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UNIVERSITÉ DE LA REUNION

Ecole Doctorale Sciences, Technologies et Santé

UMR Processus Infectieux en Milieu Insulaire Tropicale (PIMIT)

Université de La Réunion/INSERM1187/CNRS9192/IRD249

THÈSE

Pour obtenir le grade de

Docteur de l'Université de La Réunion

Disciplines : Ecologie, Santé et Environnement

Rôle de l'écologie et des dynamiques de populations du Petit Molosse de La Réunion dans la circulation d'agents infectieux

Par

Samantha AGUILLON

Soutenue publiquement le 15 décembre 2023 devant le jury composé de :

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The two most important days in your life are the day you are born
and the day you find out why

~ Mark Twain ~

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Résumé

Les chauves-souris sont des mammifères fascinants, tant par leur extraordinaire diversité spécifique et morphologique, que pour leur capacité à héberger et tolérer des virus qui peuvent engendrer des épidémies chez l'homme. A l'heure où de plus en plus de maladies émergent dans le monde, les processus écologiques qui influencent la circulation des agents infectieux chez les hôtes naturels, notamment les chauves-souris, demeurent mal connus. En prenant comme modèle d'étude le Petit Molosse de La Réunion (*Mormopterus francoismoutoui*) et trois de ses agents infectieux (paramyxovirus, herpesvirus et bactéries leptospires), nous avons analysé comment l'écologie et la dynamique des populations de chauves-souris influencent la transmission de leurs agents infectieux. Grâce à un suivi des populations sur le terrain, nous avons tout d'abord caractérisé la phénologie du Petit Molosse et mis en évidence des variations saisonnières marquées des effectifs dans les gîtes et de leur composition selon l'âge, le sexe et le statut reproducteur des individus. Ces résultats nous ont permis de proposer un nouveau cycle biologique pour cette espèce endémique de l'île de La Réunion. En utilisant des approches de phylogénie et de génétique des populations à partir de biopsies de l'aile, nous avons étudié et daté les événements de colonisation de l'île, et également montré la diversification *in situ* de cette espèce avec l'évolution de lignées ancestrales, co-existant aujourd'hui en sympatrie au sein d'une métapopulation. Enfin, l'analyse spatio-temporelle de l'infection, à l'échelle de la population (gîte) et des individus (grâce aux recaptures d'individus), suggère que les paramyxovirus et bactéries leptospires (excrétés dans les urines) ainsi que les herpesvirus (excrétés dans la salive), établissent une infection persistante avec une excrétion intermittente. Nos analyses mettent également en évidence que la reproduction, notamment la gestation chez les femelles et l'accouplement chez les mâles, ainsi que l'âge et les interactions entre agents infectieux, sont des déterminants majeurs des dynamiques saisonnières d'excrétion. Ainsi, l'approche éco-épidémiologique utilisée dans cette thèse apporte des informations précieuses pour mieux prédire, par la suite, comment les chauves-souris répondent aux perturbations des écosystèmes et les conséquences en cascade sur l'infection et les risques d'émergence de zoonoses.

Mots-clés : chauve-souris, île tropicale, phénologie, structure génétique, dynamique d'infection

Abstract

Bats are fascinating mammals because of their great specific and morphological diversity, as well as for their ability to harbour and tolerate viruses that can cause epidemics in humans. Nowadays, an increasing number of diseases are emerging around the world and the ecological processes influencing the circulation of infectious agents in natural hosts, like bats, remain poorly understood. Using the Reunion free-tailed bat (*Mormopterus francoismoutoui*) and three of its infectious agents (paramyxovirus, herpesvirus and *Leptospira* bacteria) as a study model, we analysed how the ecology and population dynamics of bats influence the transmission of infectious agents. Based on field monitoring of bat populations, we first characterized the phenology of the Reunion free-tailed bat and highlighted marked seasonal variations of roost size and of composition according to age, sex and reproductive status. These results allowed us to propose a new reproductive cycle for this species endemic to Reunion Island. Using phylogeny and population genetics based on wing punch samples, we studied and dated the colonization events of the island, and also showed the *in situ* diversification of this species with the evolution of ancestral lineages, co-existing nowadays in sympatry within a metapopulation. Finally, the spatio-temporal analysis of the infection at the scale of the population (roosts) and individuals (via bat recaptures) suggests that paramyxoviruses and *Leptospira* bacteria (excreted in urine), and herpesviruses (excreted in saliva), all establish persistent infections with intermittent excretion. Our analyses highlight that reproduction in females (gestation), but also in males (mating), as well as age and the interactions between infectious agents, are major determinants of seasonal excretion dynamics. Thus, the eco-epidemiological approach used here provides valuable information to better predict, in the future, how bats respond to ecosystem disturbances and the cascading consequences on infections and the risks of emerging zoonoses.

Keywords: bats, tropical island, phenology, genetic structure, infection dynamic

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Liste des publications scientifiques

Article 1 :

Aguillon S., Le Minter G., Lebarbenchon C., Hoarau A.O.G., Toty C., Joffrin L., Ramanantsalama R.V., Augros S., Tortosa P., Mavingui P. & Dietrich M. 2023. A population in perpetual motion: Highly dynamic roosting behaviour of a tropical island endemic bat. *Ecology and Evolution*, 13(2), e9814. <https://doi.org/10.1002/ece3.9814>

Article 2 :

En révision dans le journal *Global Ecology and Conservation*.

Disponible en pré-print : <https://doi.org/10.1101/2023.06.22.546033>

Aguillon S., Castex C., Duchet A., Turpin, M., Le Minter G., Lebarbenchon C., Hoarau A.O.G., Toty C., Joffrin L., Tortosa P., Mavingui P., Goodman S.M. & Dietrich M. Stuck on a small tropical island: wide *in-situ* diversification of an urban-dwelling bat.

Article 3 :

En préparation. Journal visé : *PNAS*.

Aguillon S., Turpin M., Le Minter G., Lebarbenchon C., Hoarau A.O.G., Toty C., Duchet A., Joffrin L., Ramanantsalama R.V., Tortosa P., Mavingui P. & Dietrich M. Fine-scale spatio-temporal monitoring of an island endemic bat reveals how individual and population heterogeneity shapes triple co-excretion dynamics.

Article 4 :

En préparation. Journal visé : *Biology Letters*.

Aguillon S., Turpin M., Le Minter G., Lebarbenchon C., Hoarau A.O.G., Mavingui P. & Dietrich M. Bat things comes in threes: within-host dynamics of herpesvirus triple infection in bats.

Liste des annexes

Annexe 1 :

Matériels supplémentaires pour l'article 1.

Annexe 2 :

Matériels supplémentaires pour l'article 2.

Annexe 3 :

Matériels supplémentaires pour l'article 3.

Annexe 4 :

Matériels supplémentaires pour l'article 4.

Annexe 5 :

Résumé de l'article en préparation :

Toshkova N., Aguillon S., Turpin M., Lebarbenchon C. & Dietrich M. How urban-dwelling bats cope with urbanization? Sex-dependent plasticity of stress, microbiome and pathogen excretion.

Lexique

ADN	Acide désoxyribonucléique
EBLV	Lyssavirus de chauve-souris européenne
HSV	Herpesvirus
LEPTO	Bactéries <i>Leptospira</i> (<i>i.e.</i> bactéries leptospires)
MERS	Syndrome Respiratoire du Moyen-Orient
OMS	Organisation Mondiale de la Santé
RABV	Virus de la rage
SIR	Sensible - Infectieux - Rétablis
SIRS	Sensible - Infectieux - Rétablis - Sensible
SILI	Sensible - Infectieux - Latent - Infectieux
SNP	Polymorphisme nucléotidique
SRAS	Syndrome Respiratoire Aigu Sévère
PMV	Paramyxovirus
UICN	Union Internationale pour la Conservation de la Nature
VIH	Virus de l'Immunodéficience Humaine

Introduction générale

I. L'émergence de maladies infectieuses

1. Histoire et impacts des pandémies

Historiquement, l'émergence de maladies infectieuses est un phénomène sporadique qui se produit depuis des siècles et qui reste difficile à prédire, mais avec d'importantes conséquences pour la santé globale, y compris humaine. Lorsqu'une épidémie touche une grande partie de la population et s'étend sur un continent ou à l'échelle mondiale, on parle alors de pandémie. Une des plus grande pandémie humaine reportée dans l'histoire de l'Europe est celle de la peste noire au 14^{ème} siècle. Cette pandémie débuta avec l'arrivée d'un navire qui transportait à son bord des rats porteurs de puces infectées par la bactérie *Yersinia pestis*, responsable de la peste. La peste noire se propagea alors rapidement dans l'Europe où elle fit environ 25 millions de morts (Glatter & Finkelman, 2021). Plusieurs siècles plus tard, en 1918-1920, la grippe espagnole due au virus *Influenza A H₁N₁*, sera responsable d'une pandémie qui provoquera entre 30 et 50 millions de morts (Johnson & Mueller, 2002).

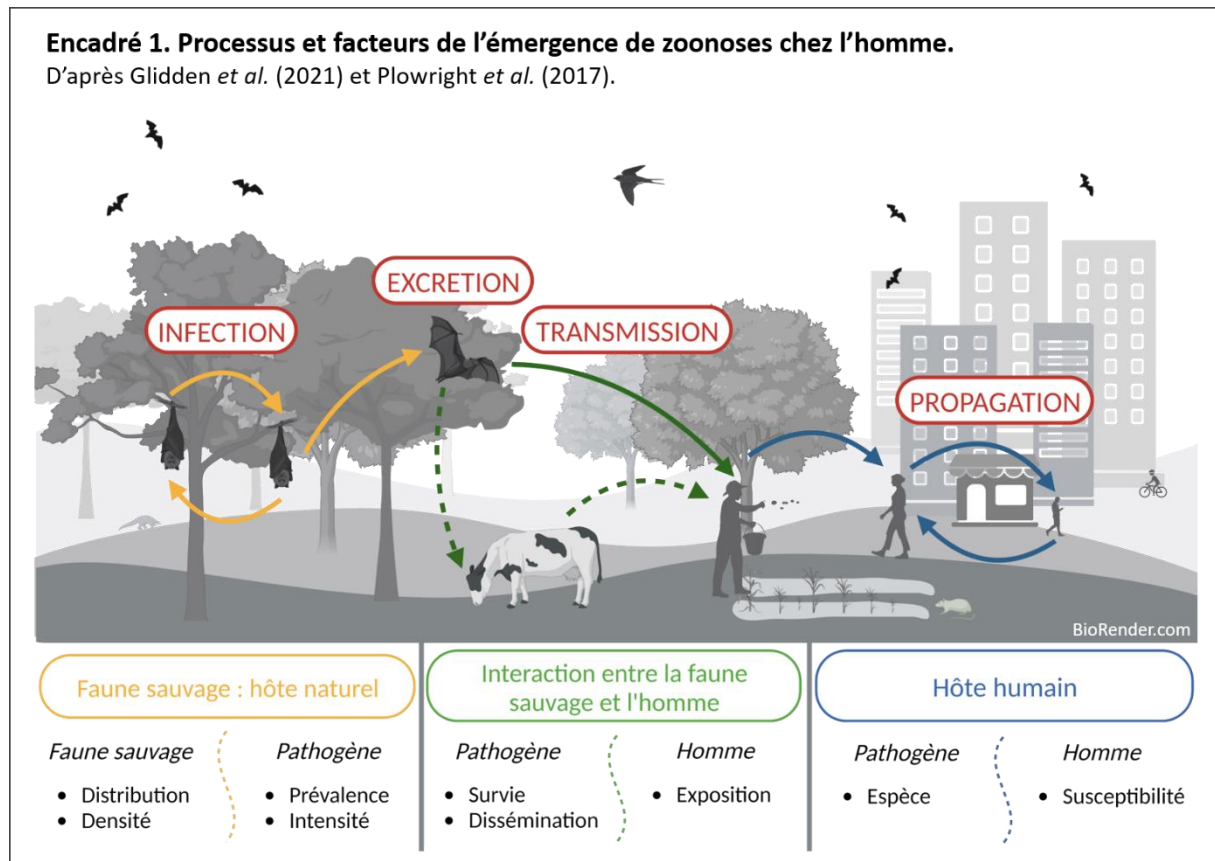
Plus récemment, l'apparition de nouveaux virus tels que le SARS-CoV (2003) et le MERS-CoV (2012) n'ont cessé d'alerter sur les risques d'apparition de virus de plus en plus virulent (Imperato, 2016 ; Mathis *et al.*, 2015 ; World Health Organization, 2023a). Ceci est également vrai lorsqu'une augmentation en fréquence et en incidence des épidémies de virus déjà connus se produit, comme pour celles dues aux virus Zika, Ebola ou Marburg (dont la dernière épidémie s'est terminée en juin dernier). Ainsi, en 2019, l'émergence du nouveau coronavirus SARS-CoV-2 qui provoque la maladie Covid-19, a bouleversé la santé humaine au niveau mondial. En effet, selon l'Organisation Mondiale de la Santé (OMS), plus de 760 millions de cas positifs ont été détectés en 2023, depuis son émergence en décembre 2019, avec un taux de mortalité de 9% (World Health Organization, 2023b). En plus d'avoir un impact important sur la santé humaine, tant physique que mentale (Kontoangelos *et al.*, 2020), la pandémie Covid-19 a eu de graves conséquences économiques, obligeant les pays à mettre en place des politiques nouvelles afin de réduire la propagation du virus (Sarkodie & Owusu, 2021). Au-delà des impacts directs pour la population humaine, les pandémies peuvent également toucher la santé animale comme l'atteste celle due au virus *Influenza A H₅N₁*, qui provoque la grippe aviaire et circule actuellement dans de nombreuses régions du monde (Charostad *et al.*, 2023). De nombreux foyers du virus H₅N₁ ont été identifiés chez les volailles domestiques et les

oiseaux sauvages, ce qui a entraîné l'abattage de plusieurs millions d'oiseaux d'élevage et causé des événements de mortalité de masse dans l'avifaune sauvage, mais aussi chez des centaines de mammifères (phoques, otaries, visons) (Charostad *et al.*, 2023 ; Venkatesan, 2023).

