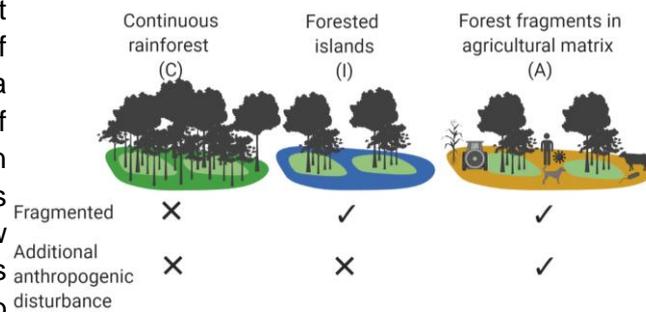


Recent Research Highlights

Effects of human encroachment into wildlife microbiomes

The gut microbiome is a symbiont consisting of a vast community of microbes and their genes, which play a key role in host health. Disturbances of this gut microbial community has been linked to many autoimmune diseases and infections, highlighting just how important a healthy gut microbiome is for the health of its host. Changes to

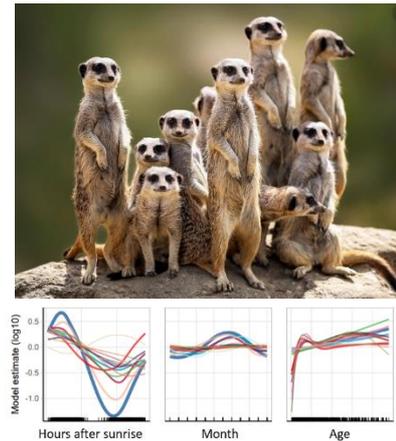


wildlife gut microbiomes due to anthropogenic disturbances, such as habitat fragmentation, can disrupt natural gut microbiota homeostasis and make animals vulnerable to infections that may become zoonotic. In this study, we used the gut microbiome as an indicator for health in order to understand how zoonotic diseases can be transferred from wildlife to humans in the Anthropocene. Although anthropogenic disturbances are known to negatively impact the gut microbiome in a process known as dysbiosis, it is still unclear which anthropogenic factors drive these changes. Our study design in the Panama Canal area allows for the differentiation between habitat fragmentation *per se* and living in close proximity to humans, their domesticated animals, and agriculture. We found that 384 spiny rats (*Proechimys semispinosus*) living in fragmented habitats close to humans had greatly altered gut microbiomes, whereas spiny rats living in protected, fragmented habitats did not. This means that not habitat fragmentation *per se* but contact with humans in the driving factor in changes to the gut microbiome. These findings are significant because spiny rats are generalist species expected to be more resilient to environmental changes. Instead, their microbiomes displayed two patterns of change: The structural composition of the microbes that make up the gut microbiome became more irregular (heterogeneous) and shifted away from its natural composition. They also harbored more bacterial species that have been found in domesticated animals and are known to cause disease in livestock. These changes seem to imply two things: The spiny rats are adapting to changes in their environment using the flexibility offered by gut microbiomes, however these adaptations are not occurring fast enough to keep up with the rate of change in the Anthropocene. Understanding that wild animals are struggling to cope with changes in the Anthropocene should serve as a warning that we may see more zoonosis-driven pandemics in the future.

Gloria Fackelmann, Mark A. F. Gillingham, Julian Schmid, Alexander Christoph Heni, Kerstin Wilhelm, Nina Schwensow & Simone Sommer (2021) Human encroachment into wildlife gut microbiomes. *Communications Biology*.
[wmf2021-asm.ipostersessions.com/Default.aspx?s=DF-7D-32-81-C3-F4-FD-35-24-9C-17-87-AF-F9-B3-6D](https://www.nature.com/articles/s42003-021-21211-1)

Meerkat gut microbiomes exhibit strong daily oscillations that do not decay with age

Gut bacterial communities are often incredibly dynamic over time, which makes them challenging to study. Microbes can fluctuate over the scale of hours, weeks, months and years, in response to things like food intake, seasonal climate, and host ageing, yet so far, we have not been able to disentangle these processes. We investigated gut microbiome dynamics in wild meerkats (*Suricata suricatta*) over a 20-year period to compare diurnal, seasonal, and lifetime processes in concert. We found that almost all common genera exhibited diurnal oscillations, and daily dynamics in bacterial load and diversity were stronger than seasonal and lifetime effects. Diurnal oscillations were driven by changes to the genus *Clostridium*, were better explained by light-dark cycles than foraging schedule, and did not decay with age. Across life, specific genera were associated with ageing and condition, and were more tightly linked to biological rather than chronological senescence. Our findings highlight that daily oscillations in the microbiome are probably more common than previously thought, and demonstrate that accounting for circadian rhythms is crucial for future gut microbiome research.



