Feature Review

The Reproductive Microbiome: An Emerging Driver of Sexual Selection, Sexual Conflict, Mating Systems, and Reproductive Isolation

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All multicellular organisms host microbial communities in and on their bodies, and these microbiomes can have major influences on host biology. Most research has focussed on the oral, skin, and gut microbiomes, whereas relatively little is known about the reproductive microbiome. Here, we review empirical evidence to show that reproductive microbiomes can have significant effects on the reproductive function and performance of males and females. We then discuss the likely repercussions of these effects for evolutionary processes related to sexual selection and sexual conflict, as well as mating systems and reproductive isolation. We argue that knowledge of the reproductive microbiome is fundamental to our understanding of the evolutionary ecology of reproductive strategies and sexual dynamics of host organisms.

The Microbiome Revolution and the Reproductive Microbiome

Animals and plants live and evolve in a world dominated by microbes, and host a diversity of microbial communities in and on their bodies. A recent explosion in research on host-associated microbial communities (i.e., **microbiota** and/or **microbiomes**, see Glossary) is revolutionising biology. While earlier research typically considered microorganisms from a pathological perspective, it is now widely accepted that the relationship between host and microbes spans a continuum, from detrimental to beneficial. Through their influence on host health, physiology, development, and behaviour [1,2], microbiomes can be seen as an integral part of the host phenotype, and potentially also the host genome (e.g., the **hologenome** concept [3], but see [4]). While considerable attention has been paid to the role of oral, skin, and gut microbiomes in host ecology, evolution, and fitness, less is known about the reproductive microbiome (Box 1). This is surprising given longstanding knowledge of microbes in male and female reproductive systems (e.g., [5]), most notably in the context of **sexually transmitted infections** (STIs) [6], and more recent DNA-sequencing studies demonstrating the presence of dynamic microbial communities in reproductive tissues [7], especially the vagina [8,9]. Thus, the reproductive microbiome represents an outstanding challenge in the study of host-associated microbial communities.

Patterns of variation have been studied for some female reproductive microbiomes, due in part to an interest in understanding mother–child microbial transfer. In humans, microbiome composition varies across the female reproductive tract (e.g., vagina, cervix, uterus, fallopian tubes, and follicular fluid [9,10]). Intriguingly, the human vaginal microbiome is a relatively low-diversity environment, dominated by bacteria from the genus *Lactobacillus* [7,9], whereas, in other mammals, lactobacilli rarely constitute >1% of the vaginal microbiome [11–13]. Several hypotheses have been proposed to explain the uniqueness of the human vaginal microbiome, including differences in reproductive physiology, disease risk, and diet [14,15]. The female reproductive microbiome can be highly dynamic. For example, in primates, the vaginal microbiome changes with female age and life-history phase, menstruation, and pregnancy [7,15]. Intra- and interindividual variability has also been reported for the vaginal microbiome of mice, although factors influencing this variation are currently unclear (e.g., variation is not associated with oestrus cycling) [13]. These findings are revolutionising our understanding of the female reproductive system and reproductive health, and have changed the perception of the female reproductive tract as a sterile environment.

Less attention has been paid to the male reproductive microbiome. However, the ejaculate in a range of taxa is known to host microorganisms [16]. High-throughput sequencing studies have shown

Highlights

Although it is clear that the microbiome can have major influences on host biology, relatively little is known about the microbial communities in the reproductive systems of males and females.

The reproductive microbiome can have significant effects on the reproductive function and fitness of males and females, although these effects are rarely considered from an ecological or evolutionary perspective.

Recent research indicates that the reproductive microbiome can influence the outcomes of pre- and postcopulatory sexual selection, as well as generate conflict between the sexes.

Thus, complex coevolutionary dynamics can emerge between the host mating system and the reproductive microbiome, to shape patterns of reproductive skew in the host, microbial diversity across individuals and species, and the ecology and evolution of reproductive microbes. Consequently, reproductive microbiomes also have strong potential to influence reproductive barriers between host populations.

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Box 1. Defining the Reproductive Microbiome

In the broadest sense, the reproductive microbiome can be defined operationally as the microbiome, including bacteria, viruses and unicellular algae, protozoans and fungi, living in or on any structure, organ, fluid, or tissue of a host that typically makes contact with the gametes (or gamete analogues, e.g., pollen or gametophytes) or reproductive tract or organs of another individual through mating, spawning, or pollination. This captures the microbiomes associated with the reproductive system of a host, involved at any point in the reproductive process, from gamete production and release (e.g., ejaculation) and up to the point of fertilisation, including the microbiomes of male and female reproductive tracts, gametes, and reproductive fluids, as well as genitalia, intromittent organs and sperm transfer structures, (e.g., pedipalps of spiders, hectocotyli of cephalopods, aedeagi of insects, gonopods of millipedes, claspers of sharks and rays, gonopodium of Poeciliidae, and hemipenes of lizards and snakes), and spermatophores.

This definition includes species that release gametes into the external environment, such as external fertilisers, wind- and vector-pollinated angiosperms, fern spores released in water, and animals that release sperm packages (e.g., spermatophores) on substrate for female uptake, as well as species where fertilisation is mediated by other organisms (e.g., pollination via animal vectors and externally fertilising fish that spawn within the gills of mussels, e.g., European bitterlings). In these cases, the reproductive microbiome reflects the microbiome associated with a set of released gametes, from which there is potential for members of the microbiome to colonise gametes of the opposite sex.

While most research is currently focussed on a few host species, this broad definition enables us to develop arguments and hypotheses of broad generality. As we develop an understanding of the structural and functional diversity of reproductive microbiomes, more refined definitions may begin to emerge, with a narrower focus and more specialised to the biology of different systems. Such definitions might benefit from identifying the host and/or site of the microbiome (e.g., the human reproductive microbiome [7], the vaginal microbiome of primates [11], or the microbiome of copulatory organs of spiders [108]).

