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Opinion

Sexual Selection in Bacteria?

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A main mechanism of lateral gene transfer in bacteria is transformation, where cells take up free DNA from the environment which subsequently can be recombined into the genome. Bacteria are also known to actively release DNA into the environment through secretion or lysis, which could aid uptake via transformation. Various evolutionary benefits of DNA uptake and DNA release have been proposed but these have all been framed in the context of natural selection. Here, we interpret bacterial DNA uptake and release in the context of sexual selection theory, which has been central to our understanding of the bewildering diversity of traits associated with sexual reproduction in the eukaryote world but has never been applied to prokaryotes. Specifically, we explore potential scenarios where bacteria releasing DNA into the environment could compete for successful uptake by other cells, or where bacteria could selectively take up DNA to enhance their fitness. We conclude that there is potential for sexual selection to act in bacteria, and that this might in part explain the considerable diversity in transformation-related behaviours.

Highlights

Prokaryotic 'parasex', or the uptake of free DNA coupled with the release of DNA into the environment, can be analysed in the framework of sexual selection developed for animals.

Increased investment in DNA release could be viewed as analogous to sperm competition, where males invest in sperm number to enhance fertilization success.

Fisherian sexual selection occurs when females gain indirect benefits by enhancing the attractiveness of their offspring to mates. Some bacteria preferentially take up DNA from strains harbouring specific motifs.

In some animals, males have evolved traits to coerce females into mating, which in turn selects for females to become resistant. We discuss whether this process could apply to bacterial DNA release and uptake.

We review recent evidence that recipient cells can actively initiate DNA uptake by using molecular cues to lyse donor cells.

Sexual Selection

The animal world is replete with conspicuous forms, colours, and behaviours that seem at odds with maximizing survival. Darwin was the first to realize that the fitness costs of such traits could be outweighed by their positive effect on reproductive success [1]. In contrast to individuals competing for resources, which is part of 'conventional' natural selection, individuals can also compete for mating opportunities, for which he coined the term **sexual selection** (see Glossary) [2]. Sexual selection can take two main forms [2]. Sexual competition involves individuals of the same sex competing for access to individuals of the other sex. In animals, this is usually male–male competition and can lead to the evolution of traits that help males win fights to secure fertilizations (but it can also lead to selection for males able to circumvent fights, or in some taxa for competition between females for males [3]). Mate choice, the other mechanism of sexual selection, involves choosing between suitors. In animals, this typically involves females choosing between males who compete for their attention or in other ways attempt to coerce females into mating.

In recent decades it has become clear that sexual selection extends beyond brightly coloured peacocks or clashing bighorn sheep and is now known to also act in plants [4] and fungi [5]. Andersson in his seminal book *Sexual Selection* wrote 'Demonstrations of sexual selection in bacteria may not be soon to come, but competition over mates in principle can occur also in unisexual organisms that exchange genetic material' [3]. Apart from this quote, we have failed to find any other mention in the literature on the potential action of sexual selection in bacteria. At first, this seems unsurprising, as the **parasexual** processes bacteria engage in are very different from eukaryotic sex. However, one main bacterial parasexual process, **transformation**, shares a key characteristic with meiotic sex, namely that it is a mechanism of genetic transfer that is under control of the cell (and does not rely on infectious elements such as plasmids or bacteriophages). During transformation, free DNA from the environment is taken up through transporters in the cell membrane(s) after which it can be recombined into the chromosome [6–9], or potentially act as templates for repair or serve as a source of nutrients (Box 1).

A range of species are known to release DNA into the extracellular environment through secretion or **autolysis** [10] and it could be hypothesized that this behaviour has evolved to provide DNA for uptake by other cells (Box 1). Assuming that benefits arising from the recombination of foreign DNA form an integral part of transformation [11] (Box 1), it can be expected that bacteria exhibit a degree of choosiness when it comes to uptake and recombination of foreign DNA. This is because a large proportion of environmental DNA will originate from very distantly related organisms or will have been subject to physical and chemical degradation (e.g., by UV radiation) over time [12], and for

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Box 1. Confounders

The uptake and release of DNA discussed in the main text in the context of sexual selection could, in many cases, be subject to natural selection instead. It is crucial to separate the relative contribution of different selective forces acting on these two processes, although we note additional natural selection aids, rather than hinders, sexual selection.

Competence

There is evidence for several model species that fitness benefits are associated with the physiological changes involved in competence that are unrelated to DNA uptake [70]. However, as uptake of DNA originating from outside of the cell (and often its protection against degradation within the cell) is an integral part of transformation, we assume that it forms a key component of its evolutionary maintenance.

