evolution of genetic architecture (so that each sex can express the shared genome in a sex-specific manner). In these cases, the lack of ornamentation may reflect naturally selected costs of female displays. However, sexual selection can also be important in the evolution of female ornamentation. For example, in the African cichlid fish *Pelvicachromis taeniatus,* female ornamentation reveals important information about female quality to mates — for example, readiness to spawn, fecundity, offspring fitness — and is under strong sexual-selection by male choice, and by natural selection through female–female resource competition too*.*

What are the wider evolutionary consequences of mutual mate choice? Mutual mate choice is probably underappreciated but can facilitate sympatric speciation and help to maintain species barriers after population divergence has occurred. Mutual mate choice may also affect the mean fitness of a population. It can increase population level fitness if, for example, males better provision preferred females and so increase the contribution of these females to the next generation. Alternatively, mutual mate choice may decrease mean population fitness if male harassment harms high-quality, preferred females and so reduces their reproductive output relative to that of low quality females.

Does mutual mate choice apply to peacock flies? While the peacock fly display has been attributed to mutual mate choice, how likely is this to be the case? Many of the criteria for mutual mate choice are probably met in peacock flies. Female fecundity often varies in insects as a function of size and, if males dance to attract females, then the costs of male mating may be high. That said, and assuming female dance is energetically expensive, it is rather surprising to find a male insect picking females on the basis of an energetically demanding female display. Most male insects pick mates on the basis of traits that signal female fecundity, such as size, mating status or age, and, while the elaborate peacock-fly dance and colouring seem likely to reveal information on female quality,

it probably reduces female fecundity. However, some male insects do prefer mates that are likely to produce high quality offspring rather than lots of them. For example, male cockroaches, *Blattella germanica,* prefer to court unrelated females. Given that peacock flies have even been reported to display towards a stray entomologists finger, might the primary function of this dance in females be related to competition over territories?

In short, we do not know for sure, but the fact that dances occur in places that are not likely to be ideal resource patches (like car windscreens), a sexual role seems likely. To improve our understanding of male mate-choice, female competition and mutual mate choice, more research into these somewhat ignored "non-traditional" behaviours is needed, and peacock flies seem a good place to start.

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Natural competence for transformation

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While most molecular biologists are familiar with the artificial transformation of bacteria in the context of laboratory cloning experiments, *natural* competence for transformation refers to a specific physiological state in which prokaryotes are able to take up genetic material from their surroundings. Occasionally, such absorbed DNA is recombined into the organism's own genome, resulting in natural transformation (Figure 1). As a consequence, natural competence for transformation is considered a primary mode of horizontal gene transfer (HGT) in prokaryotes, together with conjugation (direct cell to cell transfer of DNA via a specialized conjugal pilus) and phage transduction (DNA transfer mediated by viruses). HGT plays a major role in bacterial evolution, and past research has demonstrated that HGT, including natural competence for transformation, contributes to the emergence of pathogens and the spread of virulence factors. Indeed, Frederick Griffith discovered natural competence for transformation in 1928 while he was investigating the exchange of pathogenic traits in pneumococci. Due to the increase in the abundance and spread of multidrug-resistant microbes, research on HGT is even more important today than ever before.

Natural competence — a developmental program in prokaryotes
Natural competence for transformation is considered a developmental program in prokaryotes. Although natural competence has been described in both bacteria and archaea, the majority of knowledge is derived from studies of pathogenic, environmental, and laboratory model bacteria, which I will focus on in this Primer.