An important challenge in the study of reproductive microbiomes is the limited knowledge that we have of these microbial communities. Currently, it is generally unclear how reproductive microbiomes are established or maintained, as well as the function they may have in relation to host mating dynamics and reproductive processes. Furthermore, due to the prevalence of microbiome mixing and chance events (see Box 2) it is unclear to which degree reproductive microbiomes represent native assemblages or more transient, random assemblages, and, in some instances, there is significant contribution from a range of sources, such as the external environment (e.g., microbiome of gametes released into the environment and external genitalia) and other body sites of the host. In many instances, the structure of reproductive microbiomes may be the result of both of random and deterministic processes, which may lead to spatiotemporal dynamics in these microbiomes. Consideration of these ecological processes may also lead to greater clarity in our understanding and definition of reproductive microbiomes in the future.

that human ejaculates are typically characterised by low biomass microbial communities [7], thus, requiring special care during sample collection and processing to avoid erroneous results [17]. In humans, the semen microbiome is more diverse (greater species richness) and exhibits greater **species evenness** than that of the vagina [7], although the two communities do share taxa. A reproductive microbiome has also been described from the seminal fluid of mice [18] and the human penis [19].

Here, we review evidence for the potential impact of reproductive microbiomes for host fertility and fitness, and discuss the functional implications of these impacts for **sexual selection**, **sexual conflict**, **mating systems** and strategies, and **reproductive isolation**. We also consider the microbial perspective (Box 2), because a full understanding of microbial community assembly, succession, and function requires consideration of the fitness interests of members of the microbiome. While reproductive microbiomes occur across the animal and plant kingdoms, and indeed are likely to have critical implications for both animal and plant hosts, most empirical work to date has focussed on dioecious, sexually reproducing animals with internal fertilisation. Therefore, we focus most of our discussion on these taxa, although the arguments proposed will have broad generality.

Box 2. Microbial Evolutionary Ecology of the Reproductive Microbiome

A modified metacommunity framework is useful for understanding what shapes the composition of the microbiome [109]. Metacommunity dynamics assume that the composition of a local patch (i.e., a microbiome) is shaped by both local interactions and immigration from other microbiomes, as well as the external environment. Immigration from other microbiomes is likely to have a particularly important role in shaping reproductive microbiomes, given their inevitable large scale and, often frequent, mixing as a result of transmission during mating. Mixing will increase with increasing mating rates or increases in multiple mating by individuals, and is also greater in females than males. Coupled with the fluctuating selection pressures imposed both by changes in microbiome composition and within-host physiological status, the structure (and function) is likely to vary considerably through time [88]. The propensity for extremely rapid evolution of microbes is also likely to have a major role in shaping these ecological dynamics, which in turn will drive evolutionary change [110].

Arguably, one of the most important implications of a highly compositionally dynamic microbiome is that a 'holobiont or hologenome' perspective, which assumes that the host and (at least parts of) the microbiome are a unit of selection, is unlikely to be useful [4]. The mixing of microbiomes between sexual partners, as well as potentially acquiring members of the microbiome from other external environments, means that most members of the microbiome are unlikely to be vertically cotransmitted with the host genome and, hence, will create potential evolutionary conflict between members of the microbiome and hosts, notably with respect to rates of sexual encounters.

The mixing of microbiomes will also affect how the structure and function of the reproductive microbiome covary with host genotype, which can have a key role in, for example, the extent to which the microbiome might affect the evolution of host mate choice. From a purely ecological perspective, increased mixing of microbiomes will breakdown this covariance. However, mixing will have less predictable effects on the covariance between host genotype and microbial genotype. Microbes are likely to rapidly adapt to local host conditions (local adaptation; LA), increasing host–microbe genetic covariances. While high levels of migration will genetically homogenise populations, reducing LA, increased genetic variation resulting from immigration can also enhance LA by increasing the rate of adaptation [111]. These effects of migration on LA will be reinforced by adaption to co-occurring community members, which can limit the invasion of foreign microbial communities [112]. If certain microbial taxa have key roles in determining microbiome function (although we do not yet know if this is the case), such microbe–host genetic covariances may have important implications.

Microbial community composition and function can also be greatly affected by chance events, further reducing the specificity of microbiomes to their host genotypes [113]. Reproductive microbiomes, particularly vaginal, are regularly disturbed as a consequence of menstruation, enhancing the importance of priority effects [i.e., the dominance of early (re)colonising species] in shaping community assembly [114]. Rapid (co)evolution of microbial populations can also lead to divergent community assemblies, as a result of the stochasticity of mutational events, and can potentially reshape entire microbial communities [115].

The phenotypic consequences of the reproductive microbiome on their host may mismatch host genotype even more than community composition. First, functional equivalence between taxa can weaken structure–function relationships [116]. Second, microbiomes are complex ecosystems, often dominated by strongly competitive interactions [117]. These interactions may have a key role in shaping the phenotypic effects of the microbiome on its host. For example, interference and exploitation between microbes can be highly temporally varying traits as a result of coevolution, and can have an important impact on pathogenesis through the reductions in population and community size [118]. Finally, some traits selected in response to competition [119] can also directly affect hosts.

Impact of the Reproductive Microbiome on Host Reproductive Success

A diversity of **sexually transmitted microbes** (STMs) has long been known to have deleterious effects on the health and survival of a range of host taxa [6,20,21] (Table 1). Mounting evidence indicates that the reproductive microbiome can also have significant effects on the reproductive function and performance of males and females. These impacts are frequently (but by no means always) negative, which we suggest likely reflects a combination of both traditional emphasis on pathology and/or disease and the ecological and evolutionary processes that shape reproductive microbiome structure and function (Box 2).