DNA Uptake

Hypotheses for the benefits of DNA uptake can be broadly divided in two categories: one where foreign DNA is viewed as a source of genetic novelty, and the other where it is not. Genetic novelty hypotheses traditionally have been based on the expectation that the integration of homologous as well as nonhomologous DNA originating from genetically distinct cells facilitates positive selection [11,71]. The generation of genetic variation to improve the response to natural selection is known as the 'sex' hypothesis and has received some empirical support [72,73]. An exciting new hypothesis posits that transformation facilitates negative selection because it results in the recombination-assisted deletion of nonhomologous DNA, specifically costly selfish genetic elements, [50]. One main hypothesis for transformation which is not based on genetic novelty is the 'food' hypothesis, where foreign DNA is used as a source of energy or building blocks [34]. Another main explanation not based on genetic novelty is the 'repair hypothesis', where foreign DNA serves as a template for the repair of double-stranded DNA breaks [8,11,70]. None of the abovementioned hypotheses are mutually exclusive. However, for sexual selection to be possible, transformation needs to at least be partly selected to provide genetic novelty.

DNA Secretion

Apart from serving to donate DNA to genetically dissimilar cells, secreted DNA could also be used for biofilm formation [74].

Autolysis

Apart from serving to donate DNA to genetically dissimilar cells, autolysis could also serve to provide nutrients for clonemates or simply be the result of starvation. In addition, lysis by phages or predators will result in the nonadaptive release of DNA into the environment.

Selective Killing

Producing toxins to target genetically dissimilar cells could function to release DNA as a substrate for uptake and recombination but is likely to be selected for as a form of interference competition to kill competitors and free up resources. 'Pherotypes' underlying differential killing can also influence other important phenotypes, such as biofilm formation in *Streptococcus* [75].

successful recombination extracellular DNA needs to contain tracts of sufficient homology and physicochemical quality.

DNA released by bacteria is ubiquitous (e.g., [13]), whilst uptake by recipient cells is usually infrequent and piecemeal. The costly release of genomic DNA could be thought of as being analogous to male behaviour, with genomic DNA released into the environment akin to sperm casting in marine invertebrates. When only a portion of available free DNA finds its way into recipient genomes, uptake and chromosomal incorporation of foreign DNA could be likened to the female function, which is often the limiting factor in sexual reproduction [3]. We therefore posit that bacterial DNA release and uptake is analogous to **anisogamy** found in many sexual eukaryotes, where the basis of sexual selection is formed by males producing copious small sperm that compete for fertilization of a limited number of larger egg cells [3]. This contrasts with many eukaryote microbes such as yeast and algae which are **isogamous**, a scenario with limited to no scope for sexual selection. Further, by combining DNA release and uptake processes in a single cell, bacteria can be compared with **hermaphrodites**, a sexual system found in most plants and in many types of animal (and which does not preclude sexual selection [14,15]).

Glossary

Anisogamy: sexual reproduction via the fusion of dissimilar gametes.
Autolysis: the destruction of a cell by its own enzymes.
Chemical manipulation: the production of molecules that negatively affect the fitness of target cells and positively affect the fitness of producer cells.
Competence: the physiological state in which bacteria engage in genetic transformation.
Female choice: any biases which restrict mating to a specific subset of (rather than all) possible sexual partners.
Female resistance: when mating is costly, any trait that reduces a female's number of mating partners. As a consequence, female resistance can be considered a form of female choice.
Fisherian sexual selection: indirect selection on female preference caused by genetic linkage to a directly selected male ornament; leads to self-reinforced runaway selection.
Good genes sexual selection: indirect selection on a female preference for male traits that enhance the survival or fecundity of offspring.
Hermaphrodite: an individual capable of performing both male and female functions.
Isogamy: sexual reproduction occurring via the fusion of equally sized gametes.
Kind discrimination: a form of kin discrimination based on the recognition of a shared genetic locus mediating a social trait, rather than overall shared genomic ancestry.
Mating type: a genotype that can only engage in (para)sexual processes with other mating types.
Molecular drive: the nonrandom inheritance of a genomic locus.
Parasex: the transfer of genetic material through mechanisms other than meiosis. In bacteria, 'transformation' is a parasexual mechanism under control of the recipient cell; conjugation and transduction are two other main parasexual mechanisms mediated in part by the cell and in part by mobile genetic elements (plasmids and bacteriophages).
Sexual conflict: sexual conflict occurs when the two sexes have

In this article, we define sexual selection in its most general sense, namely as 'any competition for access to conspecifics assisting in the reproduction of genetic information'. We consider four distinct scenarios of transformation-mediated DNA uptake coupled with DNA release through the prism of sexual selection theory. It is our aim to identify testable predictions that could shed light on the wide diversity of transformation-related behaviours observed in bacteria beyond conventional discussions based solely on natural selection.