Natural competence is genetically encoded and thereby distinguishes itself from artificial permeabilization of the bacterial cell envelope in

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laboratory settings. Indeed, a plethora of genes that are involved in competence regulation and the DNA-uptake process have been identified. Interestingly, some bacteria couple the production of DNA-uptake machinery with other physiological responses, such as growth arrest or interbacterial competition, resulting in large competence-associated regulons. In *Bacillus subtilis*, for example, only approximately 10% of the bacterial population enters competence at high cell density or at the onset of stationary phase (Table 1). This bistable response is a consequence of gene-expression noise combined with a positive-feedback loop (an auto-regulatory circuit). The resumption of growth is, interestingly, significantly delayed in these competent cells when they are diluted into fresh growth medium. This is in contrast to their non-competent siblings, which rapidly resume growth. Specific competence proteins have been identified in *B. subtilis* that prevent chromosome replication and cell division, and therefore are causative for the observed growth delay in these competent bacteria. Recent data also indicated that competent individuals have decreased rRNA synthesis, which halts their growth and renders them tolerant to antibiotics. Entry into competence occurs concomitant with growth arrest and the ability to take up exogenous DNA, and may represent a bet-hedging strategy that *B. subtilis* utilizes to adapt to changing environmental conditions. Furthermore, some organisms, such as *Vibrio cholerae,* the causative agent of cholera*,* merge diverse environmental signals into the competence regulatory network. This scenario leads to a temporal and spatial heterogeneity with respect to competence induction that may be beneficial for the population as a whole.

Distribution of natural competence and species-specific triggers

There are a wide variety of prokaryotic species that are naturally transformable. Experimental data show that more than 80 species including both Gram-positive and Gram-negative bacteria are naturally competent. However, many competence genes, and especially those that encode the DNA-uptake
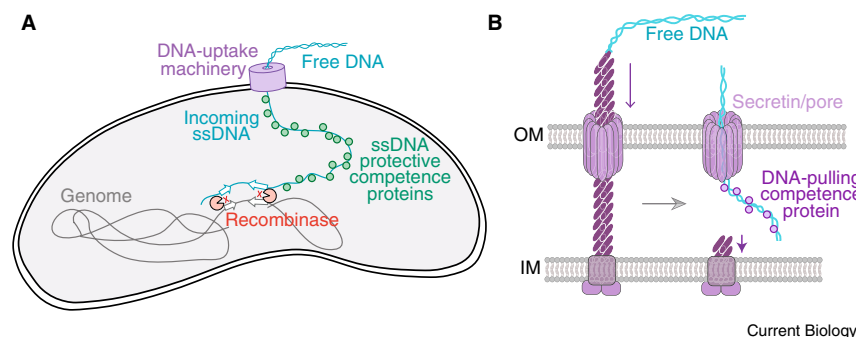

Figure 1. Overview of the DNA-uptake and incorporation processes and the DNA-uptake machinery in naturally competent bacteria.
(A) Free DNA serves as substrate for the DNA-uptake apparatus, which eventually shuffles single-stranded DNA (ssDNA) into the cytosol of the competent bacterium. This ssDNA is protected from degradation by specialized competence proteins, which also contribute to the recruitment of the recombinase. The latter then mediates the integration of the newly introduced DNA into the genome. (B) Detailed view of a representative DNA-uptake apparatus of a naturally competent Gram-negative bacterium. The central part of such nanomachines is composed of a type IV pilus structure, which, upon retraction, might open the outer-membrane secretin pore. Additional competence proteins then bind the DNA and may further pull it into the cell via a Brownian ratchet mechanism.

apparatus (see below), are even more widely distributed within the bacterial kingdom. Whether these genes are still functional and contributing to the survival, fitness, and potentially the evolution of the host is largely unknown. This lack of knowledge may be because the competence program is often not constitutively expressed in naturally competent bacteria, but rather is only switched on during certain growth phases or in response to specific environmental cues (Table 1). One example of signal-specific competence induction can be found in the pathogen *V. cholerae*. Although this bacterium has been studied for more than a century, the discovery of its potential for natural competence and transformation was fairly recent. *V. cholerae* enters competence upon reaching high cell density, which it measures through quorum sensing of a specific autoinducer that serves as a competence pheromone while growing on chitinous surfaces. Such surfaces are encountered and colonized by the pathogen in its natural habitat, the aquatic environment.