Glossary

Cloaca: the posterior orifice that functions as the external opening for the digestive, reproductive, and urinary tracts.

Dysbiosis: an imbalance in the composition of the microbiome. For example, disruption of the vaginal microbiome (i.e., bacterial vaginosis) in humans results in a reduction in lactobacilli and an overgrowth of anaerobic bacteria.

Holobiont: the host organism together with all its microbial symbionts, including transient and stable members.

Hologenome: the combined genomes of the host and its microbial symbionts.

Intralocus sexual conflict: a genetic, negative, intersexual fitness correlation generated by opposing selection pressures acting on a trait expressed in both sexes, and typically caused by individual alleles that enhance the fitness of the carrier when expressed in one sex and reduce it when expressed in the opposite sex

Mating system: spatiotemporal patterns of distribution of sexual partners within a population.

Microbiome: extends the microbiota concept by emphasising the 'metagenome' (i.e., the totality of genetic material that comprises the collective DNA of a microbiota, as well as the products of any gene contained within the metagenome); often used interchangeably with microbiota in the scientific literature. We use the more inclusive term 'microbiome' throughout this review.

Microbiota: usually taken to mean the community of microorganisms living in a given environment.

Reproductive isolation: occurs when two populations produce fewer viable and fertile offspring than expected from their relative abundance in a locality.

Sexual conflict: a divergence in the fitness interests of males and females within a population over reproductive decisions or the outcome of their reproductive interactions.

Sexual selection: selection for traits conferring an advantage in intrasexual competition over the fertilisation of gametes produced by the other sex. Sexual selection operates through intra- and

Table 1. Examples of the Effects of Reproductive Microbiomes on Reproductive Traits and Fitness, as well as General Health, in Males and Females[a]

Host	Impact	Refs
Males		
Impact on reproductive traits and fitness		
Mammals		
Humans, *Homo sapiens*	*In vitro* incubation with *Escherichia coli* led to reductions in sperm motility, sperm agglutination due to bacterial adherence to sperm cells, and morphological damage, including damage to the plasma membrane	[24,25]
	Incubation with bacteria (*E. coli* and *Bacteroides ureolyticus*) led to significant increases in the percentage of sperm with DNA damage	[99]
	E. coli led to agglutination of 40–75% of motile sperm	[70]
	Presence of *Lactobacillus* and *Gardnerella* was associated with high-quality sperm samples, while *Prevotella* and *Bordetella* were associated with low-quality sperm samples	[79]
	Bacteria are more prevalent in semen samples from infertile than fertile males	[5,100]
Mouse, *Mus musculus*	*Staphylococcus aureus* reduces sperm motility, viability, morphology, and sperm ATPase levels	[59]
Boar, *Sus scrofa*	Presence of *E. coli* in semen samples led to sperm agglutination and reduction in litter size; *E. coli* concentrations were positively correlated with degree of sperm agglutination and negatively correlated with litter size	[101]
Sheep, *Ovis aries*	Incubation with *Campylobacter fetus fetus* led to significant reduction in sperm motility and percentage of viable sperm, and an increase in percentage of morphologically abnormal sperm	[102]
Aves		
Chicken, *Gallus gallus domesticus*	*In vitro* incubation with six different bacteria (*Salmonella enterica*, *E. coli*, *Campylobacter jejuni*, *Clostridium bifermentans*, *Lactobacillus acidophilus*, and *Bifidobacterium animalis*) caused significant reductions in sperm motility	[78]
	Presence of *L. acidophilus* in semen used for artificial insemination in chickens significantly reduced sperm motility and reduced hen fertility	[103]
Turkey, *Meleagris gallopavo*	*In vitro* incubation with six different bacteria (*S. enterica*, *E. coli*, *C. jejuni*, *C. bifermentans*, *L. acidophilus*, and *B. animalis*) caused significant reductions in sperm motility	[104]
Insects		
Cricket, *Gryllus texensis*	Cricket iridovirus (CrIV) (also known as insect iridovirus type 6, IIV-6) led to complete or near-complete reduction in sperm motile function	[54]
Bedbug, *Cimex lectularius*	Exposure to polymicrobial mixture (including, e.g., *Acinetobacter*, *Alcaligenes*, *Bacillus*, *Staphylococcus*, and *Streptococcus*) increased sperm mortality by 40%	[71]

(Continued on next page)

intersexual episodes leading up to mating (precopulatory sexual selection) and, in species where females obtain semen from multiple males, sexual selection on males can continue after mating (postcopulatory sexual selection).

Sexually transmitted infection (STI): an infection that is transmitted via sexual contact (e.g., copulation). STIs can infect the reproductive system topically or cause systemic infections.

Sexually transmitted microbe (STM): any microorganism that is transmitted via sexual contact. The term 'STM' differs from 'STI' in that there is no requirement for the microbe to cause an infection. The term is broader to allow the inclusion of potentially positive, neutral, and negative effects of microbes.

Species evenness: relative abundance of each species in a population, quantifying how equal the community is numerically (e.g., a community in which all species occur at the exact same abundance would be considered perfectly even).

Sperm competition: competition between the ejaculates of two or more males to fertilise a set of ova.