Competition through DNA Release

Where there is any form of (para)sex there is, in principle, scope for individuals to increase the transmission of their genetic material at the expense of that of others through sexual selection. The release of extracellular DNA in bacteria could point at selection to direct investment into replication of genetic information by other cells (horizontal gene transfer) in addition to replication by cell division (vertical gene transfer) (Figure 1). It has been posited that the cost of DNA replication is a relatively small portion of the overall cellular energy budget [16], and so genomic DNA release with the aim of horizontal gene transfer might be a viable strategy. This is especially the case when donors have high genome copy numbers, increasing the availability of extracellular DNA after lysis, and when bacteria can accurately sense impending cell death (e.g., in the stationary phase), limiting the prospects of vertical gene transfer and making horizontal transfer relatively more favourable.

Many bacterial genera release genomic DNA during growth in liquid media [17]. Such active DNA release into the environment can be mediated through secretion, for example, via type IV secretion systems [18] and membrane vesicles (e.g., [19]) or by autolysis of a subset of the population [20,21]. When certain genotypes invest more in DNA release to increase the chance of their DNA being taken up and replicated by other cells compared with that of neighbouring bacteria, sexual selection is effectively occurring. Such a scenario, where cells 'swamp' the local environment with genomic DNA (Figure 2A), would be analogous to **sperm competition** in broadcast spawners [22], where males invest in increased sperm numbers to enhance fertilization success [23].

It has been well established that rates of recombination of donor DNA vary significantly within a species [24–26] (although it is uncertain whether this is primarily mediated by differences in DNA uptake). However, whether DNA release is equally variable among strains is much less well understood. Intraspecific variation in the quantity of DNA released is found in *Neisseria gonorrhoeae* (where it is mediated by both lysis and secretion) [20]. In addition, a wild-type *Bacillus subtilis* strain was shown to release free DNA in a lysis-independent manner whereas a standard laboratory strain evolved in clonal isolation for many generations lost this ability [27], indicating that this trait is evolvable. It will be relatively straightforward to experimentally quantify between-strain differences in DNA-release rates. These data will shed light on the variation in investment in DNA release and DNA uptake between strains. Specifically, experiments where different mutants that vary in the amount of DNA that they release are cocultured with a differentially marked strain able to efficiently take up DNA, could be used to test whether genotypes that release more DNA are more successful in having this DNA reproduced by recipient cells.

Sperm competition is not limited to investment in sperm quantity but extends to strategies to degrade the sperm of other males, strategies to make females less likely to mate again with other males [23] and even to the exploitation of rival male semen [28]. In a potential analogy, many bacteria produce extracellular DNases (Box 1), and it would be interesting to assess whether these DNases could selectively degrade DNA originating from competitors (e.g., whilst protecting self-DNA via methylation, or via temporal uncoupling of DNase activity and DNA release [29,30]).

Biased DNA Uptake

Female choice is widespread in animals, including scenarios where females prefer certain males over others [3] or where females avoid engaging in costly matings with any but the most persistent males [31]. Different benefits can be associated with such mate choice [2,32]. Females could gain direct benefits through increased fecundity or survival, or could gain indirect benefits by enhancing the

different evolutionary interests with respect to a reproductive trait. In animals for instance, males might be selected to engage in multiple matings, whereas females might be selected to limit the number of matings.

Sexual selection: has been traditionally defined as competition for access to mates leading to differences in reproductive success. In recent years, 'fertilization opportunities' (i.e., processes occurring postcopulation such as 'sperm competition') is added to 'access to mates' (i.e., processes occurring precopulation). In the context of bacteria, these differences are moot, and sexual selection could be defined in its most general sense as any competition for access to conspecifics assisting in the reproduction of genetic information.

Sperm competition: postcopulatory competition between sperm from different males, or between sperm and the female resisting fertilization. In the context of bacteria, cells donating free DNA could outcompete each other by producing more extracellular DNA, increasing the chance of successful uptake by recipients.

Transformation: the uptake into the cell of single-stranded DNA from the surrounding environment, after which this DNA can be integrated into the genome by recombination. Recombination mediated by transformation involves relatively short DNA fragments transferred from a donor strain to a recipient strain, rather than the reciprocal and complete reshuffling of two genomes during meiotic sex. In addition, bacteria can incorporate nonhomologous DNA, originating from the same or from different species, as well as homologous DNA.