2. Processus et facteurs de l'émergence des zoonoses

Plus de la moitié des maladies émergentes infectieuses sont zoonotiques, c'est-à-dire qu'elles peuvent se transmettre entre les animaux et l'homme, et la majorité de ces zoonoses (plus de 70%) provient de la faune sauvage (Jones *et al.*, 2008). Ainsi, les espèces d'animaux sauvages sont des hôtes naturels d'agents infectieux potentiellement pathogènes pour l'homme. Pourtant, en comparaison de la quantité d'agents infectieux circulant dans la faune sauvage, l'émergence de zoonoses reste un événement rare. En effet, les mécanismes qui conduisent à l'émergence de zoonoses font intervenir une série d'événements hiérarchiques qui doivent être franchis pour qu'un agent infectieux puisse passer la barrière et être transmis depuis la faune sauvage jusqu'à l'homme (Encadré 1) (Glidden *et al.*, 2021 ; Plowright *et al.*, 2017). Au départ de cette cascade d'événements se trouve la dynamique d'infection chez l'hôte naturel et l'excrétion d'agents infectieux qui dépendent de la distribution et de la densité de l'hôte, mais également de la prévalence et intensité d'excrétion de l'agent infectieux (en jaune dans l'encadré 1) (Plowright *et al.*, 2017). Pour qu'il y ait une transmission vers d'autres espèces, il faut qu'il y ait une interaction entre la faune sauvage et l'homme, c'est-à-dire une exposition à l'agent infectieux (en vert dans l'encadré 1). Différents types d'exposition existent : directe, via des fluides corporels contaminés de l'hôte naturel (salive, urine, fèces, sang), ou indirecte via une contamination environnementale (eau, terre), un arthropode vecteur (moustique, tique, puce) ou un hôte intermédiaire de la faune sauvage ou domestique (Joffrin *et al.*, 2018). Ainsi, la transmission de l'agent infectieux dépend fortement de sa survie et dissémination à l'extérieur de l'hôte naturel, mais aussi du comportement de l'homme (Glidden *et al.*, 2021 ; Plowright *et al.*, 2017). Enfin, pour qu'il y ait propagation de l'agent infectieux et apparition d'une épidémie, la dernière étape consiste au passage des barrières génétiques, physiologiques et immunologiques propre à l'hôte humain (ou susceptibilité) qui interagissent étroitement avec l'espèce de pathogène (en bleu dans l'encadré 1) (Plowright *et al.*, 2017). Au fil du temps évolutif, une circulation exclusive chez l'homme, indépendante de son hôte initial,

peut se mettre en place, comme illustrée avec l'exemple du VIH-1 (Virus de l'Immunodéficience Humaine) dont la forme simienne du virus a dérivé pour s'adapter à son hôte humain (Sharp & Hahn, 2011).



Plusieurs études rapportent que la multiplication des zoonoses observée ces dernières années est en très grande partie due aux perturbations des écosystèmes induites par les activités humaines. Ce processus d'anthropisation se traduit par un bouleversement des interactions naturelles entre la faune sauvage et leurs agents infectieux ainsi qu'une augmentation des contacts entre la faune sauvage, les animaux domestiques et les populations humaines, ce qui peut favoriser le passage d'agents infectieux chez l'homme (Bradley & Altizer, 2007 ; Ellwanger *et al.*, 2022 ; Glidden *et al.*, 2021 ; Mishra *et al.*, 2021 ; Patz *et al.*, 2004). Par exemple, en Malaisie, l'intensification des fermes d'élevages de porcs a eu pour conséquence le rapprochement des populations de chauves-souris du genre *Pteropus* et celles des porcs, qui servent d'hôtes intermédiaires au virus Nipah (famille des paramyxovirus), avant de se transmettre aux humains (Epstein *et al.*, 2008). De plus, la perte de biodiversité engendrée par les activités humaines peut affecter la transmission naturelle des agents infectieux dans les communautés d'espèces sauvages, en modifiant par exemple l'abondance

et le comportement des hôtes (Ellwanger *et al.*, 2022 ; Glidden *et al.*, 2021 ; Keesing *et al.*, 2010). Par exemple, la prévalence en hantavirus est plus importante dans les communautés de rongeurs moins diversifiées en espèces, potentiellement du fait que la probabilité de rencontre entre un individu infecté et un individu susceptible est plus grande (Suzán *et al.*, 2009). De plus en plus d'études démontrent que les changements climatiques peuvent également favoriser l'émergence de zoonoses (Ellwanger *et al.*, 2022 ; Glidden *et al.*, 2021 ; Short *et al.*, 2017 ; Sipari *et al.*, 2022). Par exemple, au Québec, l'augmentation des cas de maladie de Lyme serait liée à l'expansion de l'aire de répartition de la tique, vectrice de la bactérie, à cause de la hausse des températures qui rapprocherait les populations de tiques et les populations humaines (Germain *et al.*, 2019).

Cette pression anthropique sur les écosystèmes étant en constante augmentation, il est très probable que l'émergence de zoonoses s'intensifient à l'avenir (Jones *et al.*, 2013). Outre la mise en place de mesures post-épidémiques (protocole sanitaire, nouveau vaccin), la gestion efficace des zoonoses dépend donc aussi de l'avancée des connaissances écologiques et épidémiologiques sur les facteurs clés à l'origine des émergences.

II. Zoonoses et chauves-souris : une relation intrigante

1. Ecologie des chauves-souris

Les chauves-souris, de l'ordre des Chiroptères, possèdent la deuxième plus grande richesse spécifique chez les mammifères (après les rongeurs), avec plus de 1470 espèces décrites à ce jour (Simmons & Cirranello, 2023). Elles sont si diversifiées qu'elles représentent presque un quart de toutes les espèces de mammifères réunies. Ce sont aussi les seuls mammifères qui possèdent la capacité de voler, ce qui leur a permis de coloniser l'ensemble des continents, à l'exception des régions polaires, depuis environ 52,5 millions d'années (Simmons *et al.*, 2008). Les chauves-souris occupent ainsi des gîtes dans des habitats naturels très variés, comme des arbres ou des grottes, mais également de nature anthropique comme des constructions humaines (pont, bâtiment, cave souterraine). Au travers de ces différents modes de vie, les chauves-souris arborent une diversité remarquable tant morphologique que de par leur régime alimentaire. En effet, certaines espèces se nourrissent d'insectes (insectivores) ou de poisson (piscivores), d'autres de fruits (frugivores), de nectar de fleurs (nectarivores) ou

même de sang (hématophages). Grâce à ces régimes alimentaires très variés, les chauves-souris jouent un rôle crucial dans les écosystèmes, en fournissant des services écosystémiques tels que la pollinisation, la dispersion des graines et la régulation des populations d'arthropodes parfois ravageurs de cultures (Kunz *et al.*, 2011).

Pour permettre le vol, de nombreuses adaptations morphologiques et métaboliques ont été sélectionnées au cours de l'évolution des Chiroptères. L'une de ces adaptations est l'allongement des os des membres antérieurs reliés entre eux par une membrane de peau fine (patagium), qui constituent les ailes (Rubalcaba *et al.*, 2022). Le vol actif chez les chauves-souris a également un coût énergétique extrêmement élevé, qui demande d'augmenter considérablement le métabolisme pour fournir l'énergie suffisante. Cependant, cela génère des quantités considérables de déchets métaboliques qui peuvent endommager les cellules et induire une entrée en sénescence prématurée (Passos & von Zglinicki, 2005). L'évolution de mécanismes physiologiques conduisant à la réduction du stress oxydatif permettrait aux chauves-souris d'empêcher les dommages cellulaires trop importants et de retarder la mort cellulaire (Brook & Dobson, 2015). De plus, en abaissant leur température corporelle et rythme cardiaque pendant les phases d'hibernation saisonnières ou de torpeur diurne, les chauves-souris sont capables de réduire significativement leur activité métabolique (Wilkinson & South, 2002). Ces caractéristiques pourraient expliquer que, bien qu'elles soient souvent de petite taille, les chauves-souris possèdent une longévité exceptionnelle en comparaison des autres mammifères (Brunet-Rossinni & Austad, 2004). Par exemple, le Murin de Brandt (*Myotis brandtii*) peut vivre jusqu'à 41 ans alors qu'il ne pèse que 4 à 8 grammes (Podlutsky *et al.*, 2005).

Certaines espèces de chauves-souris sont grégaires et forment parmi les plus grandes colonies connues chez les mammifères (partie « instinct grégaire » dans l'encadré 2). Celles-ci, de nature très hétérogène en fonction du nombre d'individus, du sexe, de l'âge et du statut reproducteur des individus, permettent de remplir différentes fonctions biologiques, comme avec les gîtes d'hibernation ou de maternité (Kunz & Fenton, 2005). De nombreuses espèces de chauves-souris utilisent ainsi un réseau de gîtes au cours de leur cycle biologique, et effectuent de nombreux mouvements intra- et inter saisons (Johnson *et al.*, 2015 ; Moussy *et al.*, 2013). Certaines espèces peuvent même migrer sur de longues distances afin de rejoindre leurs gîtes saisonniers (partie « migration » dans l'encadré 2). De plus, ces espèces se

reproduisent souvent avant l'hiver et emploient des mécanismes d'interruption du cycle de reproduction (stockage des spermatozoïdes, implantation différée de l'embryon) jusqu'à l'arrivée de l'été (Racey & Entwistle, 2000). Ensuite, à la saison chaude, les femelles en particulier, se regroupent au sein de gîtes de maternité pour la mise-bas et l'élevage des nouveau-nés. Ces comportements saisonniers d'agrégation s'accompagnent très souvent d'une ségrégation spatiale entre sexes, puisque les mâles sont peu présents dans les gîtes de maternité (partie « ségrégation sexuelle » dans l'encadré 2) (Altringham & Senior, 2006). Cela peut s'expliquer par une compétition pour les ressources ou la recherche de conditions particulières pour la mise-bas (température, hygrométrie) (Lourenço & Palmeirim, 2004 ; Sedgeley, 2001). Plusieurs études ont également mis en évidence une forte philopatrie des individus, c'est-à-dire qu'ils ont tendance à rester ou revenir à l'endroit où ils sont nés pour se reproduire. Ceci est particulièrement vrai chez les femelles qui peuvent ré-utiliser les mêmes gîtes de maternité d'années en années, comme chez le Murin de Bechstein (*Myotis bechsteinii*) où une quasi-stricte philopatrie des femelles a été observée (Kerth *et al.*, 2002). Malgré un certain nombre de suivis des populations sur le terrain, associé à des approches de capture-marquage-recapture et des outils génétiques, nos connaissances sur la complexité des

Encadré 2. Exemples illustrant les caractéristiques écologiques particulières des chauves-souris

Instinct grégaire



© Jennifer Whitney, The Texas Tribune

La *Bracken Cave* est une grotte située au Texas aux Etats-Unis, qui abrite la plus grande colonie de chauves-souris au monde. C'est plus de 20 millions de Molosses du Brésil (*Tadarida brasiliensis*) qui y dorment chaque jour (McCracken, 1989).

Migration



© Jorrit Vlot, Kasanka, Zambia

La migration annuelle de la Roussette paillée africaine (*Eidolon helvum*) est un phénomène spectaculaire. C'est entre 5 et 10 millions de chauves-souris qui peuvent parcourir plus de 2000 km afin de rejoindre le Parc National Kasanka en Zambie, pour trouver de la nourriture (Richter & Cumming, 2005, 2008).

Ségrégation sexuelle



© Gilles San Martin, Namur, Belgium

Les femelles du Murin de Bechstein (*Myotis bechsteinii*) forment des colonies de maternité exclusivement composées de femelles. Au sein de ces colonies, elles vont également se séparer en sous-groupes selon leur statut reproducteur (allaitante ou non) (Kerth & König, 1999).

comportements de fission-fusion chez les chauves-souris et le fonctionnement de ces métapopulations restent encore limitées (Moussy *et al.*, 2013 ; Rossiter *et al.*, 2012 ; Russo *et al.*, 2005).

Les chauves-souris sont des espèces dont la conservation représente un enjeu important puisque 15% sont considérées menacées selon l'Union Internationale pour la Conservation de la Nature (UICN). Aucune donnée n'est disponible pour 18% d'entre elles, alarmant sur le besoin de mettre en place des plans de conservation adaptés et de promouvoir la recherche sur les chauves-souris (Frick *et al.*, 2019). Par exemple, l'utilisation de pesticides et d'insecticides provoque la raréfaction de nombreux insectes dont les chauves-souris insectivores se nourrissent, réduisant ainsi la capacité alimentaire du milieu et pouvant entraîner jusqu'à la mort des individus (O'Shea & Clark, 2002). Les activités humaines telles que l'urbanisation ou l'agriculture entraînent la perte et la fragmentation des habitats des chauves-souris et même si certaines espèces arrivent à s'adapter, d'autres moins résilientes peuvent subir un déclin de leur population (Jung & Threlfall, 2018). De plus, la fragmentation des habitats via la construction de route et de parc éolien entraîne de fréquentes collisions menant à la mortalité des chauves-souris (Fensome & Mathews, 2016 ; Kuvlesky *et al.*, 2007). Des agents pathogènes peuvent également nuire à la santé des chauves-souris, comme par exemple le champignon *Pseudogymnoascus destructans* qui provoque le syndrome du museau blanc, et entraîne des événements de mortalité de grande ampleur dans les colonies de chauves-souris américaines pendant l'hibernation (Hoyt *et al.*, 2021). En France, des mesures de conservation sont mises en place afin de protéger ces animaux indispensables à l'équilibre des écosystèmes, avec par exemple l'article L. 411-1 du code de l'environnement qui classe toutes les chauves-souris françaises comme espèces protégées.

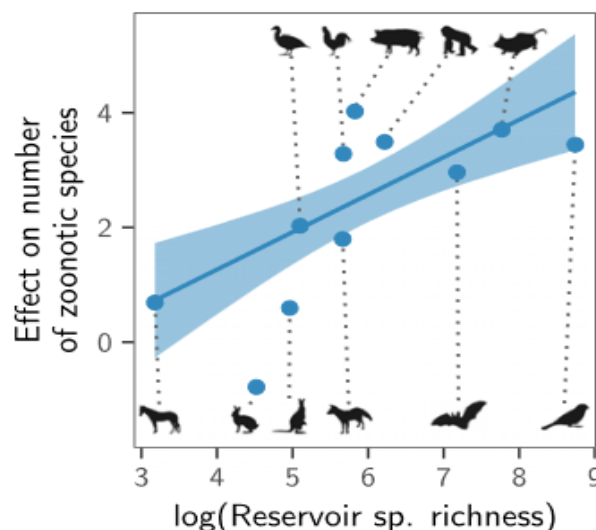
2. Les chauves-souris : des hôtes naturels d'agents infectieux zoonotiques

Les chauves-souris fascinent également les chercheurs pour leur capacité à héberger et tolérer des virus qui peuvent engendrer des maladies graves chez d'autres espèces, y compris l'homme (Brook & Dobson, 2015). C'est par exemple le cas pour les *Lyssavirus* responsables de la rage qui semblent peu impacter la survie des chauves-souris (Amengual *et al.*, 2007 ; O'Shea *et al.*, 2014a ; Robardet *et al.*, 2017), mais qui sont quasiment fatals chez d'autres espèces de vertébrés dont l'homme. Cette tolérance aux agents infectieux serait liée à leur

capacité unique à voler, qui aurait favorisé l'apparition au cours de l'évolution, de mécanismes de réparation de leurs tissus, limitant ainsi le développement d'une inflammation incontrôlée et une meilleure tolérance aux infections (O'Shea *et al.*, 2014b).

Les chauves-souris sont en effet porteuses d'une grande diversité d'agents infectieux, celle-ci étant expliquée par l'importante richesse spécifique des hôtes chiroptères (Figure 1) (Mollentze & Streicker, 2020). On retrouve par exemple des bactéries (*Pasteurella*, *Salmonella*, *Escherichia* et *Yersinia*) (Mühldorfer, 2013), des nématodes (Santos & Gibson, 2015), des endoparasites (*Trypanosoma*, *Plasmodium*) (Hamilton *et al.*, 2012 ; Schaer *et al.*, 2013) et une grande diversité de virus (Chen *et al.*, 2014). Certains de ces virus sont à l'origine d'épidémies chez l'homme (Figure 2), comme par exemple la maladie à virus Marburg (famille des filovirus) qui a encore sévit en Afrique en 2023 et qui a pour origine directe des chauves-souris frugivores du genre *Rousettus* (Towner *et al.*, 2009). Les chauves-souris sont également des espèces réservoirs d'une grande diversité de coronavirus, dont certains sont à l'origine des épidémies de SRAS (Syndrome Respiratoire Aigu Sévère, virus SARS-CoV) et MERS (Syndrome Respiratoire du Moyen-Orient, virus MERS-CoV) (Krishnamoorthy *et al.*, 2020). Bien que cela soit encore débattu, le virus du SARS-CoV-2 pourrait également avoir comme origine les chauves-souris (Krishnamoorthy *et al.*, 2020).