Table 1. *Continued*

Host	Impact	Refs
Impact on health		
Humans, *H. sapiens*	*Chlamydia trachomatis* infections can cause urethritis and epididymitis	[105]
	Neisseria gonorrhoeae infections can cause urethritis	[105]
Females		
Impact on reproductive traits and fitness		
Mammals		
Humans, *H. sapiens*	Presence of *Lactobacillus* spp. in ovarian follicular fluids was associated with higher rates of embryo transfer and improved pregnancy outcomes in IVF procedures in both fertile and infertile women, whereas presence of a range of bacterial species (e.g., *Propionibacterium* and *Streptococcus* spp.) was associated with poor embryo transfer and negative pregnancy outcomes	[7]
	Endometrial microbiome with <90% *Lactobacillus* spp, and >10% other bacteria was associated with reduced embryo implantation, as well as reduced rates of pregnancy, ongoing pregnancy, and live births	[7]
	Low vaginal microbial richness and diversity associated with risk of preterm birth	[7]
	Variation in both placental and amniotic fluid microbiome associated with adverse pregnancy outcomes (e.g., preterm birth and foetal growth restriction)	[7]
	Syphilis, caused by *Treponema pallidum*, can cause infection of foetus in pregnant women, as well as stillbirth or infant death	[105]
Chimpanzee, *Pan troglodytes*	Females infected with Simian Immunodeficiency Virus, SIVcpz, were less likely to give birth and had a higher rate of infant mortality compared with uninfected females	[106]
Aves		
Kittiwake, *Rissa tridactyla*	Eggs laid by female kittiwakes infected with *Corynebacterium* were less likely to hatch than those laid by uninfected females	[107]
Impact on health		
Mammals		
Humans, *H. sapiens*	*C. trachomatis* and *N. gonorrhoeae* infections can cause pelvic inflammatory disease, chronic pelvic pain, ectopic pregnancy, tubal infertility, and morbidity. Chlamydial infections may also be linked to cervical cancer	[105]
	Trichomonas vaginalis infection is associated with preterm birth and vaginitis	[105]

[a]This is a nonexhaustive list. Nonetheless, the examples are generally reflective of the available empirical literature.

Evidence in Males

That microbes can have a role in sperm dysfunction is becoming increasingly recognised (Table 1). However, most studies have examined bacteria and, in particular, the effects of only one or a few bacterial species. While studies of individual taxa are informative, it is imperative to study the microbiome as a whole to unravel its biological relevance for male sperm quality and fertility. For example, different studies of the same bacterial species can provide conflicting evidence on its relationship with male infertility (e.g., *Chlamydia trachomatis* [22]). Such inconsistencies may be due to different methodologies, variation in sperm:bacterial cell ratios [23,24], or, crucially, to the fact that the pathogenicity of a species may change drastically depending on its ecological interactions with other species of the microbiome.

The impact of bacteria on sperm function may result from direct sperm–bacteria cellular interactions (Figure 1, Table 1). For example, adhesion of *Escherichia coli* to sperm cells leads to sperm agglutination and destruction of the sperm plasma membrane, with negative consequences for sperm motility and ultrastructure (e.g., [24,25]). Alternatively, the release or active secretion of bacterial membrane proteins (e.g., porins or lipopolysaccharides) have been shown to impair sperm function, possibly through the inhibition of macrophagal function [26] or induction of excessive reactive oxygen species (ROS) production [22]. Additionally, the presence of bacteria can increase local leucocyte levels, which can in turn impact sperm function and integrity via the formation of ROS [27]. Thus, the reproductive microbiome may represent an important part of the free radical theory of male infertility. Finally, bacterial infections may also stimulate the production of antibodies that cross-react with sperm and cause sperm agglutination and immobilisation [28].

The consequences of microbially induced sperm dysfunction for male reproductive success are less clear. In humans, bacterial infections are associated with male infertility (Table 1), although whether these are the causative factor is often unclear. Nonetheless, some studies have demonstrated reductions in reproductive output when bacteria are present in ejaculates (Table 1). Overall, microbes appear to be a biologically relevant cause of sperm dysfunction, and microbially induced reproductive dysfunction may impose significant fitness costs on individuals. Finally, dynamic changes in the reproductive microbiome may cause temporal variation in sperm function, which may help explain the relatively low intramale repeatability observed in some sperm traits (e.g., [29]).

Evidence in Females

In placental mammals, the presence of microbes during pregnancy has long been associated with infection, inflammation, and pregnancy complications. However, we are increasingly realising that microbial communities are a typical feature of the female reproductive system [9] and can influence, both beneficially and detrimentally, a range of reproductive processes and outcomes (Table 1). The impact of the microbiome for female reproductive health and fertility is best understood for the human vaginal microbiome. A 'healthy' vaginal microbiome is typically dominated by *Lactobacillus* spp. [8,9], oxygen-tolerant anaerobes that exert a range of health-promoting effects, including the formation of a physical barrier against pathogen adhesion, the stimulation of host defences, and the production of lactic acid, which exhibits antimicrobial activity against a range of vaginal pathogens [10,30]. Combined, these effects limit the growth of pathogenic bacteria and the colonisation of STIs and yeast infections [15,30], providing potential fitness benefits to the host. Conversely, modification of the vaginal microbiome (i.e., vaginal **dysbiosis**) is associated with a range of adverse conditions, including preterm birth, pelvic inflammatory disease, an increased risk of STIs, and infertility [10]. The female reproductive microbiome may also influence the overall health of an individual. For example, changes in the vaginal microbiome may be associated with a variety of gynaecological cancers [31]. Finally, the microbiome of follicular fluid and the endometrium, as well as the placental and amniotic fluid microbiomes, have a range of effects on female reproduction [7,10] (Table 1).

While some of these microbial effects have been attributed to specific bacterial taxa (e.g., *Lactobacillus* spp.), ecological interactions among taxa in the vaginal microbiome are likely to influence

microbiome function [8]. This may also apply to bacteria usually recognised for their pathogenicity (e.g., *Gardnerella vaginalis* [32]). A degree of functional redundancy among the various dominating *Lactobacillus* spp. in the human vagina may function as a buffer in the face of environmental change [8]. Similarly, in nonhuman primates, the protective role of lactobacilli appears to be fulfilled by other taxa (the common function hypothesis [14,15]). Vaginal bacteria have also been shown to change their gene expression profiles and, thus, the end-products of metabolism, depending upon the predominant state of the microbiome (i.e., healthy versus dysbiotic [33]). Intertwined with the emerging role of the female reproductive microbiome in reproduction, is the idea of microbial seeding of the next generation. In humans, exposure to vaginal bacteria during birth contributes to bacterial colonisation in infants. There is some evidence, at least in humans, that microbial seeding may even occur *in utero* through vertical mother–child microbial transfer [9,10]. This microbial transmission may influence foetal health via bacterial priming or the establishment of a healthy newborn microbiome through stimulation of the foetal innate immune system [10]. Together, these findings highlight the essential role of the reproductive microbiome in female reproductive health and fertility, as well as offspring health in viviparous taxa.