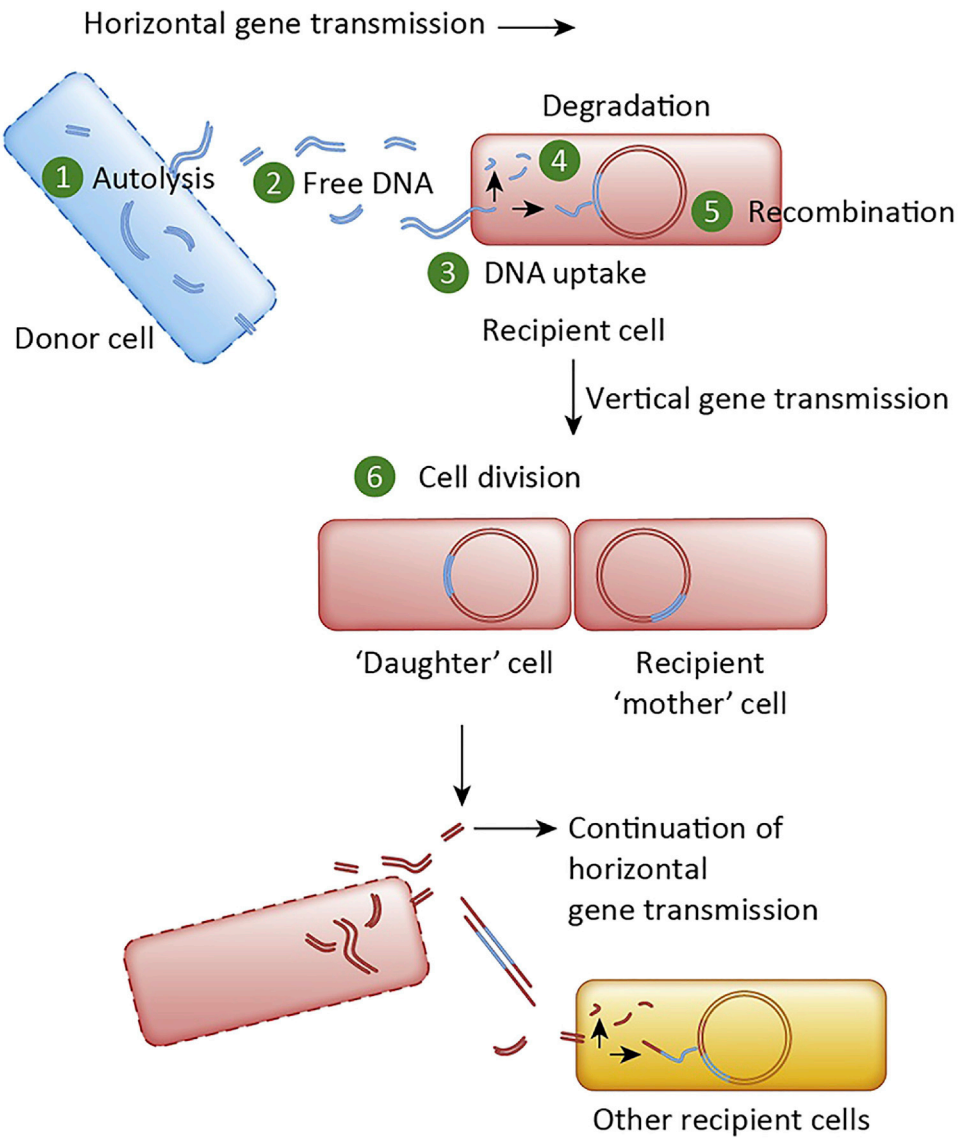

Figure 1. A Simple Schematic of DNA Release by a Donor Cell and DNA Uptake via Transformation by a Recipient Cell Resulting in Horizontal Gene Transfer.
Molecular mechanisms or the identity of cells (Gram-negative or Gram-positive) are ignored; we refer to several excellent reviews for details on the molecular biology of transformation listed in the main text. The donor cell (blue) here releases DNA via autolysis (1) into the extracellular environment (2). We note that DNA release can take place through a variety of other mechanisms including secretion (see main text). The recipient cell (red) binds free double-stranded DNA and transports single-stranded DNA into the cell (3), where it can be degraded (4) or successfully recombined into the chromosome (5). The recipient cell subsequently divides (6), reproducing the donor DNA fragment (blue) along with its genome (red) (vertical gene transfer). The cycle continues as the recipient cell turns into a donor cell, with another lineage (yellow) incorporating DNA from the previous lineages (red and blue) in its own genome (yellow).