Figure 1. Relation entre la richesse spécifique de différents groupes d'animaux (représenté en log) et



le nombre de virus zoonotiques qu'ils hébergent. La ligne centrale correspond au modèle de régression linéaire, et est entourée de l'intervalle de confiance à 95% (Mollentze & Streicker, 2020).

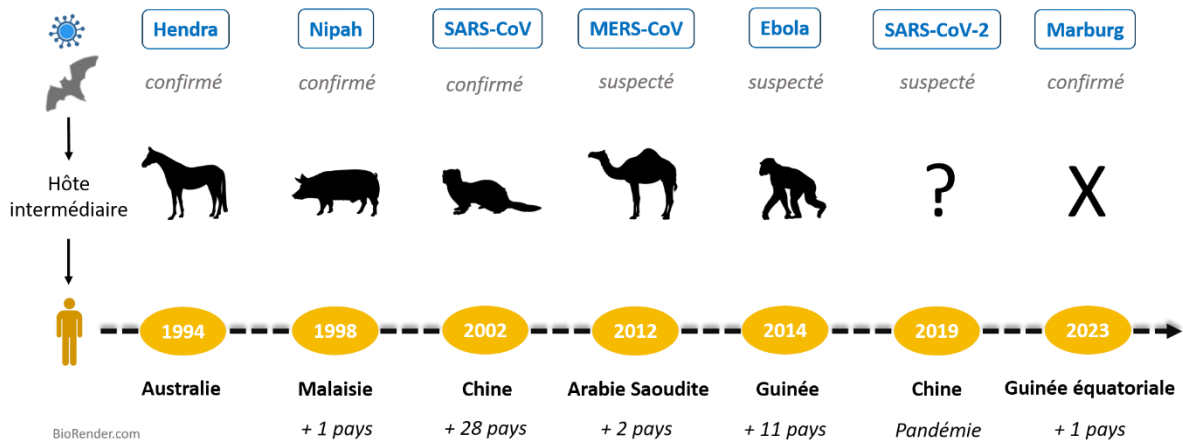


Figure 2. Exemples de maladies zoonotiques dont l'origine confirmée, ou suspectée, est la chauve-souris.

Parmi la diversité d'agents infectieux hébergée par les chauves-souris, je me suis intéressée aux paramyxovirus, aux bactéries leptospires et aux herpesvirus, du fait de leur différences structurales (virus à ARN/ADN, bactéries), de leur mode de transmission variés, et de leur potentiel zoonotique, que je détaille dans les paragraphes suivants.

Les paramyxoviridae

Les Paramyxoviridae (ou paramyxovirus) sont des virus enveloppés à ARN simple brin de polarité négative, non segmentés, d'une taille allant de 15 à 19 kb (Figure 3a). Cette large famille de virus est répartie en deux sous-familles : les Paramyxovirinae (Genre : *Respirovirus*, *Rubulavirus*, *Avulavirus*, *Morbilivirus*, *Henipavirus*) et les Pneumovirinae (Genre : *Pneumovirus*, *Metapneumovirus*) (Maclachlan & Dubovi, 2010). Les paramyxovirus infectent des hôtes vertébrés tels que les poissons, les oiseaux ou les mammifères, y compris l'homme. Globalement, les paramyxovirus de chauves-souris appartiennent aux genres : *Pneumovirus*, *Rubulavirus*, *Henipavirus* et *Morbivilirus* (Figure 3b). Drexler et collaborateurs (2012) suggèrent que l'hôte ancestral des paramyxovirus serait la chauve-souris, avec une proportion importante d'échange de virus, des chauves-souris vers d'autres mammifères et oiseaux. En effet, les virus à ARN semblent avoir une capacité plus importante à changer d'hôte, en raison de leur taux de mutation élevé qui facilite leur adaptation au sein de nouveaux hôtes (Holmes, 2009).

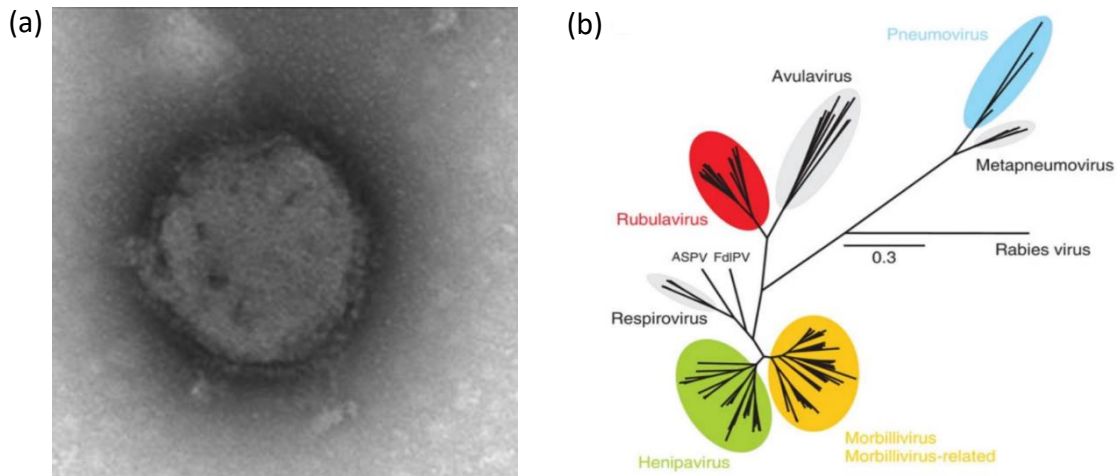


Figure 3. Diversité des paramyxovirus retrouvés chez les chauves-souris. (a) Particule de paramyxovirus-like, observé sous microscope électronique, identifié chez une chauve-souris de Madagascar (*Miniopterus griveaudi*) (Wilkinson *et al.*, 2012). (b) Phylogénie en maximum de vraisemblance reconstruite à partir du gène L des paramyxovirus. Les genres contenant des paramyxovirus de chauves-souris sont colorés en bleu, rouge, vert et jaune (Drexler *et al.*, 2012).

Les paramyxovirus représentent depuis plusieurs années une source d'inquiétude à cause des épidémies récurrentes des virus Nipah et Hendra (genre : *Henipavirus*), qui causent des symptômes respiratoires et neurologiques chez l'homme, et la mort dans 40% à 75% des cas, avec aucun vaccin disponible à ce jour (Centers for Disease Control and Prevention, 2022 ; World Health Organization, 2018). En 2012, un nouveau paramyxovirus, le virus Sosuga (genre : *Pararubulavirus*), a également causé des symptômes graves chez une biologiste travaillant sur les Roussettes d'Égypte (*Rousettus aegyptiacus*), en Uganda et Soudan du Sud, qui en sont les hôtes naturels (Amman *et al.*, 2015). Les virus Nipah et Hendra ont pour réservoir les chauves-souris du genre *Pteropus* (Chua *et al.*, 2002 ; Halpin *et al.*, 2000). La transmission à l'homme peut faire intervenir des hôtes intermédiaires comme le porc, pour le virus Nipah, et le cheval dans le cas du virus Hendra. Bien que les premières épidémies de Nipah et Hendra aient été identifiées dans les années 1990, des cas sont toujours reportés à ce jour, avec par exemple huit décès dues au virus Nipah au Bangladesh, entre le 4 janvier et le 13 février 2023 (World Health Organization, 2023c).

Les bactéries leptospires

Les bactéries *Leptospira*, de l'ordre des Spirochaetales, sont des bactéries Gram négatives, aérobies et hélicoïdales, et possèdent un génome de 3,9 à 4,6 Mb (Figure 4a). A ce jour, 64 espèces ont été identifiées et sont classées dans trois groupes selon leur pathogénicité : pathogènes, intermédiaires et saprophytes (Vincent *et al.*, 2019). Certaines de ces bactéries peuvent causer la leptospirose, zoonose bactérienne considérée comme la plus répandue au monde mais qui reste largement négligée (Bharti *et al.*, 2003). On estime à plus de 500 000 le nombre de cas sévères par an dans le monde, avec un taux de mortalité pouvant excéder les 10% (Abela-Ridder *et al.*, 2010). L'incidence chez l'homme est particulièrement élevée dans les îles tropicales (Pappas *et al.*, 2008). Une grande diversité d'animaux sauvages et domestiques peut être infectée et excréter les leptospires dans les urines, contaminant ainsi l'environnement. La transmission à l'homme se fait essentiellement via des eaux contaminées, ou par un contact direct avec un animal infecté (Bharti *et al.*, 2003). Les rongeurs, et particulièrement les rats qui sont porteurs sains, ont un rôle majeur dans l'épidémiologie de cette maladie (Mwachui *et al.*, 2015 ; Perez *et al.*, 2011 ; Zenebe *et al.*, 2013). Les chauves-souris sont également des espèces hôtes de ces bactéries, avec au moins quatre espèces détectées : *L. interrogans*, *L. borgpetersenii* et *L. kirschneri* considérées comme pathogènes et *L. fainei* appartenant au groupe intermédiaire (Figure 4b) (Dietrich *et al.*, 2015a). Cependant, aujourd'hui aucune transmission des leptospires de chauves-souris à l'homme n'a été reportée (Dietrich *et al.*, 2015a).

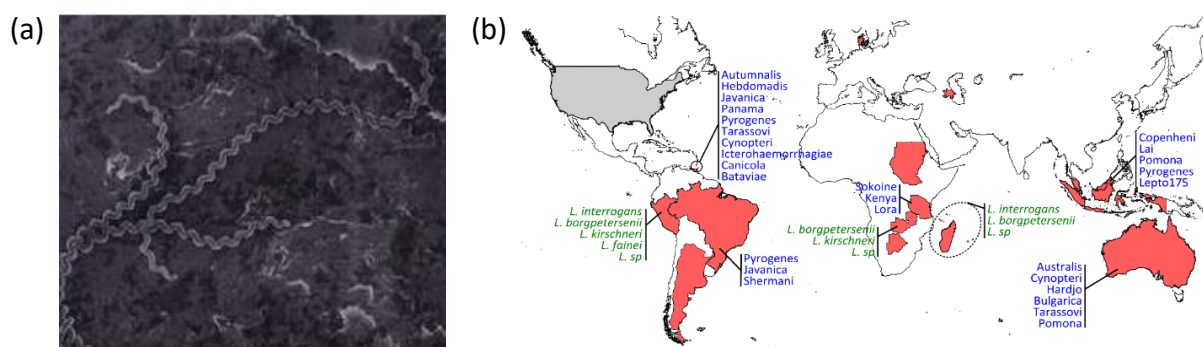


Figure 4. Diversité des bactéries leptospires. (a) Observation au microscope électronique de *L. interrogans*, serovar copenhageni (Bharti *et al.*, 2003). (b) Distribution géographique des leptospires chez les chauves-souris : les pays sont en rouge quand des résultats positifs ont été détectés, et en gris quand ce sont des résultats négatifs. La diversité des leptospires est indiquée en vert quand les données génétiques ont été utilisées pour l'identification de l'espèce, et en bleu quand ce sont des données sérologiques qui ont servies à l'identification de serovars (Dietrich *et al.*, 2015a).

Les Herpesviridae

La famille des Herpesviridae (ou herpesvirus) comporte des virus à ADN double brin, d'une taille de 125 à 290 kb (Figure 5), et sont classés en trois sous-familles : les Alphaherpesvirinae (Genres : *Simplexvirus*, *Varicellovirus*, *Mardivirus*, *Iltovirus*), les Betaherpesvirinae (Genres : *Cytomegalovirus*, *Muromegalovirus*, *Proboscivirus*, *Roseolovirus*) et les Gammaherpesvirinae (*Lymphocryptovirus*, *Macavirus*, *Percavirus*, *Rhadinovirus*) (Maclachlan & Dubovi, 2010). Les Herpesviridae peuvent infecter une grande variété d'hôtes vertébrés incluant les reptiles, les oiseaux et les mammifères, y compris l'homme (Maclachlan & Dubovi, 2010). Ils sont beaucoup étudiés chez l'homme en raison de la forte prévalence dans la population, avec par exemple environ 67% des moins de 50 ans porteurs de virus Herpès simplex de type 1 (HSV-1) (World Health Organization, 2023d). Leur forte infectiosité serait due à leur capacité à rester latent dans l'organisme et à se réactiver lors de période de stress physique ou mentale (Cohen, 2020 ; Grinde, 2013). Les herpesvirus ont plusieurs modes de transmission allant de la salive, à l'urine, avec certains qui sont sexuellement transmissibles ou transmis pendant la période périnatale (Maclachlan & Dubovi, 2010). Bien que la plupart du temps, les signes cliniques ne sont pas visibles, les herpesvirus peuvent provoquer des lésions vésiculaires ou ulcéreuses, en particulier chez les fœtus, nouveau-nés, immunodéprimés ou lors de sauts d'espèces (Maclachlan & Dubovi, 2010). Par exemple, le Cercopithecine herpesvirus 1 (CeHV-1) se transmet des macaques à l'homme, et tandis qu'il est asymptomatique chez son hôte naturel, il peut causer la mort chez l'homme (Tischer & Osterrieder, 2010).

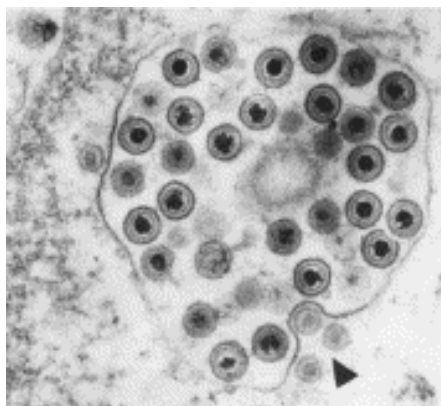


Figure 5. Observation au microscope électronique d'une cellule infectée par des particules d'herpesvirus (Maclachlan & Dubovi, 2011).

Le potentiel zoonotique des herpesvirus semble très dépendant de la sous-famille à laquelle ils appartiennent (Azab *et al.*, 2018). En effet, une étude phylogénétique suggère que la plus grande diversité en gammaherpesvirus est observée chez les primates et les chauves-souris, et que certains herpesvirus de chauves-souris aurait le potentiel de changer d'hôte mammifère (Escalera-Zamudio *et al.*, 2016). A l'inverse, l'étude de Griffiths et collaborateurs (2020) suggère une forte spécificité d'hôte des betaherpesvirus chez les mammifères bien que des exceptions soient observées. De plus en plus d'études s'intéressent aux herpesvirus de chauves-souris en tant que candidat vecteur pour un vaccin contre la rage transmissible entre chauves-souris, en raison notamment de leur forte prévalence dans la population et de leur capacité à rester latents dans l'organisme (Griffiths *et al.*, 2020, 2022, 2023).