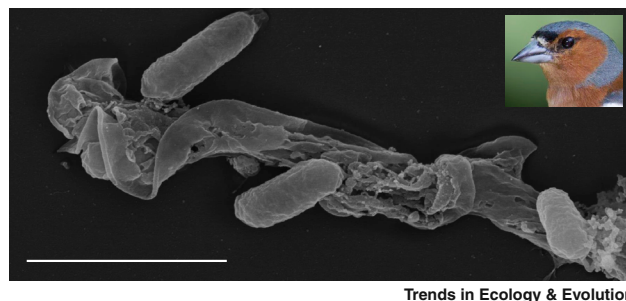## The Reproductive Microbiome and Sexual Selection

Sexual selection is a fundamental driver of the evolution of reproductive strategies, particularly in males. Here, we consider the role of the reproductive microbiome in pre- and postcopulatory sexual selection.

Precopulatory Sexual Selection: Female Mate Choice and Male–Male Competition

The importance of microorganisms in host mating behaviour has generally been studied from the perspective of the role of parasites or pathogens in female mate choice. Hamilton and Zuk [34] proposed that elaborate male ornaments indicate parasite resistance and females choosing males with more elaborate ornaments gain genetic benefits when their offspring inherit parasite resistance. Subsequent models of parasite-mediated sexual selection suggest that mate choice benefits females directly by allowing them to avoid infections when male phenotypes indicate the current parasite load, disease status, or contagion risk of the male [35–37]. In principle, STIs may mediate these mechanisms; however, evidence for female preference for uninfected males is scarce [20,38–40].

In vertebrates, the honesty of sexual signalling may be mediated by testosterone, which controls the expression of many sexual ornaments and courtship behaviours, while simultaneously suppressing part of the immune system [41]. Thus, sexual ornament expression might also reflect microbial infections in the reproductive tract or the ability of a male to combat pathogenic reproductive microbes. However, evidence for either of these scenarios is currently scarce and contradictory. For example, in male rufous-collared sparrows, *Zonotrichia capensis*, plasma testosterone levels are positively (rather than negatively) correlated with **cloacal** bacterial diversity [42]. Moreover, in some instances, STIs appear to increase the attractiveness of males [20,43], which may reflect a strategy of the microbiome to manipulate host behaviour to increase infection rates (Box 2).

Female mate choice might respond to overall microbial load, rather than to the presence of a specific infectious species. Females may be more likely to experience vaginal dysbiosis or increased activation of immunological responses in the reproductive tract when the microbial load of an ejaculate is high. Secondary sexual traits might then advertise the microbial load or ability of a male to control microbial growth within reproductive tissues and fluids. The semen of male mallards, *Anas platyrhynchos*, with more colourful bills is better able to kill bacteria in *in vitro* assays relative to males with less colourful bills, suggesting that females are able to use bill colouration to avoid mating with males with high ejaculate bacterial loads [44]. Additionally, the phenotype of primary sexual traits may also reflect infections. STIs are often characterised by distinctive symptoms, such as genital warts or discharges, and genital inspection behaviours associated with precopulatory courtship may function to discriminate against infected partners [45]. Olfactory cues can also be shaped by host-associated microbiomes (e.g., scent gland secretions in hyenas [46] or underarm odour in humans [47]), and

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Figure 1. Sperm–Bacteria Cellular Interactions.
Scanning electron micrograph of bacteria associated with the head of a damaged sperm cell from a chaffinch, *Fringilla coelebs* (inset). Scale bar = 20 μm. In addition to damaging sperm cells, microbes may, in principle, strategically associate with sperm to avoid immune responses and more effectively negotiate the female reproductive tract. Photo credits: sperm, Melissah Rowe; chaffinch, Andreas Trepte (www.photonatur.net).

recent studies suggest that olfactory cues associated with mate choice are produced by the resident microbiome [48,49]. Thus, odours mediated by the reproductive microbiome might also influence mate choice decisions.

Mate choice to avoid infected partners has been suggested to ultimately lead to the evolution of reduced virulence of STMs, thus weakening selection on mate choice [20]. However, recent theoretical work indicates that coevolution between mate choice and STIs can lead to complex dynamics, which can preserve mate choosiness and STI virulence [50]. Alternatively, some STMs may be beneficial to females [51]. One potential example is *Lactobacillus*, which can limit the growth of other bacterial species, including potential pathogens, via the production of lactic acid [30].

The role of microbes in precopulatory male–male competition is less well explored. Reproductive microbiomes might influence the intensity of sexual selection via male–male competition if, for example, the reproductive microbiome influences mating behaviour of individuals, either increasing or decreasing mating activity or the propensity for mating, and, thus, the number of males competing for access to females or the population level operational sex ratio. There is evidence that STIs, and pathogens transmitted via sociosexual contact, can both decrease [52,53] or increase [40,54] male mating activity.