Known inducers of competence in other naturally competent bacteria include high cell density, antibiotic stress, DNA damage, intracellular growth, absence of preferred carbon sources and general starvation (Table 1). For example, studies of

the human pathogen *Streptococcus pneumoniae* have demonstrated that certain antibiotics and DNA-damaging or replication-inhibiting agents trigger the onset of competence. It was therefore suggested that competence induction replaces the canonical SOS stress response, which is missing in this and a few other naturally competent bacteria, and that absorbed DNA might contribute to DNA repair and potentially to the acquisition of antibiotic-resistance genes or virulence determinants (see below). Notably, while much is known about the induction of competence in a few model organisms (Table 1), the natural trigger remains unknown for most putatively competent bacteria, which surprisingly includes one of the best-studied bacterial species, *Escherichia coli*.

The competence-induced DNA-uptake machinery

While competence regulons can encompass more than 100 genes, only a subset is required for the transformation process. This subset includes the genes that encode the DNA-uptake apparatus, the proteins that mediate protection of the incoming DNA within the bacterial cytoplasm, and the proteins that initiate recruitment of the recombination enzyme (Figure 1). The DNA-uptake machinery allows

Table 1. Model organisms for studying natural competence for transformation.

Bacterium	Year of first experimental demonstration of natural competence and transformation	Competence inducer or growth phase specificity
Gram-positive bacteria		
Bacillus subtilis	1958	Induced at onset of stationary phase or high cell density Bistable regulation (only ~10% of the population enters competence)
Streptococcus pneumoniae	1928	Short window in exponential phase Inhibited in stationary phase Induced under stress conditions (antibiotic treatment, DNA damage)
Gram-negative bacteria		
Acinetobacter baylyi	1969	Constitutive, but occurs at low or high frequencies depending on growth phase
Haemophilus influenzae	1951	Nutrient downshift or starvation
Helicobacter pylori	1990	Constitutive, but occurs at low or high frequencies depending on growth phases Further increased upon DNA damage
Neisseria gonorrhoeae/ Neisseria meningitidis	1953	Constitutive
Vibrio cholerae	2005	Chitinous surfaces and high cell density

the translocation of genetic material across the cell envelope and is usually composed of more than 10 different proteins that are conserved among competent species (aside from the expected differences between Gram-positive and Gram-negative bacteria due to the different nature of their cell envelopes).

The central part of the DNA-uptake complex is an extendable and potentially retractable type IV pilus, which shares homology with type II secretion systems. However, in contrast to the type II secretion systems, which push substrates from the periplasmic space out of the bacterial cell, the DNA-uptake pilus is thought to work in the opposite manner. The retraction of the pilus might result in the opening of a secretin pore in the outer membrane of Gram-negative organisms (Figure 1), or it may free space in the dense peptidoglycan mesh of Gram-positive bacteria, allowing the entry of the incoming DNA. Recent studies also suggest that the central DNA-uptake pilus, including certain incorporated minor pilin subunits,

might contribute to the initial binding of free genetic material as well as the species-specific sorting of DNA — the latter of which happens in the case of a minority of bacteria including *Haemophilus influenzae* and some *Neisseria* species. Once pilus retraction occurs, additional periplasmic, membrane-attached or cytoplasmic competence proteins pull the DNA further into the cell, concomitantly with its compaction. The incoming DNA that arrives in the cytoplasm is single stranded and is immediately decorated with protective proteins to prevent its degradation. Finally, the RecA recombinase is recruited and polymerizes on the single-stranded DNA, which, after a homology search, results in homologous recombination (Figure 1). Illegitimate recombination at positions of strongly divergent sequence has also been reported in naturally transformable bacteria, but such events are extremely rare. Interestingly, although translocation across the inner membrane and the recombination process are strictly conserved in naturally competent

bacteria, exceptions have been reported in the mechanisms by which incoming DNA crosses the outer membrane. Indeed, macromolecular nanomachines other than the *bona fide* type IV pilus might contribute to this initial step. For example, in *Helicobacter pylori*, DNA uptake is mediated by a type IV secretion system unrelated to type IV pili.