3. Déterminants de la transmission de l'infection chez les chauves-souris

Pour certains agents infectieux, il a été montré que l'apparition d'épidémies saisonnières chez les chauves-souris (quand la prévalence de chauves-souris infectées augmente drastiquement) coïncide avec l'émergence de zoonoses chez l'homme. En effet, lors de ces périodes de forte prévalence et d'excrétion exacerbée dans les populations de chauves-souris, la transmission des agents infectieux à d'autres espèces est plus susceptible de se produire (Plowright *et al.*, 2015). C'est par exemple le cas pour les épidémies saisonnières au virus Marburg chez la Roussette d'Egypte (*R. aegyptiacus*) en Uganda et République Démocratique du Congo, où des pics d'infection sont observés pendant la période d'élevage des juvéniles et coïncident avec la transmission du virus à l'homme (Amman *et al.*, 2012). De même, pour le virus Hendra en Australie, transmis par des chauves-souris du genre *Pteropus* aux chevaux puis à l'homme, où des pics d'infection chez les chauves-souris, et les épidémies chez les chevaux et l'homme, se produisent en hiver (Becker *et al.*, 2023). C'est pourquoi, il est crucial d'étudier les dynamiques d'infection dans les populations de chauves-souris en milieu naturel afin de mieux comprendre les mécanismes de transmission à d'autres espèces.

La transmission d'agents infectieux au sein des populations de chauves-souris a historiquement été étudiée via des approches sérologiques et de modélisation, ciblant les *Lyssavirus* (virus de rage, famille des rhabdovirus) et les *Henipavirus* (famille des paramyxovirus). Plus récemment, des approches mesurant directement l'excrétion des agents infectieux (détection des acides nucléiques par PCR) s'est élargie à d'autres familles,

notamment les coronavirus. Ces études montrent que la circulation d'agents infectieux dépend de facteurs individuels (propres à l'hôte) et populationnels (entre hôtes). A l'échelle des populations, la taille et la distribution des colonies, ainsi que la connectivité entre elles (régie par la dispersion des individus et les patrons d'agrégation), peuvent influencer les contacts entre individus et donc la transmission des pathogènes (Plowright *et al.*, 2015). Cependant, selon l'espèce de chauve-souris étudiée, les résultats divergent, et soulèvent ainsi la question d'une transmission densité-dépendante, ou non, chez les chauves-souris. En effet, la séroprévalence en EBLV-1 (*European Bat Lyssavirus*) chez plusieurs espèces de chauves-souris en Espagne est positivement corrélée à la taille des colonies (Serra-Cobo *et al.*, 2013), alors qu'au Pérou, de telles corrélations ne sont pas retrouvées chez les chauves-souris vampires (*Desmodus rotundus*) infectées par le virus de la rage (RABV) (Streicker *et al.*, 2012). Chez cette dernière, une étude combinant l'analyse génétique de l'hôte et du virus suggère une transmission spatiale sexe-dépendante, avec un rôle majeur de la dispersion saisonnière des mâles qui permettraient la propagation du RABV entre des populations de femelles isolées (Streicker *et al.*, 2016).

Parmi les facteurs individuels, de nombreuses études ont montré que la structure d'âge au sein des populations de chauves-souris est un élément clé des dynamiques d'infection. Cependant, les dynamiques d'infection observées sont variables selon l'agent infectieux considéré. Par exemple, des études sans capture d'individus, consistant à collecter des échantillons biologiques (fèces, urine) sous les colonies, ont montré des prévalences en paramyxovirus et bactéries leptospires moins importantes après la naissance des juvéniles, suggérant qu'ils ne sont pas ou sont peu infectés durant les premiers mois de leur vie (Dietrich *et al.*, 2015b). La capture de Roussettes d'Égypte (*R. aegyptiacus*) a également permis de montrer que les juvéniles étaient peu infectés au virus Marburg les trois premiers mois de leur vie (Amman *et al.*, 2012). Au contraire, pour les coronavirus, des études reportent un taux d'infection plus important chez les juvéniles (Cappelle *et al.*, 2021), qui s'infectent rapidement après la naissance (Hoarau *et al.*, 2023 ; Joffrin *et al.*, 2022). De plus, des études sérologiques ont démontré la présence d'anticorps (probablement d'origine maternelle) qui persistent pendant des durées variables chez les nouveau-nés et juvéniles (Baker *et al.*, 2014 ; Brook *et al.*, 2019). Par exemple, une étude suggère que la protection maternelle aux *Henipavirus* est variable selon des paramètres qui dépendent de la mère (intensité d'infection) mais aussi du

nouveau-né (âge du fœtus à la mise-bas) (Epstein *et al.*, 2013). Ainsi, avec la perte progressive des anticorps maternels, les juvéniles deviennent une population naïve, qui, du fait de leur dispersion, pourrait favoriser la propagation des agents infectieux (Amman *et al.*, 2012 ; Hoarau *et al.*, 2023 ; Joffrin *et al.*, 2022 ; Mortlock *et al.*, 2021 ; Pawęska *et al.*, 2018).

La reproduction est un facteur très étudié puisqu'il est connu chez les mammifères comme pouvant favoriser la susceptibilité à l'infection, en particulier pendant les périodes de gestation et d'allaitement (Lloyd, 1983). Chez les chauves-souris, il a été montré que les périodes de gestation des femelles peuvent coïncider avec une augmentation de la séroprévalence du virus Hendra chez le renard volant à lunette *Pteropus conspicillatus* (Breed *et al.*, 2011). Cela semble être également le cas chez d'autres espèces de chauves-souris frugivores, où les femelles gestantes et allaitantes ont une séroprévalence au virus Hendra, Nipah ou encore Ebola, plus importante que celles non reproductrices (Plowright *et al.*, 2008 ; Pourrut *et al.*, 2009 ; Rahman *et al.*, 2013). De même, une étude réalisée sur les urines de Roussette d'Égypte (*R. aegyptiacus*) a montré des pics d'infection aux paramyxovirus pendant la période de gestation (Mortlock *et al.*, 2019). Ce mécanisme est moins décrit chez les mâles et semble de plus être variable, comme illustré en 2008 par Plowright et collaborateurs, qui ont constaté que la séroprévalence au virus Hendra, chez le petit renard volant (*Pteropus scapulatus*), est plus importante chez les mâles adultes pendant l'accouplement, mais ils n'ont pas retrouvé ce résultat l'année suivante. Enfin, Pourrut et collaborateurs (2007) suggèrent que les épidémies saisonnières au virus Ebola chez l'homme coïncident avec une augmentation de la séroprévalence chez les femelles gestantes de roussettes à tête de cheval (*Hypsignathus monstrosus*).

En dépit de ces informations, les mécanismes d'infection des chauves-souris sont encore mal connus et trois scénarios sont ainsi encore débattus (Plowright *et al.*, 2016). Ils dépendent de l'agent infectieux considéré et ne sont pas forcément exclusifs, pouvant intervenir en parallèle dans une même population. Le premier scénario *Sensible-Infectieux-Rétablis* (SIR) correspond à une infection transitoire des individus qui restent protégés grâce à une réponse immunitaire persistante (Figure 6a). Ce scénario est difficile dans la pratique à identifier, car il faudrait un suivi des individus sur le long terme. Cependant, il semble être adapté à l'infection au virus Marburg dont les pics d'infection se produisent, de manière synchronisée, seulement après la naissance des juvéniles susceptibles à l'infection (Amman *et*

al., 2012). Dans le cas du second scénario *Sensible-Infectieux-Rétablis-Sensible* (SIRS), la réponse immunitaire s'atténuerait avec le temps, permettant une réinfection des individus par la suite (Figure 6b). La disparition progressive des anticorps peut être un processus plus ou moins long selon l'agent infectieux. Par exemple, des études de modélisation sur plusieurs espèces de chauves-souris en Europe, basées sur des données de recaptures d'individus, suggèrent que les anticorps, produits suite à l'infection par les virus EBLV-1 et EBLV-2, peuvent persister pendant plusieurs années (Amengual *et al.*, 2007 ; Colombi *et al.*, 2019 ; Horton *et al.*, 2020 ; Robardet *et al.*, 2017). Au contraire, il semble que la durée de l'immunité soit beaucoup plus limitée pour le virus Nipah (Epstein *et al.*, 2020). Le dernier scénario *Sensible-Infectieux-Latent-Infectieux* (SILI) correspond à une phase d'infection accrue suivi d'une infection chronique ou latente qui se réactive plus tard, lors de période de stress notamment (Figure 6c). Par exemple, les herpesvirus sont connus pour avoir la capacité de rester latents dans l'organisme humain et de se réactiver lors des périodes de stress (Grinde, 2013). L'infection par les herpesvirus chez les chauves-souris semble également suivre un scénario SILI puisque lorsque l'on modélise plusieurs scénarios, le modèle qui explique le mieux les données récoltées chez le Vampire commun (*D. rotundus*) est celui qui inclut la latence (Griffiths *et al.*, 2023). Cette hypothèse est également largement avancée pour expliquer la persistance des *Henipavirus* chez les chauves-souris (Epstein *et al.*, 2020). En effet, basé sur de la modélisation, le modèle le plus adapté aux données observées chez la Roussette paillée africaine (*Eidolon helvum*) est celui qui inclut une ré-infection des individus, avec une possible latence (Glennon *et al.*, 2019).

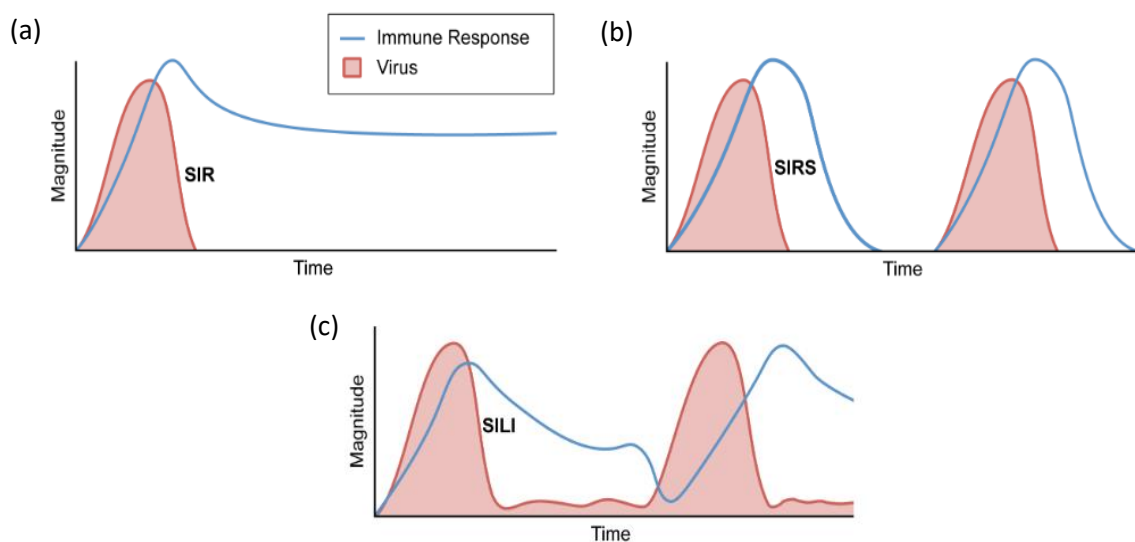


Figure 6. Dynamiques d'infection attendues chez les chauves-souris selon trois scénarios. (a) Sensible-Infectieux-Rétablis. (b) Sensible-Infectieux-Rétablis-Sensible. (c) Sensible-Infectieux-Latent-Infectieux (Plowright *et al.*, 2016).

De plus, le stress peut entraîner la ré-excrétion d'agents infectieux, comme c'est par exemple connu pour les herpesvirus qui restent latents et se réactivent lors de période de stress physique ou psychologique (Grinde, 2013). Il a ainsi été suggéré que le stress nutritionnel, lié à une réduction de la disponibilité des ressources en fruits due à la déforestation en Australie, pourrait avoir un impact sur la prévalence d'infection du virus Hendra chez les petits renards volants (*P. scapulatus*). Chez cette espèce, les individus en moins bonne condition corporelle sont plus infectés (Plowright *et al.*, 2008). De plus, Becker et collaborateurs (2023) ont récemment montré que l'amplitude des pics d'infection au virus Hendra chez les renards volants noirs (*Pteropus alecto*) était exacerbée après une récente pénurie alimentaire liée au climat (température, précipitation) et directement corrélée à la fréquence des épidémies observées chez les chevaux et l'homme. Cet exemple illustre ainsi très bien comment la modification des habitats liés aux changements environnementaux et à l'urbanisation peut impacter les dynamiques d'infection chez les chauves-souris et le risque d'émergence.

La plupart des études citées au-dessus se sont focalisées sur l'étude d'un unique agent infectieux, or, avec le développement des outils moléculaires et notamment le séquençage haut-débit des communautés microbiennes, de plus en plus d'études reportent des co-infections au sein d'un même individu chez les chauves-souris (Anthony *et al.*, 2013 ; Baker *et al.*, 2012 ; Davy *et al.*, 2018 ; Hoarau *et al.*, 2021). Cependant, les études sur les co-infections se sont concentrées sur des variants au sein d'une même famille virale (*e.g.* Mortlock *et al.*, 2019 ; Peel *et al.*, 2019) et peu se sont intéressées aux interactions entre différents groupes d'agents infectieux (bactérie, virus à ADN/ARN, champignon) (*e.g.* Davy *et al.*, 2018). Les interactions entre agents infectieux, tels que la compétition pour les ressources ou indirectement via la réponse immunitaire de l'hôte, peuvent avoir des conséquences en réduisant ou augmentant la susceptibilité de l'hôte, et donc en limitant ou facilitant ses futures infections (Hoarau *et al.*, 2020 ; Ruiz-Aravena *et al.*, 2022 ; Telfer *et al.*, 2010). Par exemple, l'étude de Davy et collaborateurs (2018) suggère que l'infection aux coronavirus augmente la

sévérité de la maladie du syndrome du museau blanc provoquée par le champignon *Pseudogymnoascus destructans* chez la petite chauve-souris brune (*Myotis lucifugus*). Ainsi, il est important d'examiner les patrons spatio-temporels simultanés de multiples infections, aux échelles individuelle et populationnelle, afin de mieux comprendre les mécanismes sous-jacents qui influencent les dynamiques d'infection dans les populations de chauves-souris.

4. Limites des études spatio-temporelles et intérêt des écosystèmes insulaires

Afin de décrypter cette hétérogénéité et complexité des populations de chauves-souris, le suivi des populations dans leur milieu naturel est indispensable (Plowright *et al.*, 2019 ; Serra-Cobo & López-Roig, 2017). Il requiert à la fois un échantillonnage spatial pour capturer les variations entre colonies, mais aussi temporel pour analyser la dynamique au cours du cycle biologique complet. Cependant, ce type d'approche spatio-temporelle à une échelle de temps assez fine reste rare (*e.g.* Eby *et al.*, 2023 ; Epstein *et al.*, 2020 ; Robardet *et al.*, 2017 ; Serra-Cobo *et al.*, 2013), comme souligné récemment pour l'investigation des *Henipavirus* et *Filovirus* (Becker *et al.*, 2019). Ceci s'explique en partie par la nature très mobile et le caractère discret des chauves-souris qui les rend difficile à localiser et à étudier. De plus, les études épidémiologiques demandent un effort d'échantillonnage important pour pouvoir estimer des prévalences d'infection de manière fiable, et donc d'avoir accès à des espèces où il est possible d'étudier un grand nombre d'individus. Enfin, le suivi des populations de chauves-souris sur le terrain mobilise un grand nombre de personnes et beaucoup de temps, et donc des contraintes budgétaires et de personnel qualifié, qui rend compliqué les études sur le long terme (Becker *et al.*, 2019).