offspring's genetic quality **(good genes sexual selection)** or their attractiveness to mates **(Fisherian sexual selection)**. In contrast to meiotic sex, transformation-mediated genomic changes do not only indirectly affect daughter cells, but also directly affect the mother cell. The uncoupling of

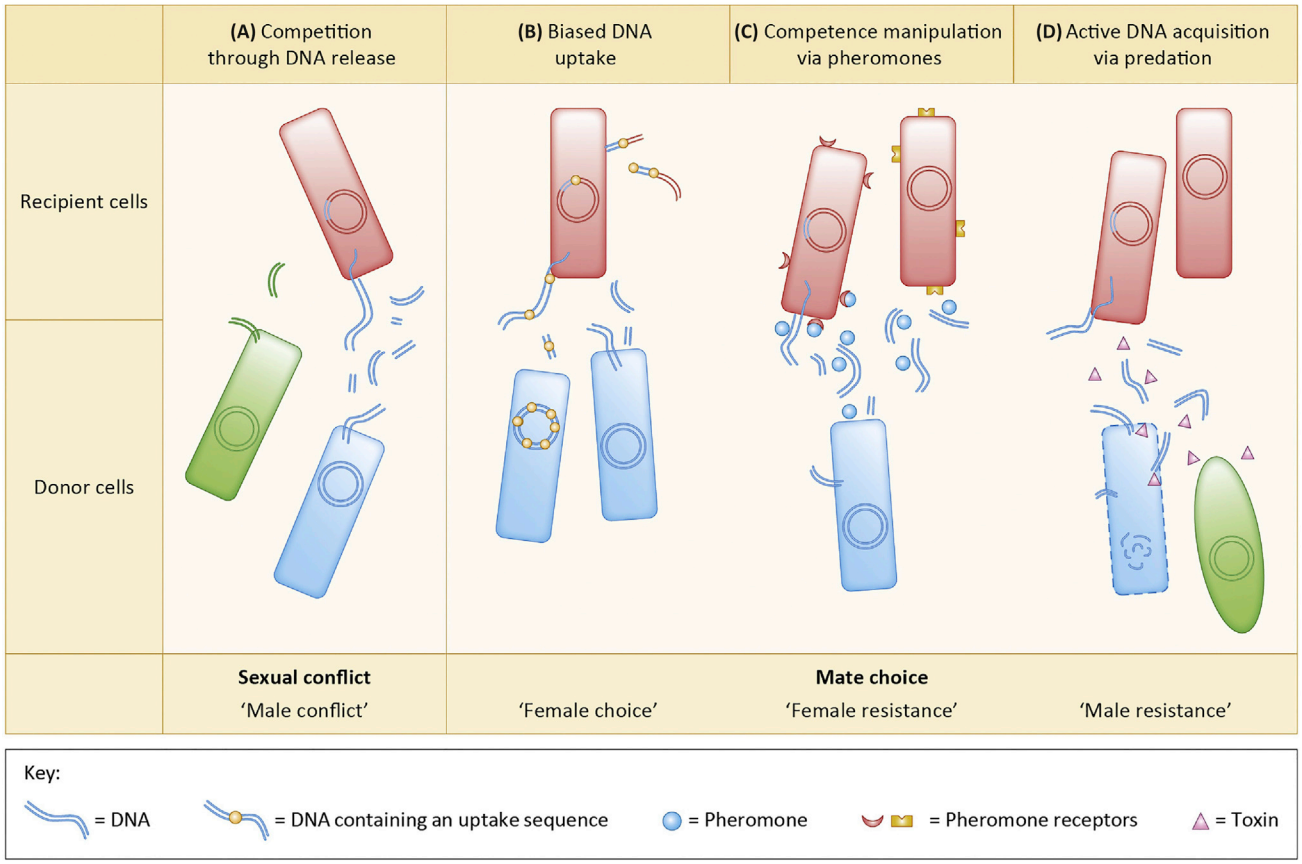

Figure 2. Four Scenarios of DNA Uptake by Transformation Coupled with DNA Release in Bacteria.
Bacteria that take up DNA (recipient cells) are red; bacteria that donate DNA (donor cells) are blue or green. DNA strands are the same colour as the cell they originate from. (A) Competition through DNA release. A green and blue cell release a small and large amount of DNA, respectively, leading primarily to the uptake of blue DNA by the recipient cell. This can be viewed as being analogous to sexual conflict, specifically sperm competition where males invest in increased sperm number to enhance fertilization success. (B) Biased DNA uptake. A recipient cell has a random bias uptake towards donor DNA containing uptake sequences (yellow circles), resulting in uptake sequences accumulating in the recipient genome and in the extracellular DNA pool as the result of subsequent DNA release by the recipient cell. This can be viewed as mate choice, specifically where females choose males based on an arbitrary characteristic (Fisherian sexual selection). (C) Competence manipulation. A blue cell releases DNA and a pheromone (blue circles), inducing competence in a recipient cell with a matching receptor (left) but not in a potential recipient cell with an altered receptor (right). This can be viewed as mate choice, specifically where males coerce females to mate. (D) Active DNA acquisition via predation. A recipient cell produces a toxin (red triangles) lysing a related, but genetically different, strain (blue), thus providing DNA for uptake by the toxin producer, whereas unrelated cells (green) (as well as related cells that produce immunity factors) are not lysed. This can be viewed as mate choice, specifically where females coerce males to mate.

recombination and reproduction in bacteria during lateral gene transfer thus means that sexual selection frameworks based on direct and indirect benefits overlap. We will therefore approach female choice-like processes in bacteria from first principles instead.

The composition of free DNA released by donor cells can vary and influence uptake efficiency of recipient cells; for instance, differential methylation patterns can affect uptake success in different species through a variety of mechanisms (e.g., [33]). However, it seems improbable for bacteria to assess immediate benefits of free DNA based on nucleotide composition. Any explanations for selective DNA uptake to gain benefits from its use as food [34], or through the uptake of 'good genes' (directly by the cell engaging in transformation as well as indirectly by daughter cells) are therefore unlikely (although we cannot rule them out).