**Natural competence for transformation — what is it good for?**
The benefit that the absorption of foreign DNA brings to the competent bacterium is an important and widely discussed topic. The DNA could, on one hand, serve a nutritional purpose (Figure 2). Indeed, several competent bacteria enter competence once they reach high cell density or encounter nutrient-limiting conditions. DNA uptake might alleviate such starvation stress by providing new building blocks, as *de novo* biosynthesis of nucleotides is costly. However, species-specific DNA uptake, as happens in some competent organisms, as well as competence-induced protection of incoming single-stranded DNA do not fully support a nutritional purpose. On the other hand, the incoming DNA might be used as genetic material, which, upon recombination, could lead to the repair of the bacterium's genome, the curing of selfish mobile genetic elements, or the acquisition of new genes and operons (Figure 2). Distinguishing between these outcomes and elucidating the primary purpose for the maintenance of natural competence in certain species is challenging. Moreover, the purpose of competence likely differs from one organism to another and might even be context dependent. Nonetheless, based on the fact that DNA uptake can and does result in the transformation of the respective competent bacteria, natural competence is considered to be one of the three major modes of HGT in bacteria.

Natural transformation as a mode of horizontal gene transfer
Research on natural transformation dates back almost 100 years. A seminal work by Frederick Griffith published in 1928 first described this phenomenon (Figure 3). Griffith's experimental setup, which led him

to postulate the existence of the *transformation principle,* was based on the following observation: non-virulent *S. pneumoniae* (a rough strain) could convert to a virulent pathogen when co-inoculated with a heat-killed virulent *S. pneumoniae* (a smooth strain) and injected into mice. The absence of disease symptoms after monoculture inoculation was due to the lack of a specific virulence factor in the rough strain and the heat inactivation in the smooth strain. However, in addition to the ability of the mixed culture to kill the mice, Griffith was also able to re-isolate live virulent (smooth) bacteria from the infected animals. We are now able to understand what occurred (Figure 3). *S. pneumoniae* belongs to the group of naturally competent bacteria, and the avirulent strain was therefore able to absorb and recombine the genetic material that was released from the virulent heat-killed pneumococci. Consequently, the competent bacterium acquired genes that encoded the biosynthetic proteins for one of the major virulence factors of *S. pneumoniae*, the polysaccharide capsule. This capsule makes pneumococcal colonies appear smooth and protects the pathogen from the host's immune system. Importantly, the *transformation principle* reported by Griffith prompted a search that eventually led to the discovery by Oswald Avery, Colin MacLeod, and Maclyn McCarty that genes are made of DNA, and paved the way for modern molecular biology.