Les écosystèmes insulaires sont considérés depuis longtemps comme des « laboratoires naturels » pour étudier les processus écologiques et évolutifs (Warren *et al.*, 2015). Ils sont caractérisés par un isolement par rapport au continent et un fort taux d'endémisme (Kier *et al.*, 2009 ; Warren *et al.*, 2015). En raison de leur capacité à voler sur de longues distances, les chauves-souris sont souvent les seuls mammifères natifs trouvés sur les îles océaniques. En effet, 60% des espèces de chauves-souris vivent en milieu insulaire et 25% d'entre elles sont même endémiques de ces îles (Jones *et al.*, 2009). L'étude des chauves-souris insulaires et endémiques permet d'avoir accès à des espèces avec une distribution restreinte souvent à de petits territoires, avec des limites géographiques bien définies, ce qui facilite leur étude d'un

point de vue spatial. De plus, les petites îles océaniques ont souvent une richesse spécifique moins importante (Warren *et al.*, 2015). Le nombre limité d'espèces de chauves-souris dans ces milieux offre ainsi la possibilité d'étudier des espèces vivant en colonies monospécifiques, avec peu de contacts interspécifiques, simplifiant les mécanismes de transmission des agents infectieux. Le nombre moins important de compétiteurs ou prédateurs peut également offrir des niches écologiques vastes et favoriser l'expansion de certaines espèces opportunistes, ayant la capacité de s'adapter à différents habitats. Une forte densité d'individus au sein des colonies, comme chez les Molossidés dont les colonies peuvent atteindre le million d'individus (Betke *et al.*, 2008 ; McCracken, 1989 ; Mcfarlane *et al.*, 2015), est un avantage certain facilitant leur échantillonnage à une échelle populationnelle. Ainsi, la présence de chauves-souris endémiques des îles, abondantes dans ces systèmes simplifiés et bien définis, représente une opportunité rare pour la mise en place d'études spatio-temporelles à une échelle fine.

III. Modèle d'étude : Le Petit Molosse de La Réunion

1. Biologie d'une chauve-souris endémique et urbaine

Le Petit Molosse de La Réunion (*Mormopterus francoismoutoui* ; Goodman *et al.*, 2008) est une petite chauve-souris (~ 6 g) insectivore de milieu tropical, endémique de l'île de La Réunion, appartenant à la famille des Molossidés (Figure 7). L'île de La Réunion, peuplée de 860 000 habitants (Institut National de la Statistique et des Etudes Economiques, 2020), est un petit territoire volcanique (2512 km²) situé dans le sud-ouest de l'Océan Indien, dans l'archipel des Mascareignes. La Réunion se situe à proximité de deux îles qui abritent des espèces voisines du genre *Mormopterus* : l'île Maurice à 175 km au nord-est, qui héberge l'espèce *Mormopterus acetabulosus* et Madagascar à 950 km de la côte ouest où vit *Mormopterus jugularis*. L'île de La Réunion a émergé *de novo* il y a environ 3 millions d'années (Cadet, 1980) et possède un relief important, avec le Piton des Neiges qui constitue le point culminant (3070 m) et un volcan encore actif, le Piton de la Fournaise. La Réunion possède un climat tropical composé de deux saisons : la saison chaude et humide s'étend de novembre à avril (été austral) et la saison sèche s'étend de mai à octobre (hiver austral). L'île a été colonisée par l'homme au milieu du 17^{ème} siècle et subit depuis d'importantes modifications de ces écosystèmes, à travers la déforestation pour l'agriculture intensive et l'urbanisation, et l'introduction

d'espèces exotiques invasives (Lagabrielle *et al.*, 2009). Le Petit Molosse de La Réunion est ainsi le seul mammifère endémique de l'île, et partage le territoire de l'île de la Réunion avec deux autres espèces de chauves-souris beaucoup moins abondantes : une chauve-souris insectivore, le Taphien de Maurice (*Taphozous mauritianus*) et une chauve-souris frugivore, la Roussette Noire (*Pteropus niger*).

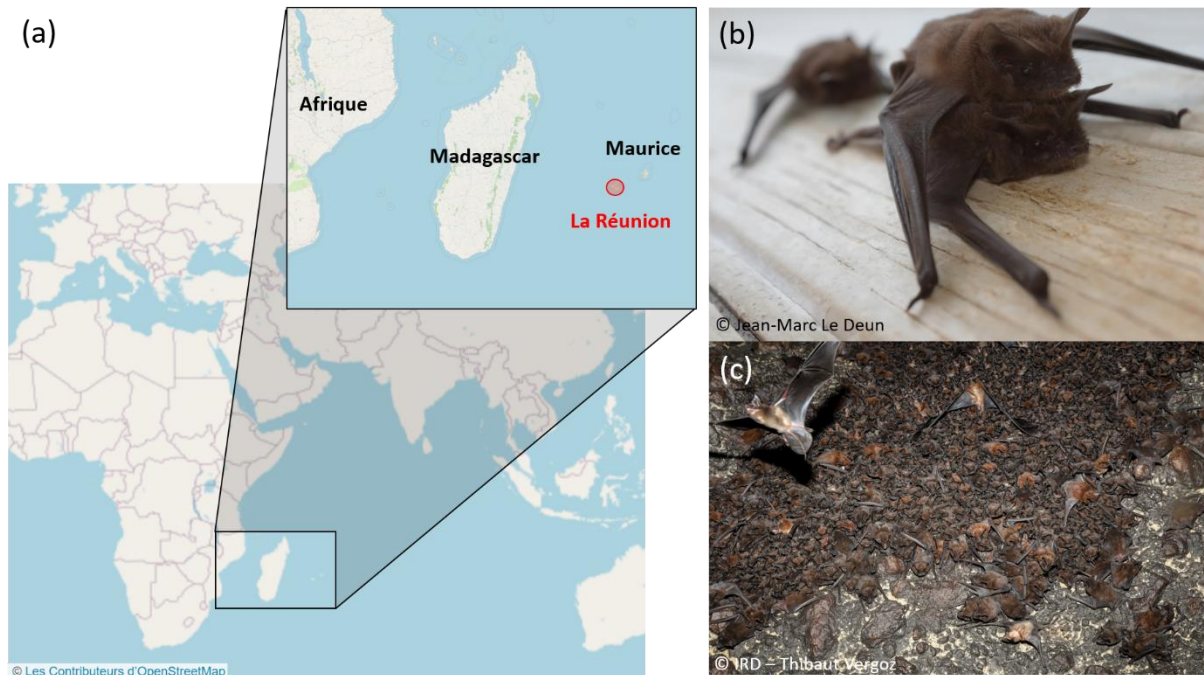


Figure 7. Localisation de l'île de La Réunion et photographies de Petits Molosses de La Réunion (*Mormopterus francoismoutoui*). (a) La carte a été créée sur Qgis (QGIS Development Team, 2022), à partir d'un fond de carte de OpenStreetMap, sous la [Licence Open Database](#). (b) Quelques individus nichant derrière un volet de maison. (c) Plusieurs milliers d'individus occupant une grotte en milieu naturel.

Le Petit Molosse est largement distribué sur l'île, et de manière très abondante, dans les parties basses et urbanisées de l'île (Augros *et al.*, 2015 ; Barataud & Giosa, 2013 ; Moutou, 1982). Les gîtes sont toujours monospécifiques et se trouvent dans une diversité d'habitats naturels tels que des grottes et des fissures dans les falaises. Cette espèce s'est aussi adaptée aux habitats anthropiques où beaucoup de colonies sont observées, notamment dans des ponts et des bâtiments (Goodman *et al.*, 2008), et qui sont donc facilement accessibles. Les gîtes peuvent héberger de quelques individus à plusieurs dizaines de milliers d'individus, avec une densité impressionnante pouvant approcher 1000 individus au mètre carré (Augros *et al.*, 2015 ; Dietrich *et al.*, 2015b). De par son abondance et la diversité de ses habitats, les contacts

entre le Petit Molosse et l'homme sont courant au sein de l'île et posent des problématiques de conservation, puisque certaines colonies situées dans des lieux publics sont délocalisées (Augros *et al.*, 2015), malgré son statut d'espèce protégée.

Malgré son abondance, la biologie du Petit Molosse reste mal connue à ce jour. Toutefois, l'étude de Dietrich et collaborateurs (2015b), réalisée dans la plus grande maternité connue de l'île (~ 46 500 individus), suggère que la période de mise-bas a lieu entre décembre et février pendant l'été austral, et qu'une certaine fidélité des femelles à leur site de mise-bas existe puisque les mêmes phénomènes d'agrégation massive d'individus sont observés chaque année au moment de la gestation. Des mouvements saisonniers importants sont également suspectés puisque les individus quittent ce gîte à la fin de l'été, pour le laisser complètement vide en hiver (de juin à septembre). D'autres colonies sont répertoriées sur l'île mais on ne connaît pas leur taille (nombre d'individus), ni les types d'individus qui les composent (*e.g.* mâle/femelle, juvénile/adulte) et donc leur fonctionnement. L'installation du Petit Molosse dans de nombreux gîtes urbains pourrait avoir favorisé une expansion récente de sa population (Goodman *et al.*, 2008). De plus, la connectivité entre ces colonies et l'influence du relief montagneux de l'île sur la dispersion des individus est mal connue. Une première étude génétique, basée sur un nombre limité d'échantillons ($n = 31$), suggérait une diversité génétique assez importante et aucun isolement par la distance au sein de l'île (Goodman *et al.*, 2008).

2. Epidémiologie de ses agents infectieux

Des études précédentes ont démontré que le Petit Molosse était porteur de plusieurs agents infectieux tels que les coronavirus (Joffrin *et al.*, 2020), les astrovirus (Joffrin *et al.*, 2021), les herpesvirus (Aguillon, 2020), la bactérie *Leptospira* et les paramyxovirus (Dietrich *et al.*, 2015b). Les acides nucléiques de ses agents infectieux ont été détectés par PCR dans différents types de fluides (urine, fèces et salive), avec parfois des co-infections au sein d'un même individu (Dietrich *et al.*, 2015b ; Hoarau *et al.*, 2023). La présence de *Lyssavirus* (virus de rage) est également suspectée sur la base de résultats sérologiques positifs (Mélade *et al.*, 2016). Plusieurs de ces agents infectieux appartiennent à des familles virales et bactériennes dont certains membres sont responsables de maladies graves chez l'homme. Par exemple, les paramyxovirus et leptospires du Petit Molosse sont phylogénétiquement proches du genre des

Morbillivirus et de *Leptospira borgeptersenii*, respectivement (Dietrich *et al.*, 2015b). Cependant, jusqu'à aujourd'hui, aucun lien épidémiologique n'a été montré entre les agents infectieux hébergés par le Petit Molosse et le développement de maladies chez l'homme à la Réunion (Guernier *et al.*, 2016). Les paramyxovirus, bactéries leptospires et herpesvirus ont été retenus comme modèles d'étude puisqu'ils sont tous particulièrement prévalents chez le Petit Molosse, qu'ils appartiennent à des groupes différents (virus ADN, virus ARN, bactérie) et sont excrétés dans différents fluides pouvant donner lieu à différents modes de transmission.

Une première étude a permis de montrer des dynamiques saisonnières d'excrétion des paramyxovirus et bactéries leptospires chez le Petit Molosse. Un échantillonnage non invasif, consistant à prélever de l'urine sous une colonie de maternité, a mis en évidence un pic d'excrétion en été austral, au moment de la gestation et mise-bas des femelles, suggérant ainsi un rôle de la reproduction dans l'infection (Dietrich *et al.*, 2015b). A cette période, près de 45% et 60% des chauves-souris de la colonie étudiée excrètent des bactéries leptospires et paramyxovirus dans leurs urines, respectivement. Une seconde étude réalisée dans une seconde maternité, et basée également sur un échantillonnage non-invasif, cette fois-ci de fèces fraîches, a permis de démontrer que la transmission des paramyxovirus se poursuit en hiver (Hoarau *et al.*, 2023). De plus, cette étude suggère que la perte des anticorps maternels des juvéniles favorisent la transmission des virus. En revanche, ces études se sont limitées à l'analyse de deux gîtes de maternité (Dietrich *et al.*, 2015b ; Hoarau *et al.*, 2023), et le rôle des facteurs populationnels dans la transmission, liés aux caractéristiques des colonies (taille, sex-ratio) ou à une potentielle structure génétique entre colonies, n'ont pas été étudiés. De plus, les deux études précédentes étant basées sur un échantillonnage sans capture d'individus, le rôle potentiel des facteurs individuels (sexe, âge, statut reproducteur) dans les dynamiques d'infection du Petit Molosse n'a jamais été testé.

IV. Objectifs de la thèse

L'objectif principal de ma thèse est d'étudier comment l'écologie des chauves-souris et la dynamique de leurs populations influencent la transmission de leurs agents infectieux. En utilisant le Petit Molosse et trois de ses agents infectieux (paramyxovirus, bactéries leptospires et herpesvirus) comme modèles d'étude, ce travail s'est intéressé à (1) caractériser le cycle

biologique et la phénologie de l'hôte, ainsi qu'à étudier sa structure génétique et son histoire évolutive et (2) analyser l'influence des facteurs écologiques sur les dynamiques d'infection aux échelles individuelle et populationnelle. La présence des agents infectieux ayant été détectée dans les fluides par PCR, je parlerais d'excrétion par la suite.

Le premier chapitre vise à apporter des connaissances basiques, mais fondamentales, sur l'écologie du Petit Molosse. Nous nous sommes intéressés aux dynamiques de populations au cours du cycle biologique et à la structure génétique de ses populations pour mieux comprendre l'hétérogénéité des gîtes et leur connectivité. Le second chapitre réunit les questions sur les dynamiques d'excrétion et de co-excrétion de trois agents infectieux (paramyxovirus, bactéries leptospires et herpesvirus), à l'échelle des populations (gîtes) et individuelle (hôte). Nous avons analysé l'influence des facteurs écologiques de l'hôte sur les variations spatio-temporelles d'excrétion et de la diversité génétique virale. Enfin, ce manuscrit se clôture par une discussion générale qui se focalise sur la compréhension des facteurs qui influencent les dynamiques d'excrétion chez les chauves-souris, et apporte des perspectives d'études dans un contexte de changements globaux, santé humaine mais également de protection et de conservation des chauves-souris.

V. Suivi et échantillonnage des populations de Petits Molosses

Les questions abordées dans cette thèse font partie d'un programme de recherche plus large qui a débuté en 2018 sur le Petit Molosse, et qui a permis le suivi et l'échantillonnage spatio-temporel de nombreux gîtes sur l'île. Etant donné que les mêmes procédures sur le terrain ont été utilisées pour collecter les données présentées dans les deux chapitres, j'ai choisi de détailler et illustrer ici le déroulement du suivi qui a été réalisé sur le terrain.

1. Déclaration éthique, composition de l'équipe et protocole sanitaire

Les captures et manipulations du Petit Molosse se sont déroulées dans le cadre d'une dérogation de capture délivrée par la DEAL de la Réunion (DEAL/SEB/UBIO/2018-09), et ont été approuvées par le comité d'éthique du CYROI Réunion (n°114) et le Ministère de la Recherche (APAFIS#10140-2017030119531267).