Risely Alice, Wilhelm Kerstin, Clutton-Brock Tim, Manser Marta & Sommer Simone (2021) Diurnal oscillations in gut microbiome load and composition eclipse seasonal and lifetime dynamics in wild meerkats, *Suricata suricatta*. *Nature Communications*.

Foraging in monocultures with heavy pesticide input decreases the gut microbiota diversity of nectar-feeding bats

The health and physiology of native species can be negatively affected by agriculture, both due to habitat alteration and to pesticide use, and it is possible that gut microbiome disruption plays a role in these negative effects. We examined the association between management intensity of banana plantations and both the gut microbiota composition and body condition of one species of nectar-feeding bat (*Glossophaga soricina*, Phyllostomidae) in the Costa Rican Caribbean lowlands. We discovered that gut microbiota from bats foraging in conventional monocultures were overall less phylogenetically diverse than those from bats foraging in organic plantations or natural forests, both of which were characterized by diverse bacterial assemblages and individualized microbiota. Nonetheless, co-occurrence network complexity was higher in conventional monocultures, possibly indicating altered microbial interactions in agricultural landscapes. Bats from both banana plantations were larger and heavier than their forest counterparts, reflecting the higher and year-long food supply. With our study, we discovered that even if both banana plantations (conventional and organic) do provide a reliable food source for bats, conventional monocultures are associated with less diverse and potentially dysbiotic microbiota, while organic plantations promote diverse and individualized gut microbiota akin to their natural forest foraging counterparts. Whilst the long-term negative effects of anthropogenically-altered microbiota are unclear, our study provides further evidence from a novel perspective that organic agricultural practices are indeed more sustainable for wildlife health.



Priscilla Alpízar, Alice Risely, Marco Tschapka & Simone Sommer (2021). Agricultural fast food: Bats feeding in banana monocultures are heavier but have less diverse gut microbiota. *Frontiers in Ecology and Evolution*, 9, 746783. DOI: 10.3389/fevo.2021.746783.

Impact of habitat disturbance and fungicides on homeostasis of amphibian skin microbiomes and abundance of protective bacteria against chytrid fungus infections - using endangered and rediscovered Costa Rican amphibians as natural model organisms

Some amphibians that were thought to be “extinct” have been found decades after population declines in the late 1980s. These species appear to have evolved resistance to the pathogen *Batrachochytrium dendrobatidis* (Bd), the likely causative agent of their declines, and raises the question how selection and diversity of protective skin microbiomes enabled these endangered neotropical amphibians to survive. Our study showed that the skin microbiome of surviving species (*Lithobates vibicarius*,



Craugastor escoces, *Isthmohyla rivularis* and *I. pseudopuma*) possess putatively Bd-inhibitory activity across species (e.g., by the presence of *Pseudomonas veronii* and *Acinetobacter johnsonii*), which could be involved in resistance against Bd. We also studied the variation of the skin microbiome across the life stages of *L. vibicarius* and observed that life stage is a strong predictor of the diversity of the skin microbiome, suggesting a dynamic skin microbiome through development. Unfortunately, these amphibian populations are still not “safe” due to ongoing anthropogenic threats, caused by habitat disturbance and overuse of pesticides. Alteration of the microbiome associated with environmental changes produced by anthropogenic activities may make the host more susceptible to pathogens. Our research showed that bacterial diversity of tadpoles of *L. vibicarius* from the disturbed habitats was lower than in those from the undisturbed habitats. Adults of *L. vibicarius* from disturbed habitats exhibited greater community dispersion than those from undisturbed habitats. These observed patterns could be associated to the presence of environmental stressors in our study sites, which can perturb a stable state leaving animals more susceptible to pathogen infections even beyond Bd. We could demonstrate that exposure to a widely used fungicide, chlorothalonil, changes the skin bacterial communities of tadpoles of *L. vibicarius*, potentially disrupting this protective trait against pathogens. With this research, we gained important information of the functional importance of the skin microbiomes, and the negative impacts of anthropogenic activities, especially agrochemicals, which can be used to develop management strategies that protect the health of these endangered amphibian populations.