In retrospect, Griffith's research unambiguously demonstrated that natural competence for genetic transformation leads to the spread of virulence traits, a finding of prime importance. Since the advent of next-generation sequencing, many bacterial genomes have been sequenced, and the occurrence of recent horizontal acquisitions was found to be the rule rather than the exception. Such HGT events can have significant consequences for pathogen emergence. For example, a large outbreak of cholera that started in the 1990s in Asia and lasted over a decade, spreading fear of a new pandemic, appears to have arisen from one such event. In contrast to the ongoing seventh pandemic, which is caused by the *V. cholerae* O1 El Tor
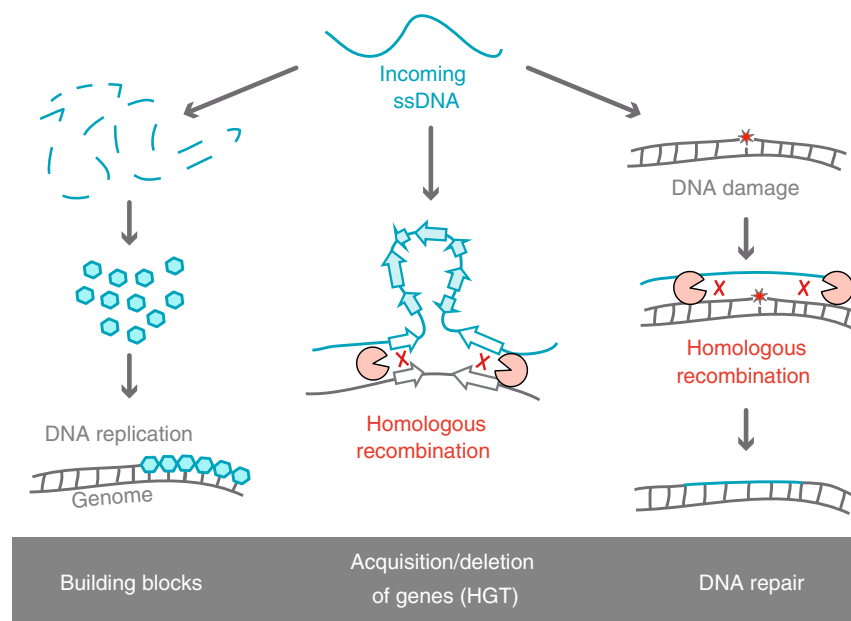
Figure 2. Potential benefits of natural competence-mediated DNA uptake in bacteria.
Upon entry into competence, bacteria might take advantage of the incoming DNA for nutritional purposes (recycling nucleotides as new DNA building blocks), for the acquisition or deletion of genes and gene clusters (HGT), for DNA repair, or for a combination of these reasons.

strain, the 1990s outbreak was caused by a new serogroup designated O139. The O139 strain differs from the El Tor strain in that it has an altered O-antigen chain and is surrounded by capsular material. Early analyses suggested that the O1 El Tor strain had acquired novel genes by HGT from another aquatic bacterium, allowing the pathogen to synthesize the new O139 O-antigen and capsule. Furthermore, it was assumed that the absence of immunity against this new serogroup contributed to its spread in Bangladesh, India, and beyond. Experimental *in vitro* evolution experiments by Blokesch and Schoolnik later demonstrated that chitin-induced natural competence for transformation fosters serogroup conversion in *V. cholerae*. Moreover, such transformation-mediated serogroup-converted strains were refractory to the predation exerted by O1-specific bacteriophages, which the pathogen frequently encounters in the aquatic environment and human intestine. Thus, natural competence for transformation is an important mode of HGT and contributes to bacterial evolution and the mosaic-like structure of bacterial genomes.

