded and replaced by a deviating strategy) [10]. For example, Oliveira *et al.* found that stable cooperation through reciprocal exchange was unlikely to emerge in evolving microbial communities; however, most bacteria and archaea live under conditions of poor genetic mixing not explored in this study [11]. In addition, cooperation in bacteria includes an interesting caveat, as many cooperative genes are carried on mobile genetic elements. Here, cooperators benefit those around them,

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and at the same time, this transfer of genes also increases the likelihood that individuals receiving the benefits become cooperators themselves.

Costs of public good genes

Public goods were conceptualized in economics as goods that are non-rivalrous and non-excludable [12,13]. This means that using them does not detract from their consumption by other individuals and that they are available for everyone to use [12]. In humans, examples of public goods include national defense, sanitation, and street lighting. In other organisms, the concept of public goods is sometimes relaxed to include common-pool resources with rivalrous consumption [14]. Public goods in bacterial populations may include extracellular digestive enzymes, siderophores for iron scavenging, antibiotics used in bacterial warfare, antibiotic-degrading enzymes, surfactants for bacterial mobility, and molecules that function in virulence, quorum sensing, biofilm formation, and light production [2,8]. Proteins can provide a public service or leaky functions even if the encoded protein itself is not secreted from the cell [15].

The nature of public goods can lead to the free-rider problem, whereby some individuals capitalize on the public goods secreted by others (i.e., cheaters) [16,17]. These non-producers enjoy a relative fitness advantage over producers as they reap the benefits of cooperation without sharing in the costs. Over time, non-producers outcompete producers in the population, undermining cooperation in the population [18,19]. Such scenarios exist in both unregulated economies of human society [20,21] and bacterial populations. For instance, the opportunistic pathogen *Pseudomonas aeruginosa* scavenges iron through siderophores, a secreted public good [22]. In long-term lung infections, some strains have been observed to mutationally lose their ability to produce siderophores, giving rise to a cheater phenotype. This consequently leads to the collapse of cooperative social behavior over time [22].

Some cellular functions incur high energetic or nutritional costs. The metabolic cost of gene product synthesis can inhibit bacterial growth [23,24], with some biosynthetic genes more costly to keep than others [25]. Strains that stop performing these costly functions and eventually lose the corresponding genes have a local advantage [15]. While gene loss due to drift is known in bacteria, especially in host-restricted taxa [26,27], genome reduction also occurs in bacteria with very large effective population sizes, such as the marine-dwelling *Synechococcus* and *Roseobacter* [28,29]. Selective pressure to lose genes could be due to general genome streamlining caused by deletion bias observed in bacterial genomes [30], a possible way to purge them of selfish genetic elements [31]. Some public good genes may even result in host death when expressed. For example, the anti-competitor toxin colicin is released through self-lysis in *Escherichia coli* [32] and *Salmonella enterica* [33]. Self-lysis also occurs in the release of anti-predator Shiga toxin in *E. coli* [34].

The Black Queen hypothesis has been proposed to explain how selection for reduced genomes impacts the dynamics of public goods in bacteria. For functions that provide an indispensable public good, a fraction of individuals retain the genes that encode these functions and thus support the entire community through leakage [15]. Leaky public goods that become available to the rest of the community will lead to functional dependencies [15,35]. Hence, while these functions are not completely lost from the community, cooperation between producers and non-producers is maintained. The Black Queen hypothesis therefore predicts an overall trend towards ‘mutual cheating’ [35], highlighting the apparent paradox in the ubiquity of public good genes in bacteria [8]. Mutual cheating may also explain the conservation of gene content and functions of microbial communities, despite taxonomic variability within communities [36,37]. However, division of labor may be a more appropriate description, especially in those cases where a keystone strain or species is no longer present in the population and all individuals in the population are dependent on some

common good produced by others (i.e., strong Black Queen hypothesis [15,35]). The stable coexistence of producers and consumers, including cheaters of cheaters, of leaky products such as siderophores has been demonstrated in *P. aeruginosa* [38,39].

HGT introduces and maintains genes in a population

Bacteria are notable in their predilection towards HGT, the acquisition of genes between organisms through mechanisms other than vertical descent from a common ancestor [40]. Horizontally transferred genes can impact the fitness of the recipient and allow it to enter new ecological niches unavailable to it through mutation alone [41–43]. Conversely, genes with neutral or nearly neutral effects may also be frequently transferred [44,45]. The probability of fixation or elimination of these neutral acquisitions in the population will be determined by genetic drift [44,46], and in the case of selfish genetic elements, on the frequency they are transferred within a population [47,48]. Whether horizontally acquired genes are positively selected or neutral, they create a remarkably diverse and constantly changing pool of novel genetic combinations for which selection to act on [40,49].