Postcopulatory Sexual Selection: Sperm Competition and Cryptic Female Choice

Increasing evidence indicates that the reproductive microbiome of males and females can have potentially fundamental roles in the dynamics of fertilisation (Figure 2). The outcome of **sperm competition** is strongly determined by the number of viable sperm inseminated or spawned by rival males and the fertilising efficiency of these sperm, which broadly reflects aspects of sperm metabolic performance, swimming velocity, and longevity [55]. Microbes can impact sperm viability and fertilising efficiency to reduce male fertility (Table 1). These effects are expected to be particularly pronounced in polyandrous species, where any reduction in fertilising efficiency will be exploited by rival ejaculates, although evidence of microbial impacts on the outcome of sperm competition is currently scarce. In the fruit fly, *Drosophila simulans*, infection by the bacterial endosymbiont *Wolbachia pipientis* affects sperm production in males and can reduce the fertilisation success of infected males in sperm competition by >10% ([56], but see [57]). Similarly, experimental removal of bacterial endosymbionts (*Wolbachia*, *Wigglesworthia glossinidia*, and *Sodalis glossinidius*) of reproductive tissue impacts reproductive gene expression in male tsetse flies, *Glossina morsitans*, including the expression of genes encoding spermatophore and seminal fluid proteins [58].

Ejaculate microbes can also influence competitive fertilisation success indirectly by triggering differential immune responses in the female reproductive tract, with sperm being collateral damage of the

female immune attack on inseminated bacteria [16]. Interestingly, cross-reactivity of sperm and bacteria due to shared antigenic determinants means that sperm-immobilising factor, the secretory protein of *Staphylococcus aureus*, both impairs sperm function and fertilisation success (Table 1) and immobilises bacteria [59,60]. In addition to important implications for assisted reproduction, such molecular mimicry between sperm and bacteria may have a critical role in female immune responses, raising the intriguing possibility that sexually transmitted bacteria may evolve molecular mimicry of sperm to dilute female immune responses. Cryptic female choice may also operate through nonimmunological responses; for example, females may preferentially eject ejaculates of males with higher microbial load. Finally, the microbiome of the female reproductive tract may impact the fertilising performance of an insemination directly. For example, a strain of *Staphylococcus warneri*, isolated from the cervix of an infertile woman, caused sperm agglutination and death *in vitro* [61].

Therefore, postcopulatory sexual selection will favour the ability of males to eliminate microbes from their ejaculates and produce sperm that can escape microbes or microbially induced female immunological responses. For example, the evolution of faster swimming sperm may allow sperm to more rapidly reach ova or female sperm storage organs, thus reducing the impact of the female reproductive microbiome and/or immunity [62], although faster sperm may have evolved because of the competitive advantage gained in sperm competition, independently of microbial effects. More convincing is the evidence for antimicrobial activity in ejaculates [44,63,64] and the presence of antimicrobial and immunity-related proteins in seminal fluid in a range of taxa (e.g., red junglefowl, *Gallus gallus* [65], *Passer* sparrows [66], *Drosophila* [67], and honeybee, *Apis mellifera* [68]). Interestingly, male junglefowl may preferentially invest in seminal fluid proteins involved in immunity over successive matings, possibly to defend depleting numbers of sperm from female antimicrobial and immune responses [69].

However, demonstrations of the impact of seminal fluid proteins on bacteria are scarce. In humans, *E. coli*-induced agglutination of washed sperm (Table 1) was significantly reduced by the addition of seminal fluid [70]. More convincingly, in the bedbug, *Cimex lectularius*, the negative effects of environmental microbes on sperm mortality (Table 1) were eliminated when bacteria were mixed with lysozyme (an antimicrobial enzyme) from chicken egg white, simulating the effect of the lysozyme-like activity of bedbug seminal fluid contained in a single ejaculate [71]. Finally, in honeybees, male seminal fluid kills the facultatively sexually transmitted fungus *Nosema apis* through seminal fluid proteins, which disrupt the life cycle of the fungus by promoting extracellular germination, and nonprotein components of the seminal fluid, which damage fungal spores [72].

Male × Female Compatibility

Genetic incompatibility between reproductive partners has long been identified as a mechanism for balancing sexual selection and a source of genetic variation in a population. Compatibility of the reproductive microbiomes of partners may have a similarly important role in sexual selection by influencing mating decisions and fertilisation success. For example, an ejaculate with a reproductive microbiome similar to that of the inseminated female could elicit a limited female immune response. Similarly, ejaculates may be preferentially retained by females if they contain bacterial species that are beneficial to females but currently scarce or absent in the female reproductive microbiome. Evidence of similar microbiomes in monogamous pairs (see later) might partly reflect such compatibilities. However, testing these ideas requires controlling for the effects of genetic compatibility. For example, while theory predicts sexual selection to favour reproduction among partners compatible at the Major Histocompatibility Complex (MHC) [73], it will be difficult to separate the effects of microbial and MHC compatibility when different MHC haplotypes are characterised by distinct microbiomes [74] (Box 2). A special and rather extreme case of incompatibility driven by bacteria is cytoplasmic incompatibility caused by *Wolbachia* (see later).
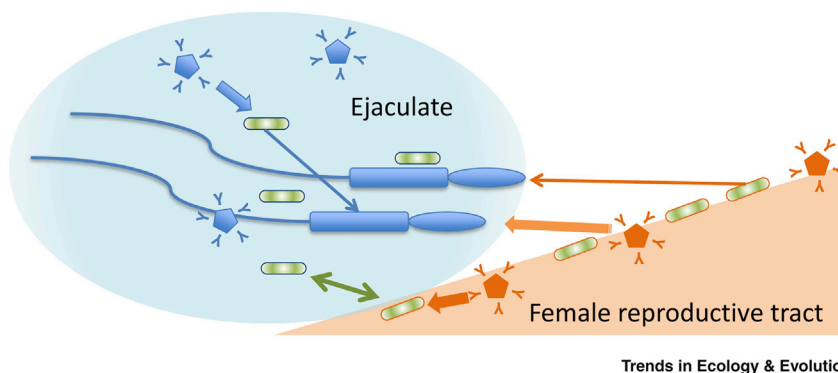
The Reproductive Microbiome and Sexual Conflict