Natural competence for transformation and the source of DNA

Compared to the two other modes of HGT, conjugation and phage-mediated transduction, natural competence for transformation is special because it is solely controlled by the DNA-absorbing competent bacterium without the need for mobile genetic elements. Moreover, free DNA is often abundant in natural habitats and animal hosts, but so are nucleases. While some bacteria cease their own nuclease production upon competence induction, surrounding prokaryotic or eukaryotic cells might nonetheless secrete these enzymes. As a consequence, researchers have speculated that free DNA is often heavily fragmented and might therefore contribute very little to the acquisition of new genes or even larger gene clusters. To overcome this problem, competent bacteria have evolved active DNA-scavenging approaches. Indeed, previous work has shown that naturally competent *S. pneumoniae* kill their non-competent siblings in a process referred to as fratricide, which was speculated to serve as a DNA repair mechanism. This is consistent
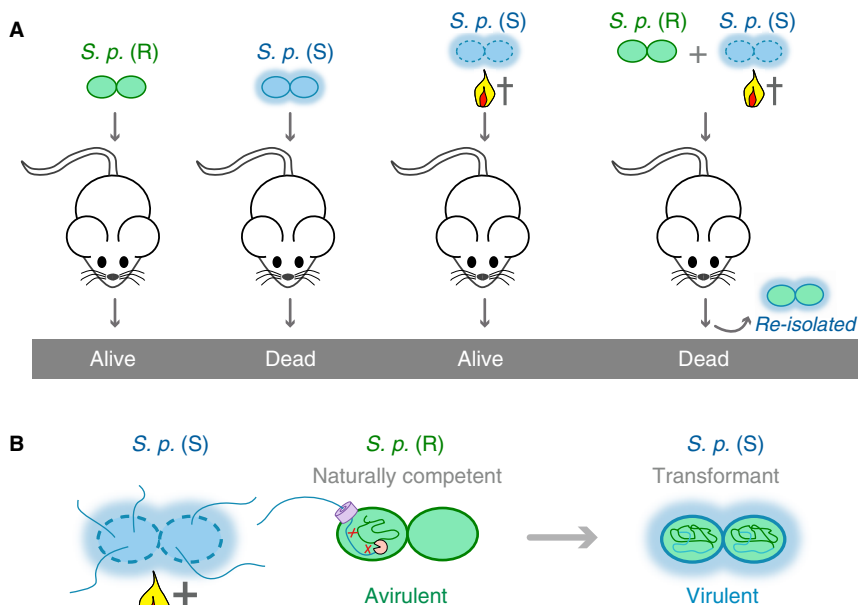
Figure 3. The discovery of natural competence for transformation via the *transformation principle* by Frederick Griffith.
(A) Inoculation of avirulent (rough; R) or virulent (smooth; S) *S. pneumoniae* resulted in the survival or death of mice, respectively. Animals did not succumb to heat-inactivated virulent strains, except if they were injected together with the live avirulent strain. (B) Molecular explanation of Griffith's experiment. The avirulent *S. pneumoniae* strain acquired virulence traits from the heat-killed virulent isolate through its ability to take up the 'transformation principle'— now understood to be DNA — as part of its natural competence program. The resulting transformants re-gained pathogenic attributes and were therefore selected in the experimental animal.

with DNA damage as an inducer of competence in this organism (Table 1). Interestingly, our recent work on competence in *V. cholerae* provided evidence for a strong coupling between kin-discriminated interbacterial predation and DNA absorption, which ultimately fosters HGT.

Perspectives
Despite the long history of research on natural competence for transformation, this process is still far from being fully understood. Indeed, while the overall composition of the DNA-uptake machinery has been described for a handful of competent bacteria, we still lack detailed structures of the overall macromolecular nanomachine as well as mechanistic insights into the DNA-uptake process. Furthermore, as discussed above, it is highly likely that the abundance and distribution of competence is currently vastly underestimated. One reason for this might be that laboratory conditions

seldom reflect the true bacterial habitat. Undeniably, in the past, many microbiology laboratories including my own have focused on single species and neglected the surrounding bacterial community. This is currently changing, and I am sure that many important lessons can be learned from natural competence for transformation if we take interbacterial cooperation and competition into consideration. Novel sequencing and imaging technologies will be extremely helpful for this endeavor. Such research will be of prime importance not only for the sake of basic science and our aim to understand general principles of HGT, transport of macromolecules, and bacterial regulatory networks, but also to shed light on the contribution of natural transformation to the spread of antibiotic resistance determinants. Recent sequencing efforts have also shown that many newly arising pathogens are hybrids that contain horizontally acquired virulence factors from other pathogens. A prominent

example of such hybrid isolates is the European *E. coli* O104:H4 outbreak strain, which affected nearly 4,000 persons in 2011, mostly in Germany and France. Interestingly, in a perspective article in EMBO Molecular Medicine in 2012 it was concluded that "Another preventive action involves the study of the exact, and up to now unknown, pathways leading to the acquisition of pathogenic traits by *E. coli*, and the selective pressures that may lead to their consolidation in a new virulent and potentially lethal strain". Research on natural competence for transformation and other modes of HGT is therefore much needed.

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