Genes are easily acquired and lost in microbes [50,51]. This interplay of gene loss and gain results in a dynamic pan-genome, with individual strains carrying distinct sets of genes [52–54]. Inter-strain variability in gene content can arise through rapid gene gain and loss in response to neutral evolution or selection to survive in variable environments. A dynamic pan-genome enables the rapid addition of potentially adaptive genes and replacement of unfavored ones in a local environment at a rate that would not be possible without mobile elements [40,55]. This is notable for cooperative genes whose fitness payoffs depend heavily on local environmental conditions, such as population composition [56,57]. For example, Cry toxins are insecticidal proteins produced as crystal inclusions during the sporulation phase of bacterial growth and are considered as public goods with a high metabolic cost to produce [58]. Rapid gain and loss, mediated by plasmids, have been observed in genes that encode the Cry toxins among *Bacillus* species [58]. HGT therefore favors cooperative genes to invade and persist in a population.

It has long been hypothesized that plasmids (extra-chromosomal replicons) are maintained in populations due to their selective advantages. These might be due to locally adaptive genes they carry or the increased mobility of conditionally adaptive traits [47,59]. However, plasmid carriage often imposes a reduction in fitness to its bacterial host [47,60]. In response, conjugative plasmids have devised unique ways to persist in their host cells, even when the two come into conflict. These include self-mobilization, partition systems, multimer resolution systems, and post-segregational killing of cells without plasmids [47,61]. To persist in the population, plasmids must therefore depend on either or both fitness cost amelioration and HGT, with plasmids having traits of both parasites and mutualists [62,63].

Plasmids can persist in the population even in the absence of selective pressures, as demonstrated in an experimental assay of conjugation plasmids in *E. coli* [64]. While costly, plasmids of different incompatibility groups are rapidly transferred, such that antibiotic resistance genes carried by these plasmids persist even in the absence of antibiotics [64]. Similarly, HGT allows antibiotic resistance genes to persist at low frequencies in the naturally competent gut microbe *Helicobacter pylori* even in the absence of antibiotics [65]. A recent mathematical model has also shown that genes with small fitness benefits that would otherwise be lost from the population without HGT persist or are rescued, despite the costs incurred by selfish genetic elements [66]. However, such scenarios occur only in spatially structured environments (Box 1), such as biofilms [66] (Box 2). The complexity of microbial communities might therefore promote plasmid persistence because of multiple sources and sinks of plasmid transfer [59,67,68].

Box 1. Dynamics of public good genes in structured populations

Since the inception of evolutionary game theory, stable cooperation has been associated with population structure [78,79]. Cooperators have to interact with other cooperators more often than they would by chance, avoiding the net fitness penalty of cooperation [100]. This depends on selection at the level of groups of cooperating individuals, whereby genetic relatedness may not necessarily be at play [101,102]. Both experimental systems and mathematical models have shown that cooperative plasmids alone are not sufficient to maintain cooperation. Some degree of population structure is therefore required [92,103].

Population structure need not be constant. Changes in population viscosity can allow for the evolution of different types of cooperation [104]. The coevolution of population structure and cooperation has been proposed and subsequently demonstrated in mathematical models of Snowdrift and Prisoner's Dilemma games [105,106], with biofilms being a proposed example of this [107,108], as elaborated on in Box 3. HGT within groups can act as a mechanism to change population structure by increasing similarity between individuals in a population, which in turn increases between-group differences and accelerates group selection. A higher rate of HGT can thus be selected both to spread more public good genes and to increase relatedness between bacteria [109]. Biased plasmid transfer increases inclusive fitness and can lead to selection for higher rates of HGT. Biased transfer can be the result of population structure or recipient discrimination [110], as in the case of kin-biased plasmid transfer in *E. coli* [111]. *B. subtilis* also possesses kin discrimination systems, where many of the genes encoding antimicrobial compounds involved in discrimination are located on mobile genetic elements [112]. These studies suggest that HGT influences the evolution of kin groups.