Une équipe de 2 à 5 personnes était mobilisée pour chaque nuit de capture, composée des membres de l'UMR PIMIT, dont des personnes habilitées à la capture (Muriel Dietrich et Gildas Le Minter). Afin de manipuler les chauves-souris, des équipements de protections personnelles ont été utilisés : masque FFP2, gants en nitrile, combinaison Tyvek et masque à cartouche pour respirer dans les environnements confinés (grotte). Les gants ont été désinfectés entre chaque chauve-souris et changés régulièrement. Tout l'équipement a été désinfecté entre les sites d'étude.

2. Sites d'études et fréquence d'échantillonnage

Au total, 19 gîtes de Petits Molosses ont été étudiés dans le cadre de cette thèse (Figure 8). Ces gîtes ont été sélectionnés car ils sont répartis sur toute l'île ce qui permet d'avoir une bonne représentation spatiale à l'échelle de l'île. Ils sont également situés dans différents types d'habitats et possèdent des effectifs très diversifiés (toujours au-dessus de 200 individus). Ils sont aussi facilement accessibles ce qui simplifie le suivi et l'échantillonnage fréquent de ces sites. Les gîtes se trouvent dans des habitats naturels tels que des grottes ($n = 1$) et des fissures de falaises ($n = 1$), mais surtout anthropiques tels que des bâtiments ($n = 7$) et des ponts ($n = 10$). Pour la suite du manuscrit, le nom des gîtes a été codé avec un code à trois lettres. Par exemple, le gîte TGI se trouve dans un transformateur électrique, le gîte TBA dans une grotte, le gîte MON dans les joints de dilatation d'un pont et le gîte PSR sous le toit d'un bâtiment (Figure 8).

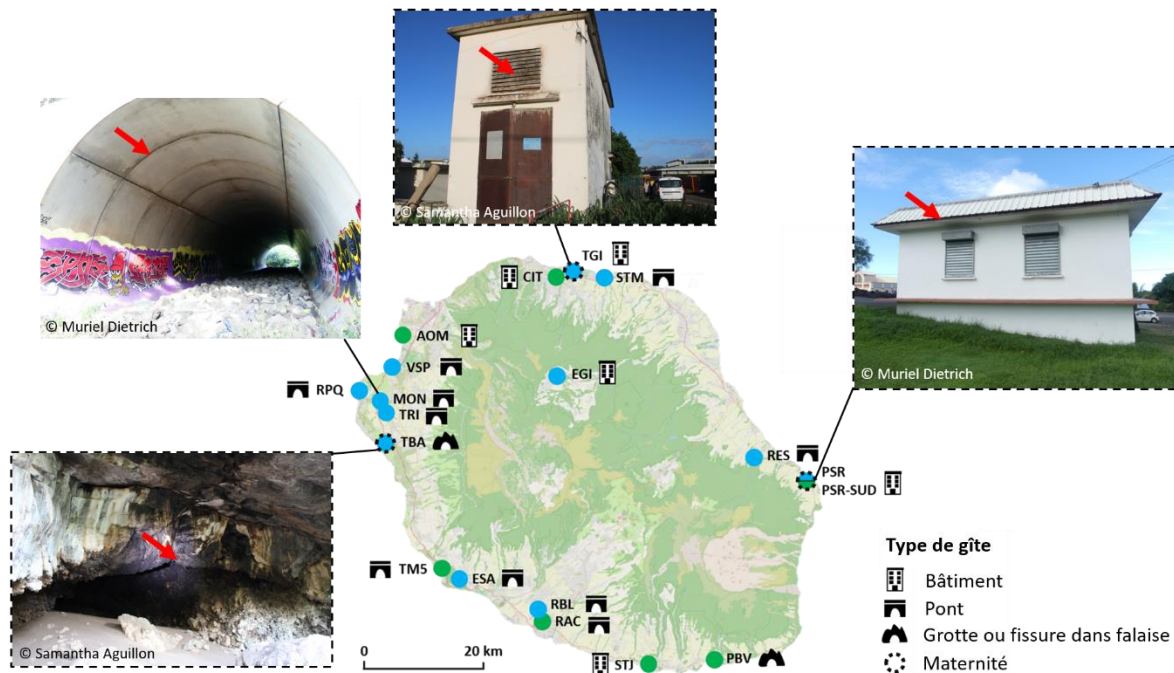


Figure 8. Localisation des gîtes étudiés pour le suivi des populations de Petits Molosses (*Mormopterus francoismoutoui*) et exemples des différents habitats utilisés. Les flèches rouges indiquent l'emplacement où se trouvent les chauves-souris à l'intérieur du gîte. Sur la carte, les gîtes en bleu ont été échantillonnés plusieurs fois au cours de l'étude, et les gîtes en vert une seule fois afin de compléter l'échantillonnage spatial. Les gîtes de PSR et PSR-SUD se situent dans le même bâtiment, dans lequel on suspecte une scission entre les mâles (PSR) et les femelles (PSR-SUD). Le gîte PSR-SUD n'a été échantillonné qu'une seule fois, car il est moins accessible que le gîte PSR qui est situé à une hauteur moins importante. Modifié d'après Aguillon *et al.*, (2023).

L'étude de ces gîtes s'est déroulée sur un peu plus de 2 ans (27 mois), d'octobre 2018 à décembre 2020. Un suivi longitudinal a été réalisé sur douze des gîtes (EGI, ESA, MON, PSR, RBL, RES, RPQ, STM, TBA, TGI, TRI et VSP ; en bleu sur la figure 8), qui ont été échantillonnés de manière fine, toutes les 6 semaines environ (Figure 9). Cependant, à cause de contraintes logistiques, de météo défavorable ou d'absence de chauves-souris dans les gîtes, certains n'ont pas été échantillonnés à la même fréquence, celle-ci variant de 5 à 17 sessions d'échantillonnage par gîte. Les sept autres gîtes (AOM, CIT, PBV, PSR-SUD, RAC, STJ et TMS ; en vert sur la figure 8) ont été échantillonnés une seule fois pour collecter plus de données d'un point de vue spatial. A chaque session, l'ensemble des gîtes a été échantillonné de manière la plus rapprochée dans le temps, sur une fenêtre de 2 à 3 semaines environ (1 gîte par soir environ, Figure 9). De plus, pour limiter le dérangement des individus pendant les périodes critiques du cycle biologique, les captures n'ont pas eu lieu pendant la mise-bas (début des

naissances à la mi-décembre), et ce jusqu'au moment où il n'y a plus de nouveau-nés ou de juvéniles non volants observés dans les maternités (mi-janvier). Sur les 27 mois de l'étude, un total de 173 sorties de captures a été effectué sur toute l'île. Pendant mon stage de Master 2 (janvier à juin 2020) et ma thèse (depuis octobre 2020), j'ai participé à 13% de ces sorties.

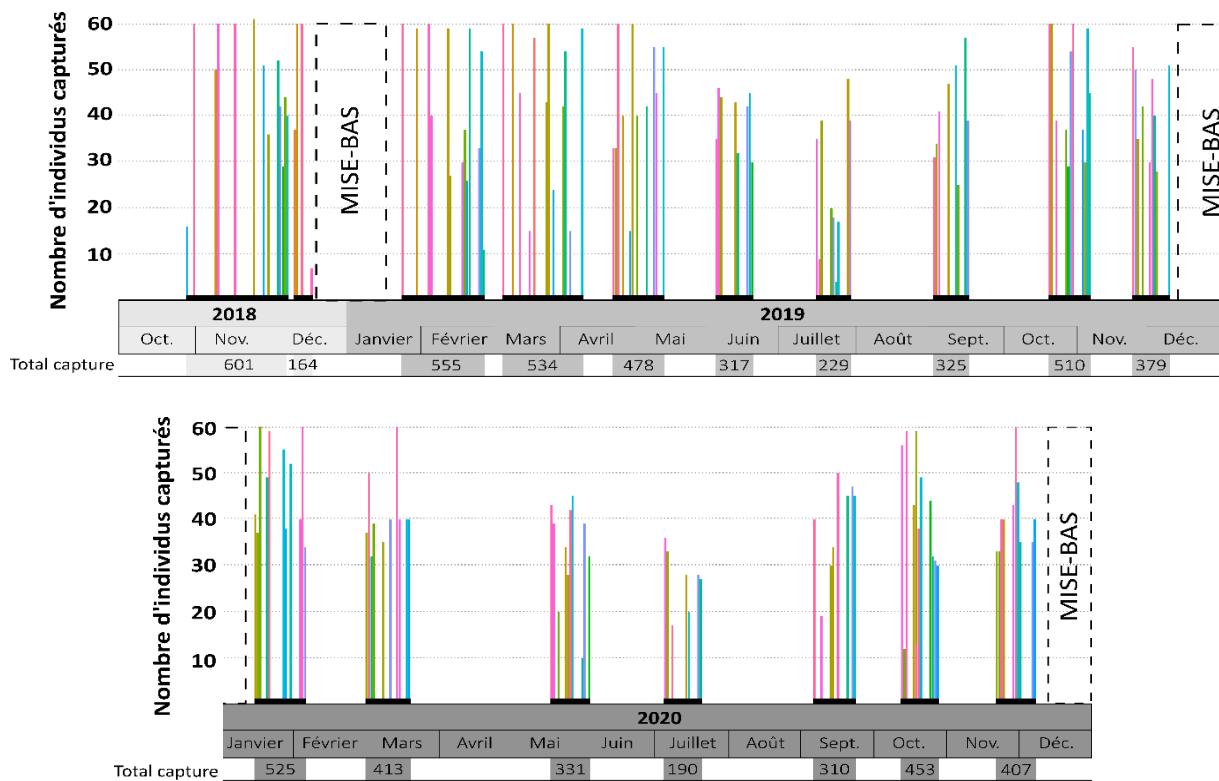


Figure 9. Fréquence d'échantillonnage des gîtes et nombre d'individus capturés. Chaque événement de capture est représenté par une barre verticale, colorée selon le gîte. Les 17 sessions d'échantillonnage sont représentées par un trait noir sur l'axe des abscisses et le nombre total de chauves-souris capturées est indiqué pour chaque session en bas du graphique.

3. Capture des chauves-souris

Techniques de piégeage

Les chauves-souris ont été capturées en majorité à la tombée de la nuit, lorsque les individus quittent leur gîte pour aller se nourrir. Nous avons utilisé principalement des pièges harpes et des filets japonais installés devant la sortie des gîtes, sans la bloquer totalement pour que les individus puissent sortir librement. Dans certains gîtes, il était difficile d'installer ces pièges (pas assez de hauteur, ou trop d'individus), c'est pourquoi nous avons parfois utilisé un filet papillon en journée en le posant délicatement sur les individus au repos (Figure 10). Après qu'un maximum de 60 chauves-souris ait été capturé, les individus ont été hydratés par voie

orale à l'aide d'une seringue stérile et placés dans de petits pochons propres faits de tissu déperlant, proches d'une source chaude (bouillote), en attente d'être manipulés sur le site de capture.



Figure 10. Différents types de pièges ont été utilisés pour capturer les chauves-souris : (a) filet papillon, (b) filet japonais et (c) piège harpe.

Relevé biométrique et morphologique

Pour chaque individu, la masse a été mesurée en utilisant une balance électronique et la taille de l'avant-bras à l'aide d'un pied à coulisse. Le sexe, l'âge et le statut reproducteur ont été relevés sur la base de critères morphologiques. L'âge a été déterminé en examinant la fusion de l'épiphyse au niveau de l'articulation des doigts, qui n'est pas soudée chez les jeunes. Les juvéniles sont facilement identifiables pendant les 6-7 premiers mois de leur vie, donc jusqu'à environ juillet. Les nouveau-nés (roses, sans poils) n'ont pas été capturés mais une inspection visuelle des gîtes pour déterminer leur présence a été réalisée.

Le statut reproducteur des femelles a été déterminé selon la visibilité et le gonflement des mamelles. Les femelles considérées comme actives pour la reproduction sont gestantes, allaitantes et post-allaitantes. Pour déterminer si une femelle est gestante, nous avons palpé légèrement l'abdomen et vérifié la présence de mamelles gonflées. Les femelles allaitantes ont les mamelles gonflées et mâchouillées (noté M2) tandis que pour les femelles post-allaitantes, on peut observer une repousse des poils autour des mamelles non-gonflées (M3). Les femelles non actives pour la reproduction ont les mamelles non visibles (M0), ou visibles mais non gonflées (M1). Le statut reproducteur des mâles a été déterminé selon le niveau de gonflement des testicules, bien que chez cette espèce, les testicules soient difficiles à observer. Trois codes ont été utilisés : testicules non visibles (noté T0), visibles (T1) et gonflés (T2), les individus T1 et T2 étant considérés comme actifs pour la reproduction.

Collecte d'échantillons biologiques

Une série d'échantillons biologiques a été collectée pour chaque individu. Tout d'abord, une biopsie a été réalisée sur chaque aile (~ 2 mm) à l'aide d'un poinçon, chacune mise ensuite dans un cryotube à sec, dont l'un a été utilisé pour réaliser l'étude de génétique de l'hôte (Figure 11). Afin de rechercher la présence d'herpesvirus, un écouvillon stérile a été introduit délicatement dans la commissure des lèvres pour prélever de la salive, et placé dans un cryotube contenant 250 µL de milieu MEM (Minimum Essential Medium Eagle). Afin d'analyser les paramyxovirus et bactéries leptospires, de l'urine a été prélevée dans le pochon étanche ou directement au niveau de l'orifice externe de l'urètre avec une pipette et des cônes stériles, avant d'être placée dans un cryotube contenant 50 µL de MEM. D'autres prélèvements ont été effectués mais non pas été utilisés dans le cadre de cette thèse (Figure 11). Des fèces fraîches ont été collectées, soit dans le pochon, soit directement sur l'individu pendant la manipulation, à l'aide de pinces propres, et conservées dans un tube à sec. Des ectoparasites (minuscules acariens situés au niveau du patagium) ont aussi été prélevés avec des pinces propres et conservés dans des cryotubes contenant 250 µL de MEM. Tous les prélèvements biologiques ont été stockés dans une glacière au froid sur le terrain, puis placés au congélateur à -80°C de retour au laboratoire quelques heures après le début des manipulations.