The reproductive microbiome may have an important role in sexual conflict. STIs have long been identified as part of the costs of mating, particularly in taxa with a cloaca, which functions in both

gamete transfer and excretion, which may be predisposed to sexual transmission of faecal microorganisms [21,75]. Asymmetries in the relative costs and infection risk likely generate divergent mating optima between males and females. First, females are at higher risk of infection compared with males [21], even in species without intromission, such as birds [76]. Second, the Darwin–Bateman paradigm suggests that that the costs of contracting an STI are more likely to be offset by reproductive gains in males than in females. Therefore, sex-specific costs of STIs may contribute to the evolution of sexually antagonistic strategies, where females evolve resistance to mating, while males evolve strategies to impose mating by circumventing female resistance. Consistent with a male-beneficial, female-detrimental scenario is the finding in garden ants, *Lasius niger*, that the sperm storage organs (accessory testes) of virgin males enhance bacterial growth, while the sperm storage organs (spermatheca) of virgin queens strongly inhibit bacterial growth [77]. The presence of antimicrobial peptides and proteins in semen may also lead to sexual conflict. For example, while these substances may protect sperm from antimicrobial attack, including from those encountered in the female, these same proteins may be detrimental to the reproductive microbiome of healthy females.

Conversely, a female-beneficial, male-detrimental pattern may be linked to the presence of *Lactobacillus* spp., which may be beneficial in the vaginal microbiome [8,10], while impairing sperm function ([78], but see [79]). A more complex scenario may occur in bedbugs, in which traumatic insemination can lead to accelerated female mortality caused by infection of environmental microbes from the male aedeagus [71], and, thus, creates potential for conflict over mating decisions [80]. Females mated to males and experimentally injected with lysozyme (simulating the antimicrobial effect of male seminal fluid [64]), did not differ in fecundity and longevity from females exposed to mating and control injections [71]. However, lysozyme-injected females and females exposed to prolonged mating were less likely to suffer reproductive senescence, possibly reflecting complex interactions between deleterious effects of insemination (possibly mediated by bacteria) and beneficial effects (e.g., courtship feeding) [71]. These patterns may be partly explained by the recent discovery that females ramp up their immune system in expectation of traumatic insemination (i.e., following a blood meal) [81].

Microbes can have a key role in intersexual coevolution; their fitness interests are likely more aligned with those of the male, particularly for microbial species that are exclusively sexually transmitted. Therefore, such microbes may evolve strategies to promote male mating interests, prevent females from detecting and avoiding infected partners, or increase attractiveness of infected males (see

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Figure 2. Potential Impact of Reproductive Microbes on Sperm function and the Dynamics of Fertilisation. Microbes contained in an ejaculate will impact sperm and will, in turn, be targeted by antimicrobial factors (blue pentagons) in the seminal fluid (blue arrows). Similarly, the female reproductive tract will respond immunologically (orange pentagons) to local microbes and, following insemination, to sperm and microbes from the male ejaculate (orange arrows). Ecological interactions between ejaculate microbes and the female microbiome will also occur (green arrow). Microbial effects are represented by line arrows, immunological responses by block arrows.

earlier). The notion that microbes can be beneficial in hosts of one sex but detrimental in that of the other, creates potential for these organisms to constrain optimal sexual dimorphism of the host through mechanisms akin to sexually antagonistic alleles in **intralocus sexual conflict**. This is particularly relevant when the risk of horizontal (e.g., sexual) transmission is sufficiently high to prevent the segregation of antagonistic microbes in the sex where they are beneficial. The evolution of vertical transmission will influence this conflict by modulating sex-specific costs [82].

The Reproductive Microbiome and Mating Systems: A Dynamic Feedback

Increasing evidence indicates that host **mating systems** can influence the ecology and evolution of reproductive microbiomes. Monogamy should promote similarity in the microbiomes of mating partners (i.e., low individual diversity and high differentiation across mating partners), while polygamy should promote high individual diversity and low differentiation across partners, although this depends on the precise structure of the sexual network and sex-specific risk of sexual transmission (Figure 3). Monogamous mating partners appear to share microbes through sexual transmission [76], which can lead to increased similarity in their reproductive microbiomes [83–85], while a positive relationship between female polyandry and the diversity of the female reproductive microbiome has been found within a population of common lizards, *Zootoca vivipara* [86], and across species of *Peromyscus* mice [87] and primates [11]. These effects could reflect both compositional changes to the female reproductive microbiome through sexual transmission of ejaculate microbes and the disruption of the female microbiome caused by intromission and insemination [84]. Specifically, while sexual activity is known to alter the microbial communities of the female reproductive tract [7,84], the female system appears to be constantly attempting to revert back to its 'natural' or 'optimal' state [84,88]. Mating systems will also have a key role in patterns of selection and evolution of reproductive microbes: monogamy and low mating rates are expected to promote within-host evolution and reduced
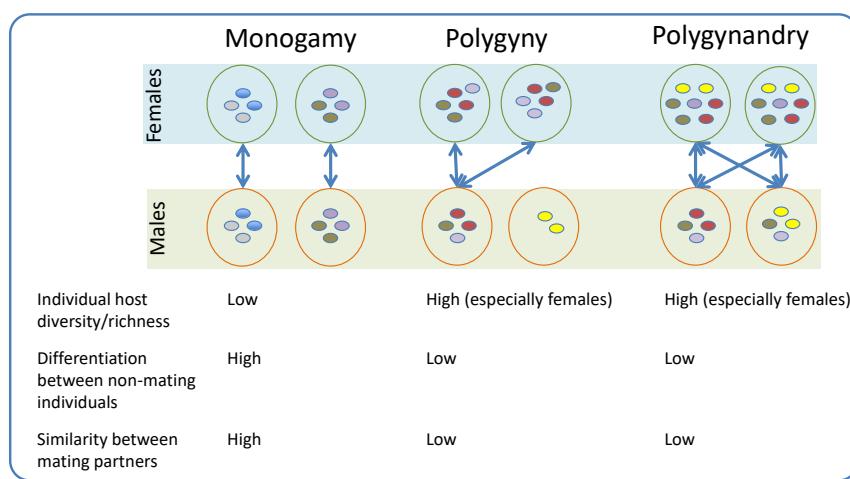