The ability to maintain genes in a population might be especially useful for traits that are difficult to re-evolve via mutation once they are lost. This applies to some cooperative traits, as in the case of *P. aeruginosa*, where the loss of the siderophore pyoverdine was not recovered, even with increased spatial structure and reduced cost of public good production [69]. HGT may also act in concert with the physical arrangement of genes in the genome to prevent gene loss. The selfish operon hypothesis posits that genes coding for weakly selected functions physically cluster together due to HGT, so they can be donated [70,71]. Selfish operon theory can explain horizontally transferred gene clusters, such as genomic islands found in a variety of species [71–73], although its relative importance is a point of debate [74,75]. Widespread HGT can thus prevent the loss of a trait or reintroduce it if lost.

Mobile genetic elements as an enforcement mechanism

Cooperation requires enforcement to evolve and thrive. Although enforcement strategies may vary, enforcement ensures that the self-serving behavior of some members of the group is reduced

Box 2. Public goods and HGT in biofilms

The population structure described in Box 1 often manifests itself in physically distinct populations. Most bacterial and archaeal cells live in biofilms or small aggregates [113]. They are therefore more likely to be physically adjacent to cells with whom they share recent ancestry and more likely to have the same genotype with respect to leaky functions. These neighborhood relations are expected to increase frequency-dependent selection on genes encoding these functions. Drescher *et al.* [114] showed that chitinase-secreting *Vibrio cholerae* can avoid the public goods dilemma by strengthening relationships between cells of the same genotype through creation of a thick biofilm, leading to larger benefits to the producers when the overall concentration of the public good decreases. While the cheater strain has a fitness advantage over the cooperators at low frequencies, it has a fitness disadvantage at high frequencies. This shows that genes encoding common goods can be under frequency-dependent selection, which could lead to local feedback loops that promote the coexistence of different cheaters.

Biofilms are also hotspots for HGT events facilitated by a variety of transfer mechanisms, including conjugation, nanotubes, natural transformation, phages, and membrane vesicles [115–117]. Biofilms also provide physical means to structure bacterial communities and therefore set physical boundaries for HGT [118,119]. Conjugative plasmids can directly induce biofilm formation in bacteria, first demonstrated in an *E. coli* laboratory strain [120]. Non-conjugative plasmids have also been shown to affect biofilm formation [121]. In turn, biofilm growth can result in a greater copy number of plasmids [122] and more persistent plasmids [117,123], raising the possibility of positive feedback loops formed by this interaction [124]. Biofilms can therefore lead to enhanced cooperation. In *E. coli*, more cooperative resistance from a plasmid-encoded β-lactamase has been reported in biofilms compared to liquid cultures [125]. Mobile elements can therefore promote cooperation not just by carrying public good genes, but by inducing environmental conditions favorable for cooperation, such as biofilms [119].

[76,77]. Through his inclusive fitness theory, Hamilton first proposed that relatedness between interacting organisms can favor cooperation even when costly to the individual [78,79]. Inclusive fitness theory has since been expanded to include general models taking individual genes into account [80,81]. Spatial structure of a population can also allow cooperation to evolve [56,57], even without considering relatedness [7,80]. Spatial self-organization of the population can also sustain cooperation through repeated colonization, even when the costs for cooperation are high [82,83]. Enforcement can also take the form of policing [84,85] or the repression of competition within groups [86,87]. Other enforcement mechanisms in bacteria include quorum sensing and antagonistic pleiotropy [9].

We propose that HGT is also an important enforcement mechanism in bacterial populations. Figure 1 shows HGT impeding the invasion of a cheater in a cooperating bacterial population. A mutant cheater strain arises in a cooperating population (Panel A) through a random loss-of-function mutation (Panel B). In the absence of HGT as an enforcement mechanism, the cheater can invade the population (Panel C) and undermine public good production, resulting in the fitness loss of the overall population (Panel D). With HGT, the mutant cheater is converted into a cooperator by reintroducing the functional allele (Panel E), thus rescuing cooperation (Panel F).

The concept of HGT as an enforcement mechanism was first hypothesized in the context of acute infection within and between hosts described using a differential equation model [88]. In this