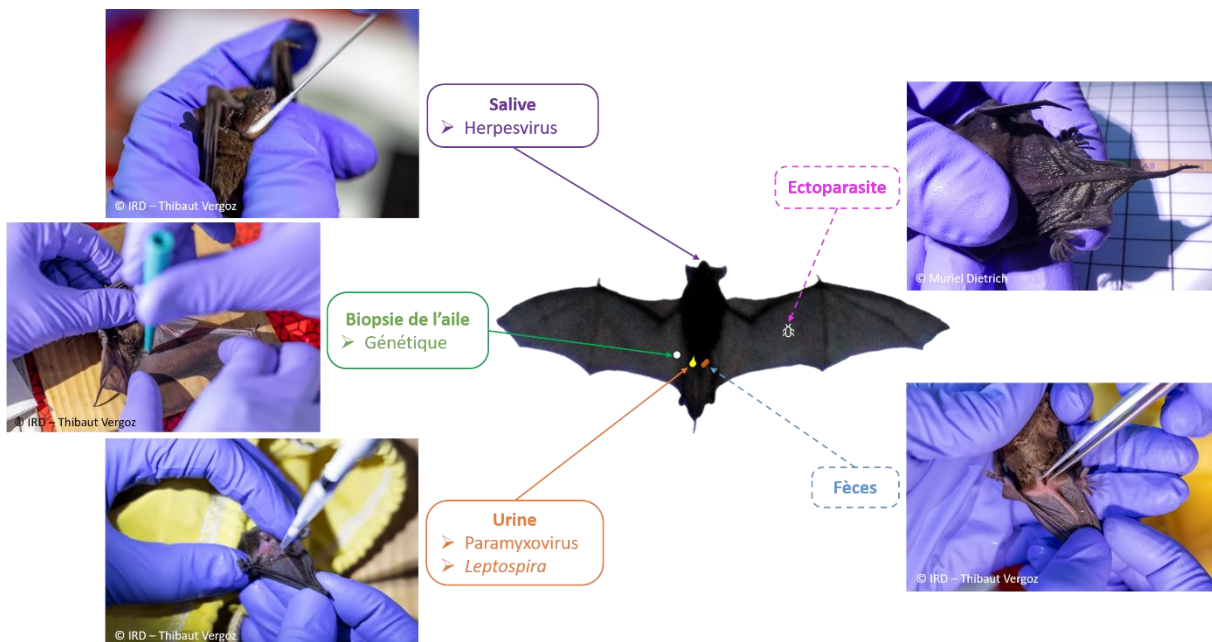


Figure 11. Prélèvements effectués chez le Petit Molosse et utilisation dans le cadre de la thèse (trait plein). Les différents prélèvements sont illustrés par une photo prise sur le terrain.

Marquage des individus

Toutes les chauves-souris capturées ont été marquées par tatouage, en utilisant un dermographe et de l'encre noire. Après avoir désinfecté puis préparé la membrane de l'aile avec une huile spéciale, un code alphanumérique unique a été inscrit sur le propatagium droit (Figure 12). Les individus ont ensuite été relâchés sur le site de capture.

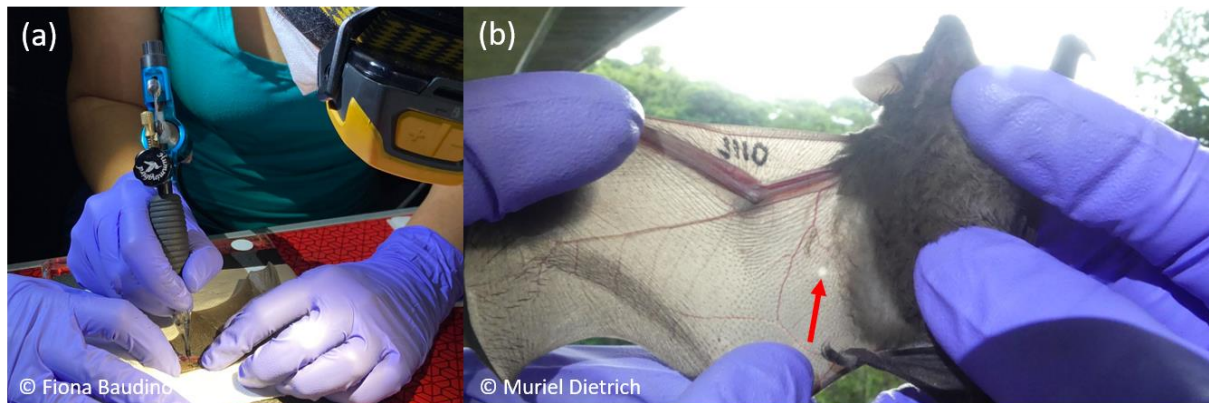


Figure 12. (a) Tatouage d'une chauve-souris. (b) Lecture du tatouage (J110) d'une chauve-souris recapturée après 41 jours. On peut également apercevoir la cicatrice de la biopsie de l'aile (flèche rouge).

4. Estimation de la taille des gîtes

Une estimation visuelle de la taille des gîtes (nombre d'individus) a été effectuée le plus souvent possible. Parmi les douze gîtes échantillonnés régulièrement, dix gîtes (ESA, MON, RBL, RES, RPQ, STM, TBA, TGI, TRI, et VSP) ont fait l'objet d'un suivi régulier de l'évolution de leur taille (au moins 5 estimations). Pour les sept gîtes échantillonnés de manière occasionnel (AOM, CIT, PBV, RAC, STJ et TM5), la taille a été estimée une seule fois le jour de la capture, à l'exception de PSR-SUD à cause de la difficulté de compter au sein du bâtiment.

La taille des gîtes a été estimée, dans le meilleur des cas, le même jour que celui de la capture, ou jusqu'à 14 jours après ou avant à cause de contraintes logistiques. Différentes méthodes ont été utilisées pour estimer la taille des gîtes (Figure 13). Quand cela était possible (conditions de luminosité favorable), l'estimation du nombre d'individus a été réalisée durant l'émergence crépusculaire des chauves-souris. Dans le cas des ponts et de certains bâtiments où les individus utilisent plusieurs sorties, l'estimation a été réalisée en journée, en comptant visuellement les individus au repos dans les joints des ponts et dans les pièces des bâtiments. Pour la grotte, le nombre de chauves-souris étant très important (plusieurs dizaines de

milliers), nous avons estimé la surface de la grotte recouverte par les individus (en m^2) au matin, et multiplié cette surface par la densité d'individus estimée visuellement (900 individus/ m^2), comme décrit dans le rapport de Héré et collaborateurs (2009).



Figure 13. Différentes méthodes d'estimation de la taille des gîtes : (a) comptage à l'émergence, (b) comptage visuel des individus au repos et (c) estimation de la surface recouverte par les individus dans la grotte.

CHAPITRE 1

Ecologie du Petit Molosse

Dans ce premier chapitre, nous avons étudié l'écologie du Petit Molosse à travers deux approches : la première est basée sur un suivi de la dynamique des populations sur le terrain pour caractériser son cycle biologique et sa phénologie, et la deuxième utilise les outils génétiques pour analyser la structure des populations et l'histoire évolutive du Petit Molosse.

I. Phénologie et dynamiques de populations du Petit Molosse

Dans la première étude, les données collectées dans 19 gîtes pendant 27 mois (6721 individus) ont été analysées pour mieux comprendre la saisonnalité d'occupation des gîtes, en particulier l'évolution de leur taille (nombre d'individus) et de leur composition (sex-ratio, âge, statut reproducteur). Une précédente étude réalisée sur le plus grand gîte connu de l'île suggérait d'important mouvements saisonniers entre gîtes, caractérisés par une importante agrégation des femelles pendant l'été austral pour la gestation et mise-bas (Dietrich *et al.*, 2015b). C'est pourquoi, nous avons également analysé comment les individus de sexe et d'âge différents utilisaient les différents gîtes, en mesurant le niveau de ségrégation entre mâles et femelles, et entre adultes et juvéniles. Nous avons également testé si ces patrons de ségrégation étaient corrélés avec les variations d'effectifs mesurées dans les gîtes. Les périodes du cycle biologique du Petit Molosse n'ayant été que peu décrites, l'observation de caractères morphologiques lors des captures d'individus a permis d'identifier les périodes et lieux de gestation, de mise-bas, d'accouplement et de dispersion des juvéniles. Les différentes périodes du cycle biologique ont aussi été mises en lien avec les variations de la condition corporelle des individus. Notre étude révèle des variations saisonnières marquées des effectifs et du sex-ratio dans les gîtes, en relation avec des patrons de ségrégation sexuelle. Cette étude a permis de proposer un nouveau cycle biologique complet pour le Petit Molosse, pour lequel nous proposons une possible interruption du cycle de reproduction entre l'accouplement et la gestation chez cette espèce tropicale. De manière intéressante, même si des femelles gestantes ont été capturées partout sur l'île, la mise-bas, elle, était ensuite localisée dans seulement quelques gîtes, suggérant ainsi une dispersion et une sélection de gîtes par les femelles gestantes au moment de la mise-bas. De plus, la capture de juvéniles volants dans l'ensemble des gîtes, quelques semaines seulement après la mise-bas, montre un sevrage et une dispersion rapide des juvéniles au sein de l'île. Tous ces résultats suggèrent une structure sociale complexe et très dynamique, avec d'importants mouvements saisonniers au sein de

l'île, et une potentielle migration hivernale en altitude chez cette espèce, qui expliquerait la baisse drastique des effectifs en hiver dans les colonies étudiées. Ces résultats sont présentés dans un article publié en 2023 dans le journal *Ecology and Evolution* (**Article 1** et Annexe 1).

Article 1

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RESEARCH ARTICLE

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A population in perpetual motion: Highly dynamic roosting behavior of a tropical island endemic bat

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Abstract

Although island endemic bats are a source of considerable conservation concerns, their biology remains poorly known. Here, we studied the phenology and roosting behavior of a tropical island endemic species: the Reunion free-tailed bat (*Mormopterus francoismoutoui*). This widespread and abundant species occupies various natural and anthropogenic environments such as caves and buildings. We set up fine-scale monitoring of 19 roosts over 27 months in Reunion Island and analyzed roost size and composition, sexual and age-associated segregation of individuals, as well as the reproductive phenology and body condition of individuals. Based on extensive data collected from 6721 individuals, we revealed a highly dynamic roosting behavior, with marked seasonal sex-ratio variation, linked to distinct patterns of sexual aggregation among roosts. Despite the widespread presence of pregnant females all over the island, parturition was localized in a few roosts, and flying juveniles dispersed rapidly toward all studied roosts. Our data also suggested a 7-month delay between mating and pregnancy, highlighting a likely long interruption of the reproductive cycle in this tropical bat. Altogether, our results suggest a complex social organization in the Reunion free-tailed bat, with important sex-specific seasonal and spatial movements, including the possibility of altitudinal migration. Bat tracking and genetic studies would provide additional insights into the behavioral strategies that shape the biology of this enigmatic bat species. The fine-scale spatiotemporal data revealed by our study will serve to the delineation of effective conservation plans, especially in the context of growing urbanization and agriculture expansion in Reunion Island.

KEYWORDS

Chiroptera, Molossidae, reproductive seasonality, Reunion Island, sexual segregation, tropical island bat

TAXONOMY CLASSIFICATION

Behavioural ecology, Phenology, Population ecology

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1 | INTRODUCTION

Islands are unique but vulnerable ecosystems. Indeed, islands shelter a higher proportion of endemic taxa than do equivalent continental areas, and this diversity is disproportionately more sensitive to both natural and anthropogenic disturbance, such as cyclonic storms, urbanization, habitat degradation, or species introductions (Brooks et al., 2002; Elton, 2000; Jones et al., 2009; McCreless et al., 2016; Myers et al., 2000). Due to their ability to disperse over water, bats are often the only native mammals on islands, especially on remote oceanic islands (e.g., the Hawaiian hoary bat *Lasiurus semotus* in Hawaii; Baird et al., 2015; Jones et al., 2009; Pinzari et al., 2020). More than half of known bat species live on islands, and 25% are endemic to islands (Jones et al., 2009). Island endemic bats provide key services to insular ecosystems, including arthropod regulation, pollination, and seed dispersal (Aziz et al., 2017; Kalka & Kalko, 2006). For example, the flying fox *Pteropus niger* plays an important role in dissemination and regeneration of native plants on the oceanic island of Mauritius in the Indian Ocean (Florens et al., 2017). Endemic bats are considered as a significant conservation concern and represent around 50% of the world's threatened bats (Conenna et al., 2017; Jones et al., 2009). The ability of bat populations to recover after natural catastrophes or human perturbations depends on their size, ability to move, and social behavior (Jones et al., 2009). Therefore, knowledge of the ecology of island bats is crucial for the delineation and success of sustainable conservation projects.

The Reunion free-tailed bat (*Mormopterus francoismoutoui*; Goodman et al., 2008) is a small insectivorous tropical bat endemic to Reunion Island, and is the most abundant bat species compared to the two others living on the island: the Mauritian flying fox (*P. niger*), a fruit bat, and the Mauritian tomb bat (*Taphozous mauritanus*), an insectivorous bat. Reunion Island is a small volcanic territory (2512 km²), that emerged about 3 million years ago (Cadet, 1980) in the south-western Indian Ocean (Mascarene Archipelago), and shaped by a very steep mountainous landscape. The tropical climate of the island is recognized by two seasons: a hot rainy season and the dry season, defined here as summer and winter, respectively. Through 350 years of human colonization, Reunion Island ecosystems have suffered from deforestation, agricultural expansion, and urbanization (Lagabrielle et al., 2009). Despite this extensive landscape fragmentation, the Reunion free-tailed bat is broadly distributed on the island, mostly in the lowland and urbanized areas (Augros et al., 2015; Barataud & Giosa, 2013; Moutou, 1982). Roosts, that are always monospecific, are home to a few hundred to tens of thousands of individuals (Augros et al., 2015; Dietrich et al., 2015). This species occupies a variety of day roosts such as caves and crevices within cliff faces, as well as anthropogenic settings such as buildings and bridges (Goodman et al., 2008). This bat follows a similar pattern to many other species of Molossidae found elsewhere in the world, that have the capacity to adapt to urban or modified habitats (Jung & Kalko, 2011).

Despite being the only exclusive endemic mammal of Reunion Island, little information is available about the biology and population

dynamics of the Reunion free-tailed bat. The size of the island population has never been precisely assessed but could reach several hundreds of thousands of individuals (Augros et al., 2015; Dietrich et al., 2015). Based on the study of the largest known maternity roost (ca. 46,500 adult individuals), Dietrich et al. (2015) confirmed that parturition occurs during austral summer, from December to February, and that the roost is empty during austral winter (June to September), suggesting seasonal movements between roosts (Goodman et al., 2008). However, the mating period remains unidentified, and social organization within and between roosts is largely unknown, as the occupancy of roosts by different types of individuals (male/female, adult/juvenile) has never been investigated. The goal of the present study was to examine the phenology and roosting behavior of the Reunion free-tailed bat. We set up a fine-scale monitoring of multiple roosts all over the island during a 27-month period in order to estimate temporal variations of roost size and composition (age and sex). We evaluated temporal trends in the segregation of individuals within roosts, relative to sex and age classes, and analyzed the effect of roost size on such patterns. We also described the reproductive phenology in this tropical bat species, and measured the influence of factors shaping body condition of individuals.

2 | MATERIALS AND METHODS

2.1 | Study sites and sampling frequency

From October 2018 to December 2020 (27 months), we monitored 19 roosts of the Reunion free-tailed bat, throughout the island (Figure 1). Roosts were investigated in various habitats including two roosts in natural settings (caves and cliffs) and 17 roosts in human constructions (7 roosts in buildings and 10 in bridges; Table 1). Roost name was coded with a 3-letter code. Longitudinal monitoring was performed across successive "sampling periods" occurring every 3–8 weeks. To monitor all roosts within a study period, each sampling period lasted for 3–4 weeks (Table S1). Monitoring included both capture-mark-release of bats and roost size estimation.