Jiménez RR, Alvarado G, Estrella J, Sommer S (2019) Moving beyond the host: unraveling the skin microbiome of endangered Costa Rican amphibians. *Frontiers in Microbiology*, 10, 2060.

Jiménez RR, Alvarado G, Sandoval J, Sommer S (2020) Habitat disturbance influences the skin microbiome of a rediscovered neotropical-montane frog. *BMC Microbiology*, 20, 292.

Jiménez RR, Alvarado G, Mena F, Ruepert C, Ballesteros E, Sommer S (2021) The fungicide chlorothalonil changes the amphibian skin microbiome: a potential factor disrupting a host disease-protective trait. *Applied Microbiology*, 1, 26–37.

Adenovirus and Astrovirus infections perturb the gut microbial community of non-human primates and bats

Non-human primates represent an excellent natural model system for studying host-pathogen-microbiome interactions, since they harbour an important source of several crucial viruses of humans and livestock, including Adenoviruses AdV - a major cause of diarrhoea in children. As in humans, a remarkably high AdV-prevalence, and inferred zoonotic potential has been reported for mouse lemurs. We examined the influence of AdV on the gut microbiome of naturally infected Malagasy mouse lemurs (*Microcebus griseorufus*). Our study shows that enteric AdV indeed disturbed the gut microbiome community composition. Beneficial genera decreased, whereas pathogen-containing genera increased after AdV-infection leading to pathobiont-like shifts in the gut microbiome. This shows that in apparently healthy lemurs, AdV infections disturb the gut bacterial homeostasis, which can increase previously suppressed

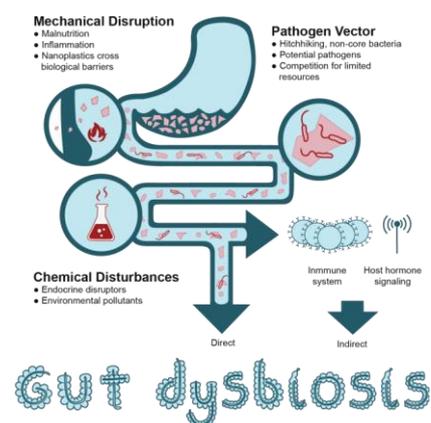


health risks by promoting co-infections. Interestingly, we observed similar effects also in a neotropical bat species (*Artibeus jamaicensis*) infected with Astroviruses, also causing diarrhoea in young and immune-compromised humans, supporting the idea that virus-microbiome interactions have important, largely neglected implications for host health. If similar processes are present in humans, the effects of enterovirus infections would cause longer-term impacts and go beyond the directly observed symptoms.

Wasimuddin, Victor M. Corman, Jörg U. Ganzhorn, Jacques Rakotondrany, Yedidya R. Ratovonamana, Christian Drosten & Simone Sommer (2019) Adenovirus infection is associated with altered gut microbial communities in a non-human primate. *Scientific Reports*, 9,13410. DOI: [org/10.1038/s41598-019-49829-z](https://doi.org/10.1038/s41598-019-49829-z).

Wasimuddin, Brändel SD, Tschapka M, Page R, Rasche A, Corman VM, Drosten C, Sommer S (2018) Astrovirus infections induce age-dependent dysbiosis in gut microbiomes of bats. *The ISME Journal*, 12, 2883-2893. DOI: [10.1038/s41396-018-0239-1](https://doi.org/10.1038/s41396-018-0239-1).

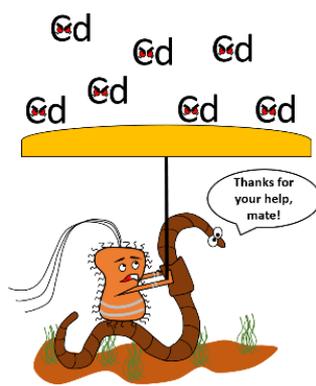
Microplastics and the gut microbiome: how chronically exposed species may suffer from gut dysbiosis



Since the mass production of plastics began in the 1950s, we have become inundated in plastic. Although this development brought with it advantages (imagine what might happen if you dropped a mobile phone made out of glass), it also started a massive and uncontrolled experiment since it takes centuries for plastics to degrade. In this review, we identified three mechanisms through which microplastics could harm one of animals' greatest symbionts, the gut microbiome, and thus, because of its vital standing in host health, how microplastics could harm wildlife health: 1) by mechanically damaging the gut lining, an essential component in maintaining homeostasis between host and the gut microbiome; 2) by acting as a pathogen vector, allowing potential pathogens to hitchhike on the surface of microplastics and thus disturbing the gut microbial community; and 3) by leaching chemicals that can interfere with normal host hormone signaling, another major regulator of the gut microbiome.