Species belonging to the Pasteurellaceae and Neisseriaceae families preferentially take up DNA from related strains and species mediated by binding of specific DNA motifs present across the genome [8]. This form of selective DNA uptake can be explained by the fact that any random uptake bias towards a particular sequence motif will result in selection for this motif, as it enhances uptake of donor DNA. Consequently, the motif will accumulate in the recipient's genome and accumulate in the pool of free DNA (after secretion or lysis of recipients) [8,35] (Figure 2B). This positive feedback loop has been described as a form of **molecular drive** [8,35]. However, the preferred uptake and recombination of a specific trait has interesting analogies with Fisherian sexual selection. This form of intrasexual selection relies on a female preference (in this case the uptake bias) for a male trait which is not necessarily related to male genetic quality (the uptake sequence motif). Females with such a preference benefit from mating with a preferred ('attractive') male as their sons inherit their father's attractiveness, which will in turn be preferred by females in the next generation, resulting in more grandoffspring. Similarly, cells which bias uptake to sequences with a preferred motif will be more likely to have their DNA accepted by recipients as a DNA donor (Figure 2B).

The spread of preferred uptake motifs is easy to envisage, but the evolution of uptake specificity is less well understood, in large part because exact mechanisms are unknown [8]. If carrying uptake sequences is desirable (for instance by promoting horizontal gene transfer), increased specificity of uptake is also expected to evolve. Moreover, uptake specificity is expected to be favoured as uptake motifs provide a cue that DNA originates from conspecifics, which are more likely to contain genetically compatible variation. As transformation results in the piecemeal uptake of foreign DNA, rather than the complete shuffling of two genomes, genetic linkages between preference and preferred trait could be expected to develop much more easily in bacteria than in eukaryotes [36], which is essential for Fisherian sexual selection. The fact that uptake biases are pervasive in a few species, seemingly absent in many others, and with no or only a few cases where biases are slight [8], is consistent with a 'runaway' scenario where sexually selected traits and preferences rapidly spread.

Competence Manipulation via Pheromones

In various animals, males have evolved offensive traits to coerce females into mating, which in turn favours females to become resistant against most mating attempts **(sexual conflict)** [37,38]. Here, **female resistance** is a form of mate choice, as it limits mating to those males who can overcome her resistance [39]. Bacteria engage in transformation during a physiological state termed **competence** [9]. The cues for developing competence vary widely between species and range from sensing the presence or absence of specific metabolites, starvation, (genotoxic) stress, or bacterial signalling [29]. In theory, a bacterial genotype could exploit existing competence-regulating signals to maximize the uptake of its DNA by other cells (Figure 2C). This in turn would exert a selection pressure on recipient cells to resist such manipulation, potentially resulting in a coevolutionary arms race. The role of bacterial signalling in competence is well known [40] and predicted to be prone to exploitation [41], but examples of intraspecific **chemical manipulation** of competence have rarely been explored.