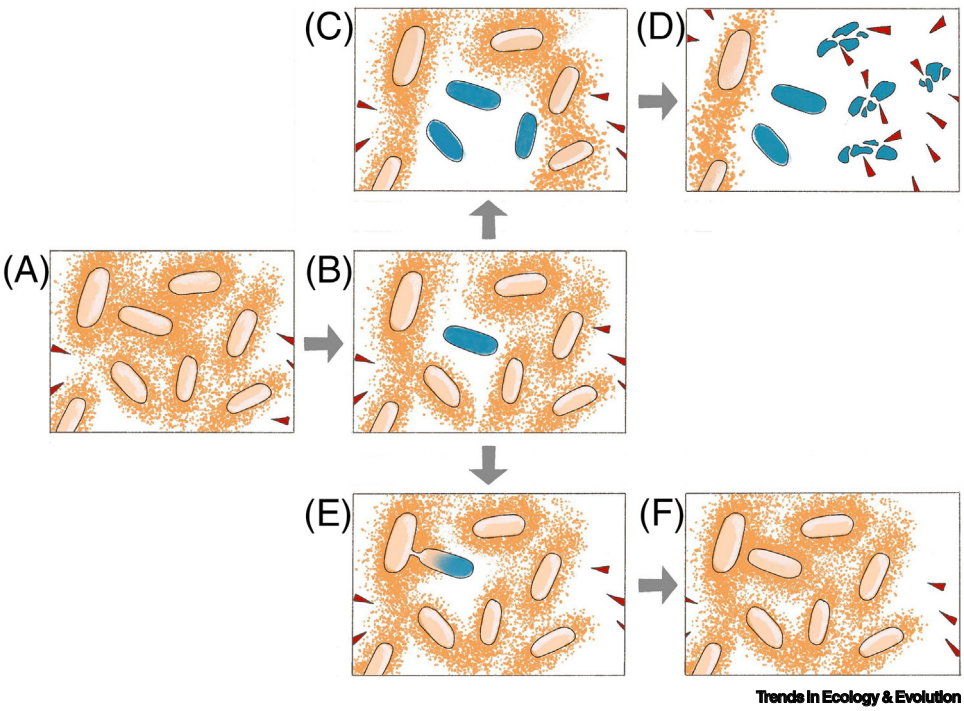

Figure 1. Cooperative bacterial population invaded by a cheater, with and without horizontal gene transfer. (A) Bacteria secrete extracellular proteins (orange dots), a public good, to protect themselves from an antimicrobial agent (red triangle). (B) A non-producing cheater (blue cell) arises in the population as a result of a loss-of-function mutation. (C) Due to its fitness advantage, the cheater strain invades the population. The presence of the public good produced by the remaining cooperators enables the survival of the cheaters. (D) In the absence of cooperators to produce enough of the public good, the cheaters are killed by the antimicrobial agent. The size of the population is significantly reduced as a result. (E) In the presence of horizontal gene transfer, cooperator strains can transfer a functional allele into the cheater strain. The loss-of-function mutation is rescued as a result. (F) In the absence of cheaters, the population remains immune to the antimicrobial agent.

model, selection for infectious transmission favored pathogens that can force cheater strains to produce the virulence factor (i.e., public good secreted extracellularly) via HGT [88]. These findings were supported by investigations in a variety of bacterial species that made use of genomic data [89,90], simulations [89,91], and experimental work [69,92]. Other cooperative traits such as virulence factors, toxins, and detoxification proteins are also frequently found on mobile elements [93], and may facilitate evolutionary rescue of some genes [94]. More recently, a larger survey of 5397 bacterial genomes across several taxa confirmed the overrepresentation of genes coding for secreted proteins on plasmids [90]. However, we note that leaky functions do not necessarily require the protein catalyzing this function to be secreted (see section on Costs of public good genes).

The role of plasmid-mediated HGT in the context of bacterial cooperation remains unsettled. Two recent preprint studies describe other factors that influence the presence and mobility of plasmids, such as virulence traits. A genomic analysis of 51 bacterial species reported that HGT might help cooperation to initially invade a population but does not help in maintaining cooperation in the long term [95]. An experimental study of a master regulator of virulence inserted into a conjugative plasmid demonstrated the emergence of cooperative virulence, but its stability depended on transmission dynamics [96]. The role of population structure and time scales being studied may explain these discrepancies. HGT might cause cooperation to appear short-term in local populations, without necessarily maintaining it across a species. Future work is needed to explore patterns of transfer of other mobile genetic elements, such as chromosomal cassettes, transposons, integrons, and phages. Since interactions between different mobile elements can facilitate their transfer, broader sampling may present a clearer picture of HGT [97,98].