Fackelmann G, Sommer S (2019) Microplastics and the gut microbiome: how chronically exposed species may suffer from gut dysbiosis. *Marine Pollution Bulletin*, 143, 193-203.

Gut microbiome as indicator of heavy metal pollution in soil



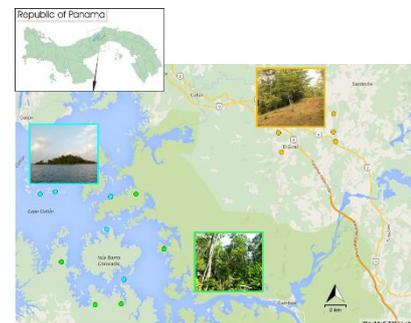
Gut microbiomes are becoming recognized as important players in organism health, with comprehension of their perturbations in the polluted environment offering new insights into the nature and extent of heavy metal effects on the health of soil biota. Earthworms are essential species in soil, which carry out the soil biological regulation by mixing organic matter and mineral particles inside their gut. Through their intimate contact with soil, earthworms are exposed to the variety of pollutants and are important indicators of soil quality. In this research we investigated the effect of heavy metal cadmium (Cd) on the earthworm (*Lumbricus terrestris*) gut microbiota. Cd exposure led to perturbations of earthworm gut microbiota with an increase in bacteria previously described as

heavy metal resistant or able to bind heavy metals, revealing the potential of the earthworm-gut microbiota system in overcoming human-caused heavy metal pollution. Furthermore, we defined several bacterial genera as bacterial indicators of exposure to Cd which could serve as biomarkers in soil biomonitoring practices. The results of this study help to understand the impact of anthropogenic disturbance on soil fauna health and will have implications for environmental monitoring and protection of soil resources.

Šrut M, Menke S, Höckner M, Sommer S (2019) Earthworms and cadmium – Heavy metal resistant gut bacteria as indicators for heavy metal pollution in soils? *Ecotoxicology and Environmental Safety*, 171, 843–853.

Human-induced landscape change disturb animal communities and species abundance pattern with significant effects on virus prevalence in wildlife

Anthropogenic environmental change can impact community traits such as species richness and population densities which have been shown to increase the prevalence of viral infections in wildlife reservoirs. It has been postulated that especially host species that are more resilient to changes of their natural habitat may increase in numbers which in turn may affect the prevalence of directly transmitted viruses. We have carried out an ecological survey of small mammal communities in three tropical landscapes differing in their degree of environmental change in Central Panama and investigated the effects of community changes on *Hepacivirus* prevalence, a virus closely related to human hepatitis C virus. The modification of continuous habitat into partly connected or isolated habitat patches during the past century was linked to changes in species richness and species assemblages, which in turn was associated with shifts in the abundance of generalist marsupial and rodent species. In particular, the spiny rat *Proechimys semispinosus* has become dominant in isolated habitat patches. Landscape-specific host density represents the most important ecological driver influencing local *Hepacivirus* prevalence. Our study provides important empirical data on how human-induced landscape changes may affect virus prevalence in wildlife and emphasizes the importance of a landscape scale approach considering the complex interactions between ecological factors driving host-virus interactions.



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Schmid J, Rasche A, Eibner G, Jeworowski L, Page RA, Corman VM, Drosten C, Sommer S (2018) Ecological drivers of *Hepacivirus* infection in a Neotropical rodent inhabiting landscapes with various degrees of human environmental change. *Oecologia*, 188(1), 289-302.

The best smellers make the best choosers: MHC-dependent mate choice is affected by female chemosensory receptor gene diversity



Sexual selection involving genetically disassortative mate choice is one of several evolutionary processes that can enhance population genetic variability and pathogen resistance. In many vertebrate species, females select mates depending on their major histocompatibility complex (MHC) genes. The products of the MHC genes are known to be drivers of pathogen resistance and sexual selection enhancing offspring genetic diversity. MHC further influences individual odor types and social communication. However, little is known about the receptors and their volatile ligands that are involved in this type of chemical communication. We investigated the effect of two groups of chemosensory receptor genes (trace-amine associated receptors (TAARs) and olfactory receptors (ORs)) on MHC-dependent mate choice. Our study is amongst the first to show a genetic link between behavior and chemosensory receptor genes. These results contribute to understanding the link between genetics, olfaction and associated life history decisions.