Perhaps the only well described example of chemical manipulation between related bacterial strains pertains to the parasexual process of conjugation. Plasmid-free *Enterococcus* strains can produce a chromosomally encoded peptide that, when detected by strains carrying so-called 'pheromone plasmids', induces an aggregation behaviour that promotes plasmid transfer to plasmid-free cells [42]. In yeast, the costly production of a sex pheromone was shown to be favoured when a **mating type** producing a pheromone ('signaller') was mixed with a low number of another mating type not producing this signal ('receiver') when competition for mates was high, but not when receivers were numerous, and competition was low [43]. However, no evidence was found of changes in mate preference in this experiment.

Experiments could be designed to screen for the existence of chemical manipulation of competence. For instance, experiments supplying a recipient strain with purified donor DNA and supernatants from other strains (potentially containing competence-inducing signals) have the power to reveal

potential differences in DNA uptake and recombination efficiency of the recipient strain depending on supernatant origin. This would show that a prerequisite for the action of sexual selection through chemical manipulation would be met. In *B. subtilis*, transformation was found to be more efficient using DNA from late exponential growth supernatants compared with DNA from cell lysis [30], which could be consistent with this scenario.

Active DNA Acquisition via Predation

Instead of manipulating other cells to take up self-DNA, cells could also take molecular cues from other cells to inform their own DNA-uptake decisions. There is now evidence that recipient cells can actively initiate DNA uptake by lysing donor cells based on genetic cues [44]. For instance, the Gram-negative species *Vibrio cholerae* [45] and *Acinetobacter baylyi* [46] utilize type VI secretion systems to lyse genetically distinct conspecifics, after which DNA thus released is taken up and recombined into the chromosome. Species of the Gram-positive genus *Streptococcus* produce competence-stimulating peptides (CSPs) that, besides initiating competence, also initiate the production of toxins that lyse related strains and species producing different CSPs, increasing the efficiency of lateral gene transfer [47,48] (Figure 2C). One potential explanation for such **kind discrimination** [49] mediated by strain-specific killing factors is that it ensures that competence would not be initiated (i) in a (near)clonal swarm, which would not introduce any genetic variation, or (ii) upon encounter of dissimilar species, which would result in the uptake of divergent DNA unlikely to result in successful recombination. Instead, coupling competence with lysis of related, but distinct neighbouring strains maximizes the probability of efficient incorporation of novel alleles and genes that have proved to function in a genomically and ecologically similar context, or of outcrossing of parasitic mobile genetic elements with homologous DNA that lack these elements [50] (Box 1).

In the framework likening bacterial sex to eukaryotic sex used throughout this article, active DNA acquisition (recipients lyse donors) equates to female coercion (Figure 2D). This stands in contrast to the much more commonly observed male coercion observed in animals (and the hypothetical scenario of male coercion in bacteria via pheromones discussed above). Female coercion might be expected when costs of male function (i.e., DNA release) are higher than that of the female function (i.e., the reproduction of fragments of non-self DNA). Female coercion is expected to result in the evolution of male resistance. Interestingly, in the case of *Streptococcus*, some strains have monospecific CSP receptors, but some have promiscuous receptors that, in addition to responding to its cognate CSP, can also sense foreign pheromones, making cells resistant to lysis by other strains [48]. The evolution of greater levels of resistance to lysis (which can be assumed to come at a greater cost) points at possible coevolution between mechanisms of cell lysis for DNA uptake and mechanisms of resistance to lysis. However, we note that it will not be straightforward to tease out the relative effects of coevolution due to sexual selection (lysing other strains to gain access to preferred DNA) or due to natural selection (lysing other strains to free up resources) (Box 1).

Broader Consequences of Sexual Selection in Bacteria

DNA release and uptake is presumed to be favoured by natural selection because these traits facilitate genetic recombination, as well as provide direct fitness benefits such as biofilm formation or food (Box 1). Although natural selection is expected to be the main driver of these processes, we argue here that it is worth examining whether sexual selection could also influence DNA release and uptake. Sexual selection could be expected to alter the rates of DNA release and uptake over and above that favoured by natural selection. This is because, under sexual selection, uptake rates do not necessarily reflect expected survival benefits of foreign DNA, but rather the prospect that foreign DNA (once incorporated) increases the rate of horizontal gene transfer of the recipient's other genes. Indeed, theory in eukaryotes predicts that sexual selection may drive transitions from partial sexuality to obligate sexuality for exactly these reasons [51]. While we do not imply that sexual selection could drive the evolution of obligate sex in bacteria, rates of bacterial horizontal gene transfer may well be higher than in taxa without sexual selection.