Figure 3. The Role of Host Mating Systems in Shaping Patterns of Reproductive Microbiome Diversity.
Hypothetical patterns of reproductive microbiome diversity across three mating systems (strict monogamy, polygyny, and polygynandry), assuming a higher risk of sexual transmission for females. Under monogamy, sexual transmission is restricted within mating pairs and this is expected to generate high similarity between partners and high diversification between pairs. Under polygyny, few males typically mate with multiple females each, while others fail to mate, and females mate with only one male. This mating system will lead to high variation across males proportional to the mating skew and females mating with the same male will share reproductive microbes. Under polygynandry, high promiscuity will mean that most individuals have multiple mates, leading to high diversity within, low diversification among, hosts . These expectations are likely to change with several ecological factors. For example, local costs of sexually transmitted infections (STIs) may promote divorce and promiscuity in monogamous species [120].

virulence, while polygamy and high mating rates will favour high transmission rates and across-host evolution, creating more scope for virulence (Box 2).

Reproductive microbiomes can also shape ecological and evolutionary changes in host mating systems. Sexually transmitted microbes may, in principle, manipulate host behaviour to increase re-mating rates and promiscuity. For example, in the butterfly *Hypolimnas bolina*, a male-killing *Wolbachia* infection leads to a female-biased population sex ratio, male scarcity, and, as a consequence, frequent sperm depletion in males, which in turn promotes female re-mating to secure sufficient sperm supplies [89]. These coevolutionary dynamics depend on, and in turn affect, the benefits of mate choice and multiple mating, and the virulence of STIs [90]. Theory predicts infection levels to diverge between sexes with increasing mating skew, and less-virulent STIs with long infection periods to be favoured in mating systems with high mating skew [50].

The Reproductive Microbiome and Speciation

Reproductive isolation between populations is a fundamental step in speciation. The idea that microbes (i.e., parasites) might drive speciation has received some attention (parasite-mediation speciation; e.g., [91,92]), although other studies have instead suggested that parasites represent a homogenising force [93]. Parasite-mediated divergent selection is proposed in the context of ecological speciation, whereby local adaptation to contrasting parasite communities may facilitate speciation via several nonexclusive mechanisms, including reduced immigrant and hybrid viability or fecundity, pleiotropy, and ecologically based sexual selection, and empirical evidence for these ideas is building [91]. Furthermore, the gut microbiome may have a role in mate choice and hybrid inviability [94,95]. Given their potential to influence mating patterns and fertilisation success, reproductive microbiomes may also have a role in speciation.

In general, reproductive isolation is promoted when mating and fertilisation are favoured by locally co-adapted, compatible microbes, while microbes with high transmission rates will likely be associated with panmixia. Empirical work investigating these ideas is currently limited. However, in fruit flies, *Wolbachia*-induced unidirectional cytoplasmic incompatibility coupled with behavioural isolation contributes to reproductive isolation between sister species [96], while in *Nasonia* wasps, bidirectional *Wolbachia*-induced cytoplasmic incompatibility may contribute to reproductive barriers [97]. Divergence in the human vaginal microbiome has been suggested among ethnic groups [8]. Similarly, for males, diet-induced changes in the semen microbiome of mice [98] suggests that dietary shifts in host populations promotes divergence in the reproductive microbiome. These examples indicate considerable potential for the reproductive microbiome to have a role in reproductive isolation and ultimately speciation.

Concluding Remarks

The widespread occurrence of microbes in male and female reproductive systems can impact, both beneficially and detrimentally, a range of reproductive traits (e.g., sperm quality) and processes (e.g., embryo implantation), as well as reproductive success (e.g., fertilisation and pregnancy loss). Although limited, current knowledge underscores the importance of understanding both community structure and the functional activity of the reproductive microbiome. While microbes of the reproductive tract likely form spatiotemporally structured, locally adapted communities, those found in fluids, such as the male seminal fluid, or associated with sperm may represent more transient assemblages with predisposition for dispersal and colonisation through sexual transmission. Thus, the reproductive microbiome can generate selective pressures on males and females, with critical implications for sexual selection, sexual conflict, and the emergence of mating systems and reproductive barriers. In turn, host reproductive strategies, mating system, and reproductive barriers will have important consequences for the evolution of reproductive microbial assemblages (e.g., microbial ecology and virulence). The study of the evolutionary ecology of host-reproductive microbiome dynamics is in its infancy and many major questions remain (see Outstanding Questions). The combination of sequencing advances, genomic resources, and fine-grained studies of host sexual behaviour will likely catalyse rapid progress in the near future.

What are the implications of reproductive microbiomes for conservation or captive breeding of threatened and endangered species? For example, does the microbiome of captive animals differ from their wild counterparts? If so, how might this affect the success of re-introduction programs?

How might changes in climate and environment influence the reproductive microbiome? For example, do extreme temperatures (e.g., heatwaves) or increasing urbanisation affect reproductive microbiomes and, if so, what are the implications of this for reproduction and disease spread?

Flower pollen represents a unique microbial habitat, and studies have shown that the pollen microbiome exhibits a high degree of species specificity. Given this, how might pollen microbiomes influence sexual reproduction and reproductive isolation in plants?

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