Santos PSC, Mezger M, Kolar M, Michler F-U, Sommer S (2018) The best smellers make the best choosers: mate choice is affected by female chemosensory receptor gene diversity in a mammal. *Proceedings of the Royal Society London B*, 285, 20182426.

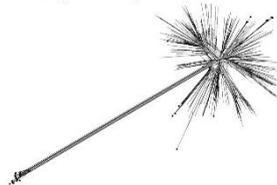
Santos PSC, Michler F-U, Sommer S (2017) Can MHC-assortative partner choice promote offspring diversity? A new combination of MHC-dependent behaviors among sexes in a highly successful invasive mammal. *Molecular Ecology*, 26, 2392-2404.

Santos PSC, Courtiol A, Heidel AJ, Höner OP, Heckmann I, Nagy M, Mayer F, Platzer M, Voigt CC, Sommer S (2016) MHC-dependent mate choice is linked to a trace-amine-associated receptor gene in a mammal. *Scientific Reports*, 6, 38490.

Extreme MHC diversity in bats with wide geographic ranges

Different hypotheses may explain extreme MHC variability. One is that such variability is attributable to adaptation to a wide geographic range and a diverse array of habitats, as found in passerine birds. Here we show that MHC class I diversity in Seba's short-tailed bats (*Carollia perspicillata*), a widely distributed, generalist, neotropical species, shows a remarkable individual and population-level diversity and length-polymorphism comparable with that reported in passerine birds. Investigation of the details of the underlying adaptive processes and the role of the high MHC diversity in pathogen resistance are important next steps for a better understanding of the role of bats in viral evolution and as carriers of several deadly zoonotic viruses.

Extreme high MHC I-diversity in a neotropical bat



Qurkhuli T, Schwensow N, Brändel SD, Tschapka M, Sommer S (2019) Can extreme MHC class I diversity be a feature of a wide geographic range? The example of Seba's short-tailed bat (*Carollia perspicillata*). *Immunogenetics*, 71, 575. DOI: [org/10.1007/s00251-019-01128-7](https://doi.org/10.1007/s00251-019-01128-7).

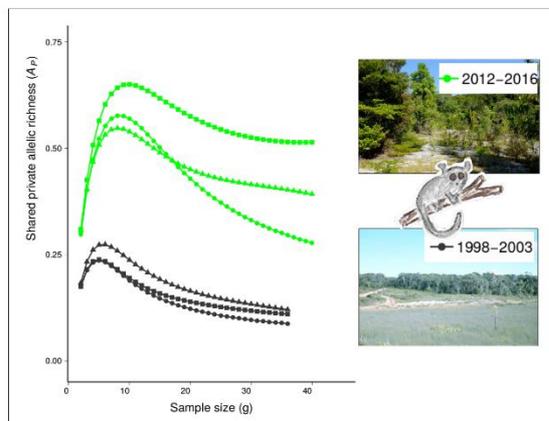
How low is functional MHC diversity in cheetahs?

Species that experienced a population bottleneck, a sharp reduction in the size of a population, often show a low genetic variability, also in adaptive immune relevant genes required to defend against parasites and pathogens. Free-ranging Namibian cheetahs (*Acinonyx jubatus*), however, despite being a textbook example of depleted genetic variability due to habitat loss, human-wildlife conflicts and historic bottlenecks, show no signs of impaired immunocompetence or health - thus contradicting the theoretical assumptions. How are cheetahs able to defend themselves against diseases? This study revealed that free-ranging Namibian cheetahs indeed possess only a low number of immune gene alleles at the major histocompatibility complex (MHC) comparable to other bottlenecked (endangered) cat species. However, these remaining alleles are functionally very divergent and might (currently) be sufficient in terms of pathogen recognition and initiation of the immune defence. Moreover, the allelic composition of cheetahs influences the level of MHC expression (so far only known from human research) which could add to functional variation and, thus, might play a role in the ability to defend against pathogens. This offers an explanation as to how bottlenecked cat species might have avoided impaired immuno-competence, despite showing low MHC allelic diversity.