Many extracellular toxins that function as public goods are also associated with mobile elements. In *E. coli*, colicin is a plasmid-encoded toxin used in bacterial warfare, where it causes lysis in both cells expressing it and neighboring cells exposed to it [32]. Since colicin results in a loss of reproductive potential, it is rarely expressed. Cells thus bet-hedge, with colicin silenced within most of them [99]. In *S. enterica* serovar Typhimurium, colicin is phage-encoded and also causes self-lysis [33]. Phage-mediated bet-hedging has been observed, with HGT hypothesized as maintaining the evolutionary stability of colicin production [33]. HGT can therefore cause phenotypic heterogeneity associated with bet-hedging due to the frequent gain and loss of traits. Another example is the Shiga toxin in *E. coli*, a phage-encoded anti-predator molecule. It is also secreted and causes self-lysis when expressed [34], thereby functioning as a public good. Due to the mobility of the phages encoding Shiga toxin, susceptible non-cooperating bacteria can be induced to produce it, enforcing cooperation in the population [34]. These examples demonstrate how the transfer of mobile elements coding for public goods can enforce their expression in phage-susceptible cells, and thus the cooperation among members of the population (Box 3).

Concluding remarks

Evidence exists for a positive relationship between gene mobility and cooperation, although primarily focused on plasmids. Direct investigations of genetic recombination and of other mobile genetic elements are lacking. Given how the prevalence of HGT itself is evolvable, such as through the construction of structured microbial communities, conceptualizing HGT as a dynamic parameter in the evolution of cooperation is warranted. The relationship between bacterial cooperation and HGT could lead to a positive feedback loop, whereby HGT and cooperation maintain each other in the population. Finally, the study of HGT as an act of cooperation in itself seems appealing, but the fitness costs of HGT itself should be investigated. The availability of whole-genome sequence data will lead to more comprehensive surveys of mobile genetic

Outstanding questions

Are the differences in the frequency of HGT and accessory gene content among bacterial taxa and among strains due to differences in their propensity for cooperation?

What are the fitness costs associated with HGT? Are these costs primarily due to the carriage of potentially harmful genes, or are they due to the act of transfer itself?

Cooperative traits increase the fitness of a group, allowing for increased population density. Does this increased fitness always translate to more frequent HGT?

To what extent do other types of mobile genetic elements, such as phages, transposons, gene cassette, and integrons, facilitate cooperation in bacterial populations?

How do different mechanisms of transfer (conjugation, transformation, transduction, homologous recombination, and illegitimate recombination) influence the cooperative behaviors between strains?

To what extent do biases in HGT partners (due to phylogenetic, geographical, or ecological proximity) enhance or hinder cooperation in a population?

Are there differences in the distribution of public goods and cooperative behaviors during asymptomatic carriage and disease?

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Box 3. Horizontal gene transfer as second-order cooperation

The free-rider problem, by which a population loses a cooperative function due to individuals having no incentive to maintain it [18,19], can be remedied in populations by enforcing cooperation [77]. This makes enforcement itself an act of cooperation and the enforcement system a public good [126]. Individuals may opt to punish non-cooperators even when doing so incurs a cost, leading to 'altruistic punishment' [127,128]. However, the fitness cost may lead to the second-order free-rider problem, whereby individuals who do not punish non-cooperators outcompete punishers. How these enforcement systems evolve is an exciting new field of study [127–129].

The production of cooperative offspring can be a form of second-order cooperation, with the comparative rates of production of cooperators and non-cooperators being an evolvable trait [130]. In cooperating populations of bacteria, population structures with high levels of relatedness select against hypermutators, favoring the continued transmission of the cooperative phenotype [131,132]. The same principles that apply to the vertical inheritance of cooperative traits should also therefore apply to HGT, with HGT acting against mutation in cooperative populations.

Mobile genetic elements, such as plasmids, often have fitness costs to their hosts. This can be due to cargo genes not being adaptive or conflicts between these elements and the hosts [133,134]. The costs may also vary depending on the mobile elements, the host, and their interactions [134,135]. The fitness changes associated with random horizontally transferred DNA fragments may range from maladaptive to neutral to adaptive [51,60,68]. The act of conjugation itself may also carry fitness costs [136]. Horizontally transferred genes can also either cooperate with or antagonize their host genomes [137]. While maintaining HGT may thus be a costly public good in some cases, such as in the case of conjugative plasmids that have evolved persistence mechanisms in opposition to their hosts, the costs may be reduced when mobile elements and their host genomes coevolve towards stable mutualism [47,68,137]. Future work is needed to better understand the second-order free-rider problem in the context of maintaining HGT.

elements and public good genes across various microbial taxa. These phenomena are mechanistically distinct yet dynamically intertwined, feeding into each other to produce the rich tapestry of bacterial sociobiology (see Outstanding questions).

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Authors' contributions

C.P.A. and I.P.A.L. developed the concept and wrote the initial manuscript. All authors read, discussed, edited, and approved the final manuscript.

Declaration of interests

The authors declare that they have no competing interests.

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