Any action of sexual selection in bacteria thus could influence both recombination rate and the level of promiscuity. As molecular evolution in many bacterial species is driven to a greater extent by horizontally rather than by vertically acquired polymorphisms [52], changes in lateral gene transfer dynamics could have important evolutionary consequences. Sexual selection is of intrinsic interest in its role generating unique adaptations, but it also plays an important role in other processes such as population divergence and speciation through assortative mating [53]. Sexual selection can also result in rapid antagonistic coevolution between relevant traits (e.g., [54]), which itself can have genome-wide effects as a consequence of genetic linkage or selection for increased mutation rates [55].

Outstanding Questions

Does competition for uptake drive rates of DNA release?

How common are (low-specificity) DNA uptake biases mediated by sequence motifs?

Can cells secrete compounds that initiate competence in other strains?

Have some species diverged into recognition types to facilitate gene transfer?

Concluding Remarks

In animals as well as in other organisms, sexual selection theory has been extremely successful in explaining morphologies and behaviours that do not make sense from the point of natural selection. Although terms such as 'assortative mating' [56] and 'mate choice' [8] have been used in the literature on bacterial transformation, it remains largely unknown whether the release and uptake of DNA is governed by some of the same underlying evolutionary forces as sex in eukaryotes, or that similarities are merely superficial. This is in part due to the fact that DNA release is less well studied than DNA uptake (i.e., competence and transformation) and the mechanistic and functional links between both processes are only beginning to be uncovered [44].

If processes analogous to sexual selection act in bacteria, they can be expected to be relatively weak. Bacteria are largely clonal and do not rely on parasexual reproduction of DNA by other individuals. Moreover, the DNA that is recombined by recipient cells consists of relatively short tracts, and not 50% of the genome as in meiotic sex (although in some cases it can involve large-scale uptake, for example, a single transformation event in *Haemophilus influenzae* can replace 1–3% of the recipient genome [57], and in *B. subtilis* >100 kB fragments can be recombined [58]). However, there are reasons to keep an open mind when it comes to the potential action of sexual selection in bacteria, of which we list three below.

First, bacteria often have very high effective population sizes favouring efficient selection, be it natural or sexual, and making the evolution of even subtle adaptations more likely. For instance, Fisherian sexual selection in particular is known to be a weak selective process [59].

Second, great variation in the genetics and physiology of transformation is found in the two dozen or so species used as model systems for transformation [29]. Considering that a trillion species of bacteria and archaea remain to be discovered [60], there is enormous potential to find new types of genome organization, physiology, ecological life-styles, and even methods of active gene transfer (exemplified by the relatively recent finding of gene transfer mediated by nanotubes [61]) that could be more permissive to forms of sexual selection than those currently known. The dynamics of lateral gene transfer processes mediated by mobile genetic elements (MGEs; plasmids and viruses) are in part controlled by the recipient cell [36]. For instance, the CRISPR-Cas adaptive immune system can recognize and restrict deleterious MGEs [62]. The 'nonrecognition' of MGEs that are on average less deleterious therefore could potentially mean that this constitutes a mechanism of (female) choice.

Third, there is a marked discrepancy between the substantial number of species known to harbour the suites of genes controlling competence and transformation, and the much smaller number of species amenable to transformation experiments in the laboratory [29,44]. Initiating competence after sensing related genotypes might be widespread among phylogenetically different bacteria but overlooked because most laboratory studies are based on single clones [44]. Recent studies on natural bacterial populations have revealed that even very closely related, coexisting strains within a species have the capacity to recognize self from non-self, and that there are likely to be many thousands of such recognition types present within species [63–66]. This divergence in recognition types is often accompanied by killing of non-self [64,67]. When such killing is associated with transformation and

concomitant recombination-based benefits, this would be analogous to the evolution of mating types [68], known to profoundly affect patterns of genetic exchange in eukaryotes. In general, faced with tremendous variation in the quality of foreign DNA, we would expect a substantial selective advantage to any mechanism that allows individuals to have a degree of choosiness in the uptake of DNA. Data on the potential nonrandomness of DNA uptake and chromosomal integration are therefore needed to inform models of lateral gene transfer evolution [69].

We hope that the identification of possible mechanisms of sexual selection in bacteria will spur on further research into the evolutionary origins of the diverse horizontal gene transfer behaviours found in prokaryotes (see Outstanding Questions).

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