Schwensow N, Castro-Prieto A, Wachter B, Sommer S (2019) Immunological MHC supertypes and allelic expression: how low is the functional MHC variability in wild endangered Namibian cheetahs? *Conservation Genetics*, 20, 65-80. DOI: [org/10.1007/s10592-019-01143-x](https://doi.org/10.1007/s10592-019-01143-x).

Corridor effects on the genetic diversity of mouse lemurs



Corridors rank top among the recommendations to counteract the effects of habitat modification and reduce the vulnerability of small populations to environmental variation and stochastic processes, such as genetic drift. Our study focuses on the effect of the establishment of corridors on neutral (microsatellites) and adaptive (MHC) genetic diversity in *Microcebus ganzhorni*, a recently discovered primate species restricted to a few patches of littoral forest in southeastern Madagascar. While we find similar patterns of neutral and adaptive individual diversity as suggested from estimates of heterozygosity and allelic richness between

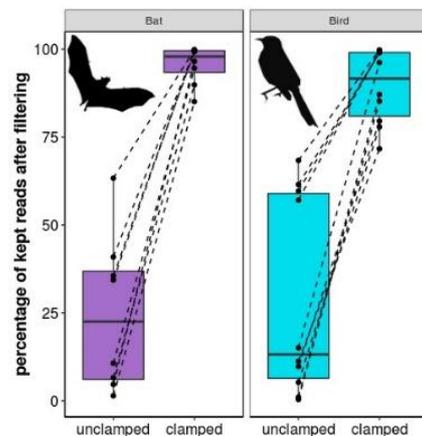
sampling periods, we provide evidence that after the establishment of corridors a larger number of shared private alleles are found at microsatellite loci. Furthermore, our results confirm the role of selection as a main driver of MHC II diversity in *M. ganzhorni*. We argue that corridors, even for animals that appear to be robust to fragmentation, might play an important role in population dynamics. This work highlights the relevance of long-term genetic monitoring providing insights into the evolutionary history and patterns of gene flow of wild populations and the possibility to tease apart the effects of drift from selection maintaining MHC adaptive variability.

Montero K, Ramanamanjato J-B, Ernest R, Rakotondranary SJ, Ganzhorn JU, Sommer S (2019) Challenges of NGS in conservation management: insights from long-term monitoring of corridor effects on the genetic diversity of mouse lemurs in a fragmented landscape. *Evolutionary Applications*, 12, 425-442.

Method development for microbiome and MHC research

Jumping the green wall: the use of PNA-DNA clamps to enhance microbiome sampling depth in wildlife microbiome research

As microbiome research moves away from model organisms to wildlife, new challenges for microbiome high throughput sequencing arise caused by the variety of wildlife diets. Normally, high levels of contamination from the host (mitochondria) or diet (chloroplast) are commonly observed in wildlife samples. This contamination “hijacks” reads thus decreasing the overall sequencing depth of wildlife samples and reducing statistical power in downstream analysis. We developed an amplification protocol utilizing PNA-DNA clamps to maximize the use of resources and to increase the sampling depth of true microbiome sequences in samples with high levels of plastid contamination. The PNA-DNA clamps are DNA mimicking molecules that bind selectively to a target sequence. We used these clamps to block the sequences of chloroplast and mitochondria in fecal samples of birds and bats. Our protocol successfully blocks the signal organelle signals and provides a 13-fold increase in bacterial sequence amplification in comparison with the Earth Microbiome Protocol.



Viquez-R L, Fleischer R, Wilhelm K, Tschapka M, Sommer S (2020). Jumping the green wall: the use of PNA-DNA clamps to enhance microbiome sampling depth in wildlife microbiome research. *Ecology and Evolution*, 10, 11779-11786.

A novel workflow to improve multi-locus genotyping of wildlife species

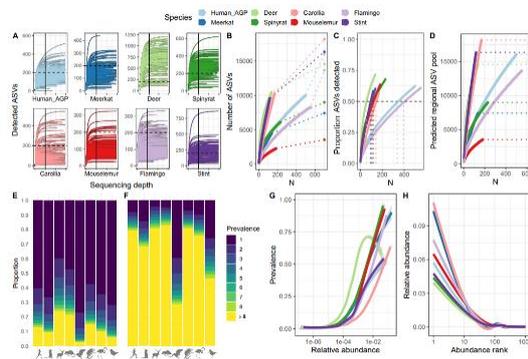


Genotyping novel complex multigene systems is particularly challenging in non-model organisms. Target primers frequently amplify simultaneously multiple loci leading to high PCR and sequencing artefacts such as chimeras and allele amplification bias. Most genotyping pipelines have been validated in non-model systems whereby the real genotype is unknown and the generation of artefacts may be highly repeatable. Further hindering accurate genotyping, the relationship between artefacts and copy number variation (CNV) within a PCR remains poorly described. We developed a novel open-source genotyping pipeline (ACACIA) to the data, and compared its performance with another, previously published, pipeline. ACACIA yielded very high allele calling accuracy (>98%). We discuss in detail the pitfalls researchers should avoid in order to reliably genotype complex multigene systems.

Gillingham MAF, Montero BK, Wilhelm K, Grudzus K, Sommer S, Santos PSC (2021). A novel workflow to improve genotyping of multigene families in wildlife species: an experimental set-up with a known model system. *Molecular Ecology Resources*, 21, 982-998.

Can the estimation of core gut microbiomes be standardized across studies?

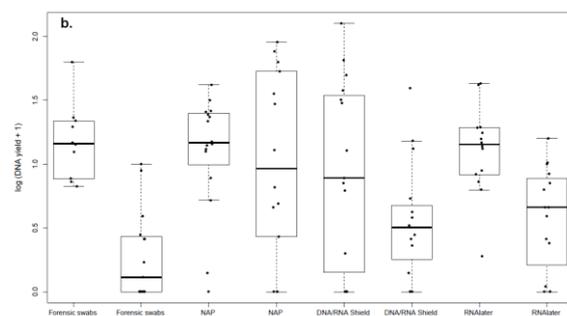
The filtering of faecal microbial datasets to retain high prevalence taxa is often performed to identify a common core microbiome that may be important for host biological functions. The common core gut microbiome is the subset of gut microbes that are particularly common across the host population, and are often identified based on prevalence (e.g. microbes that occur in over 50% of sampled individuals). However, these thresholds - though frequently used to compare results across studies and species - vary substantially across studies, and it is unclear whether gut microbial communities sourced from different host species demonstrate universal or species-specific responses to increasing prevalence thresholds. This has consequences for how comparable studies of core microbiomes are. We compared macroecological patterns in prevalence and abundance of eight gut microbial datasets from different mammal and bird species, and tested the effect of increasing prevalence thresholds on eight measures of alpha and beta diversity. Our results highlight some critical differences in macroecological patterns of rare taxa across the different datasets that can guide future sample collection and sample size, yet show that despite these differences, all microbial communities demonstrated similar responses in alpha diversity and beta dissimilarity to increasing prevalence thresholds. This suggests that most gut microbial communities exhibit similar patterns in prevalence and abundance distributions, especially of dominant taxa. Based on our results, we recommend methods that increase comparability of studies that identify a common core.



Risely A, Gillingham MAF, Béchet A, Brändel S, Heni A, Heurich M, Menke S, Manser M, Schmid J, Tschapka M, Wasimuddin, Sommer S (2021) Phylogeny- and abundance-based metrics allows for consistent comparison of core gut microbiome diversity indices across host species. *Frontiers in Microbiology*, 12, 659918. doi: 10.3389/fmicb.2021.659918

Home-made cost-effective preservation buffer stands the test against commercial preservation methods for microbiome research

The investigation of wildlife gastrointestinal microbiomes is a growing field in microbial ecology and conservation. Such studies often face difficulties in sample preservation if neither freezing facilities nor liquid nitrogen (LQN) are readily available. Thus, in order to prevent microbial community changes because of bacterial growth after sampling, preservation buffers need to be applied to samples. However, the amount of microbial community variation attributable to the different preservation treatments and potentially affecting biological interpretation is hardly known. Using sheep feces, we analyzed the effect of air-drying, an inexpensive self-made nucleic acid preservation buffer (NAP), DNA/RNA Shield™, and RNA/later®, each together with freezing or storing at room temperature prior to 16S rRNA gene high-throughput sequencing to determine bacterial communities. Overall, NAP had better preservation qualities than RNA/later® and DNA/RNA Shield™ making this self-made buffer a valuable solution in wildlife microbiome studies.



Menke S, Gillingham MAF, Wilhelm K, Sommer S (2017) Home-made cost-effective preservation buffer stands the test against commercial preservation methods for microbiome research. *Frontiers in Microbiology*, 8, 102.