2.2 | Bat capture

In total, longitudinal captures were performed in 12 roosts (from 5 to 17 sampling periods depending on the roost, Table 1). Because of time and logistic constraints, the 7 remaining roosts were only sampled once, during known periods of roost occupation. For most sampling periods, the majority of bats ($n = 4978$, 74%) were captured during the dusk emergence, reaching a maximum of 60 individuals (over one or two consecutive nights if no or only few bats were captured during the first night). We mainly used harp traps (Faunatech Ausbat) and Japanese monofilament mist nets (Ecotone) installed close to the roost exit, without completely blocking the exit of bats. Because it was not possible to set up traps at the exit of some

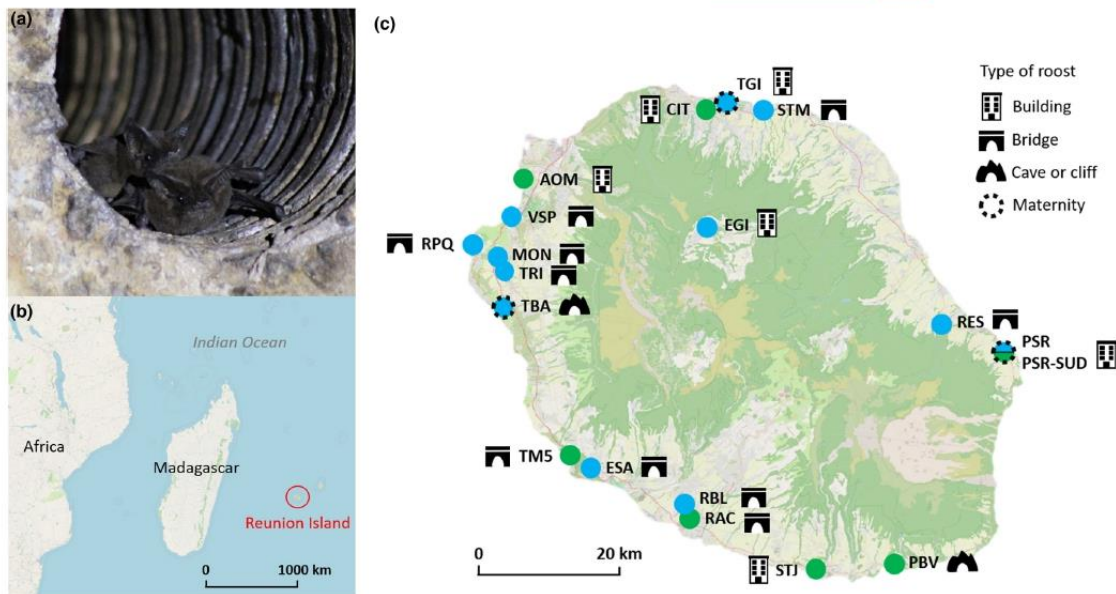


FIGURE 1 Sampling sites of the Reunion free-tailed bat (*Mormopterus francoismoutoui*). (a) A photograph of bats roosting inside a pipe in a bridge. (b, c) Location of Reunion Island and the studied roosts with the green color indicating forest areas and the pale indicating urbanized area. The twelve roosts in blue were monitored regularly over the 27-month study while the seven roosts in green were sampled only once. PSR and PSR-SUD roosts are located in the same building. Maps were created using Qgis (QGIS.org, 2022) with background of OpenStreetMap under License CC-BY-SA 2.0. Photo credit: Samantha Aguilon.

roosts, a butterfly net was also used by carefully approaching resting (nonflying) individuals during the day. After capture, bats were immediately hydrated using a sterile syringe and water, placed in a clean individual bag close to a warm source (hot water bottle), and processed at the capture site. To limit disturbance to bats at critical times of the year, trapping was not undertaken from the beginning of the parturition period (December) until the time there were no more pink-colored newborns in the maternities (around mid-January), nor on nonflying juveniles.

We visually determined the sex, age, and reproductive status of each individual. Age was determined by examining the epiphysis fusion in finger articulations that are not welded for juveniles. Juveniles were reasonably identifiable until July. After, it starts being difficult to see unfused finger joints, and some juveniles might have been classified as adults. Newborns were not captured but a visual inspection of the roosts, whenever possible, was performed to check for the presence of small size and pink-colored individuals. Nonbreeding females were classified in accordance with the visibility of nipples: nonvisible (M0) or visible but noninflated (M1). Reproductively active females were recorded as pregnant, lactating, and postlactating. Pregnancy was determined by slight palpation of the abdomen, and the presence of inflated nipples (Figure 2a). Lactating females were defined when they had chewed inflated nipples (M2), and postlactating when hair regrowth was visible around the nipples, which were noninflated and chewed (M3). Males with large testes were considered reproductively active. It should be noted that testes are difficult

to observe in this species (Figure 2b). For each bat, we also measured the forearm length with a caliper and the mass using an electronic scale for each bat. To identify all individuals, bats were tattooed using a dermograph with black ink on the right propatagium with an individual alphanumeric code. Finally, each individual bat was immediately released at the capture site after being processed.

Handling of bats was performed using personal protective equipment (FFP2 masks, nitrile gloves, as well as Tyvek suits and respirator cartridge masks inside the cave). Gloves were disinfected between each bat individual and changed regularly, and all the equipment was disinfected between sites as well. Bat capture and manipulation were evaluated by the ethic committee of Reunion Island, approved by the French Ministry of Research (APAFIS#10140-2017030119531267), and conducted under a permit delivered by the Direction de l'Environnement, de l'Aménagement et du Logement (DEAL) of Reunion Island (DEAL/SEB/UBIO/2018-09).

2.3 | Roost size estimation

A longitudinal monitoring (at least 5 different estimations) of the roost size was set up for 10 of the 12 monitored roosts (ESA, MON, RBL, RES, RPQ, STM, TBA, TGI, TRI, and VSP), because of the difficulty to count bats in PSR (no counts at all) and EGI (only 2 counts possible) buildings. For the nonmonitored roosts (AOM, CIT, PBV, RAC, STJ and TM5), roost size was estimated visually once over the

TABLE 1 Roost characteristics of the Reunion free-tailed bat (*Mormopterus francoismoutoui*).

Roost	Roost size estimation		Number of captured individuals					Mean male %		
	Habitat	Method	Minimum and maximum (number of estimations)	Number of sampling periods	Total	Male	Female		Adult	Juvenile
AOM	Building	Chamber	2560 (1)	1	57	33	24	35	22	57.89
CIT	Building	Joints	50 (1)	1	37	37	0	37	0	100
EGI	Building	At emergence	0–620 (2)	5	269	167	102	231	38	62.08
ESA	Bridge	Joints	570–3200 (15)	16	730 (+1 indetermined age)	349	381	651	78	47.81
MON	Bridge	Joints	197–1150 (16)	16	557	381	176	504	53	68.40
PBV	Cliff	At emergence	300 (1)	1	11	7	4	9	2	63.64
PSR	Building	-	-	16	615	480	135	586	29	78.05
PSR-SUD*	Building	-	-	1	52	26	26	9	43	50
RAC	Bridge	Joints	153 (1)	1	16	12	4	16	0	75
RBL	Bridge	Joints	350–1300 (16)	16	734 (+1 indetermined age)	362	372	633	100	49.32
RES	Bridge	Chamber & joints	35–420 (9)	9	301	163	138	268	33	54.15
RPQ	Bridge	Joints	150–3000 (13)	16	601 (+1 indetermined age)	240	361	573	27	39.93
STJ	Building	At emergence	200 (1)	1	39	26	13	39	0	66.67
STM	Bridge	Chamber	27–350 (13)	13	451	336	115	428	23	74.50
TBA*	Cave	Covered surface	0–100,000 (12)	13	607	127	480	372	235	20.92
TGI*	Building	At emergence	14–1270 (35)	16	641	371	270	501	140	57.88
TMS	Bridge	Joints	200 (1)	1	30	17	13	0	30	56.67
TRI	Bridge	Joints	115–251 (5)	5	164	118	46	147	17	71.95
VSP	Bridge	Chamber & joints	15–6000 (17)	17	809	506	303	724	85	62.55
Total	-	-	-	165	6721	3758	2963	5763	955	-

Note: Maternity roosts (where parturition occurred) are indicated with an asterisk (*) next to the roost name. The number of sampling periods provide information on the monitoring frequency. The male percentage is calculated over all sampling periods.

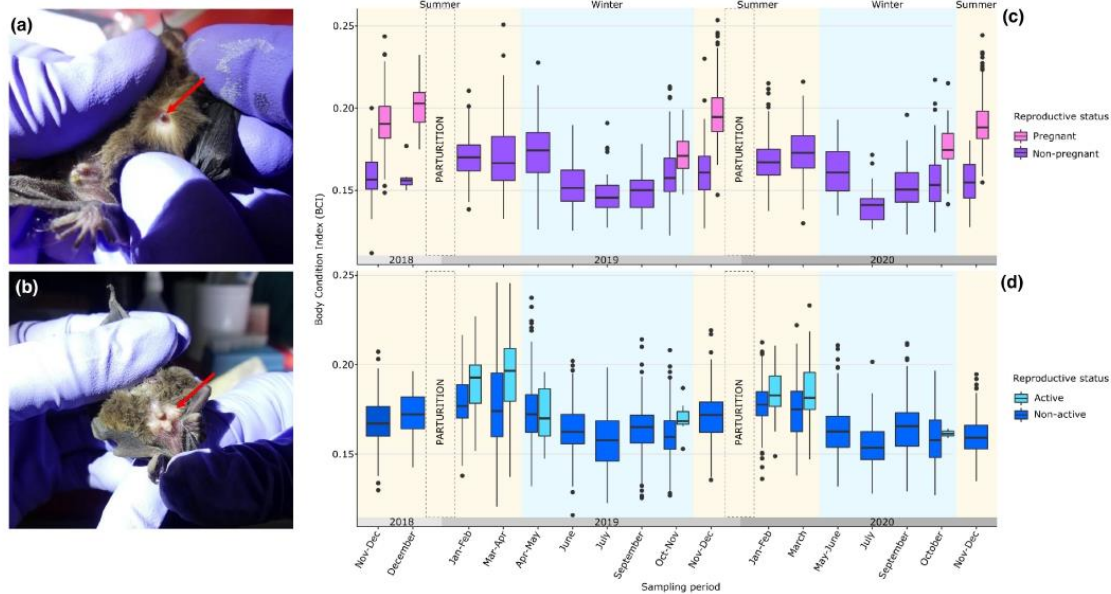


FIGURE 2 Visual indicators of the reproductive status of the Reunion free-tailed bat and temporal variation of the Body Condition Index (BCI) according to the reproductive status. (a) Pregnant female with inflated nipples. (b) Male with large testes. (c) Temporal variation of adult female's BCI according to pregnancy status. (d) Temporal variation of adult male's BCI according to reproductive status (active or not). Summer and winter periods correspond to rainy and dry seasons and are indicated by yellow and blue backgrounds. Photo credit: Muriel Dietrich.

study period, except for PSR-SUD (no count at all). Roost size estimations were conducted either the day of a capture session or up to 14 days before or after capture, because of logistic constraints. To estimate roost size, different methods were used according to roost type (Table 1). Because this species emerges during the dusk when the sunset is not over, and light condition is favorable, bats were visually counted at emergence when possible. For the cave, because counting at emergence is challenging with the high number of bats, estimation was based on the size of the bat-covered surface in m^2 , observed during the morning, multiplied by the visually assessed density of individuals ($900 \text{ bats}/m^2$), as in Héré et al. (2009). For bridges and some buildings with multiple exits, making counts at emergence was difficult and we rather visually counted during the day, when bats were resting bats and easily visible in joints and chambers.

2.4 | Statistical analyses

Variation of roost size over time was investigated using a generalized additive mixed model (GAMM) with a quasi-distribution and identity link, including the sampling period as a fixed effect, and the roost and roost size estimation method (joints or chambers/at emergence/covered surface) as random effects. When capture was undertaken over 2 days for the same roost, and thus roost size was estimated twice, we used the mean size calculated over the 2 days.

Sex-ratio (with 95% confidence interval) was first estimated globally by calculating the proportion of males captured over the study in the 19 roosts. Bias differences were tested using a χ^2 test. Temporal variation of the sex-ratio was then investigated using a linear mixed model (LMM), including sampling period as a fixed effect and roost as a random effect. We also examined the influence of roost size (expressed as its logarithm) on sex-ratio patterns using a Pearson correlation test. Finally, the sex-ratio was estimated for adults and juveniles separately, and differences from a 1:1 ratio were tested for each sampling period, using χ^2 tests.

We investigated how adults of each sex aggregate or segregate among roosts. We used the Sexual Segregation and Aggregation Statistic (SSAS), a test comparing observed aggregation/segregation patterns to a random association of males and females (Bonenfant et al., 2007), and tested whether SSAS was correlated to the sex-ratio using a Pearson correlation test. The test of SSAS estimates the confidence limits (CL) around the observed values among sampling periods, specific to each species (Bonenfant et al., 2007). By comparing SSAS values with the CL, we can determine if individuals are segregated (SSAS values above CL), aggregated (SSAS values below CL) or randomly associated (SSAS values in CL). To analyze if segregation patterns were positively correlated to the roost size, we calculated, for each sampling period, the global roost size (sum over all roosts, expressed as its logarithm), and the global SSAS and used a Pearson correlation test.

Reproductive phenology was first analyzed by identifying active periods in females (pregnancy, lactation, and postlactation) and males (large testes as a proxy of mating), as well as the presence of newborns and flying juveniles. Then, patterns of aggregation and segregation among roosts between adults and juveniles were examined using the SSAS. Age segregation was analyzed in correlation with the percentage of juveniles using a Pearson correlation test.

Sexual dimorphism occurrence was investigated first in adults, by testing the difference in forearm length between sexes, using a LMM including the roost as a random effect to account for potential geographic variation (excluding data for recaptured bats to avoid redundant data). Sexual dimorphism in juveniles was also analyzed by measuring the growth of the forearm over time using a LMM including sex and date as fixed effects (and the interaction), and roost and year as random effects (excluding recaptured bats) to control for potential difference of juveniles growth between years. The relationship between the forearm length and the mass, including adults and juveniles, was tested using a Pearson correlation test.

Subsequently, the individual body condition was estimated using the Body Condition Index (BCI) by calculating the mass/forearm length ratio. Variations in BCI were investigated using a LMM including age and sex (and the interaction) as fixed effects and roost as random effects, followed by Tukey's post hoc tests. Sampling period was also added as a random effect to accommodate for potential within-year variations. Then, BCI was analyzed in adults using a LMM in relation to the reproductive status (active or not; as a fixed effect), also including roost and sampling period as random effects. Analyses were performed in adult females and adult males, separately. Because aggregation phenomenon (increase in roost size) can represent a cost or a benefit for the fitness of individuals (Brown, 2016), we tested if the roost size could affect the body condition of reproductive individuals. We focused the analysis on the pregnancy period in females (November–December) and on the mating period for males (March, see results). BCI variation was analyzed using a LMM in relation to the roost size (expressed as its logarithm) and reproductive status (and the interaction) as fixed effects, including the roost as a random effect. We also included the year and date as nested random effects to accommodate for temporal variations of the BCI within the pregnancy and mating periods.

All analyses were performed in Rstudio v.1.4.1106 (Rstudio Team, 2020) using packages *date*, *dplyr*, *emmeans*, *ggplot2*, *lme4*, and *mgcv*. For LMM analysis, the most parsimonious model was selected

by excluding step by step every nonsignificant variable of the complete model and using an ANOVA test to compare models. All the models used are presented in Table S2.

3 | RESULTS

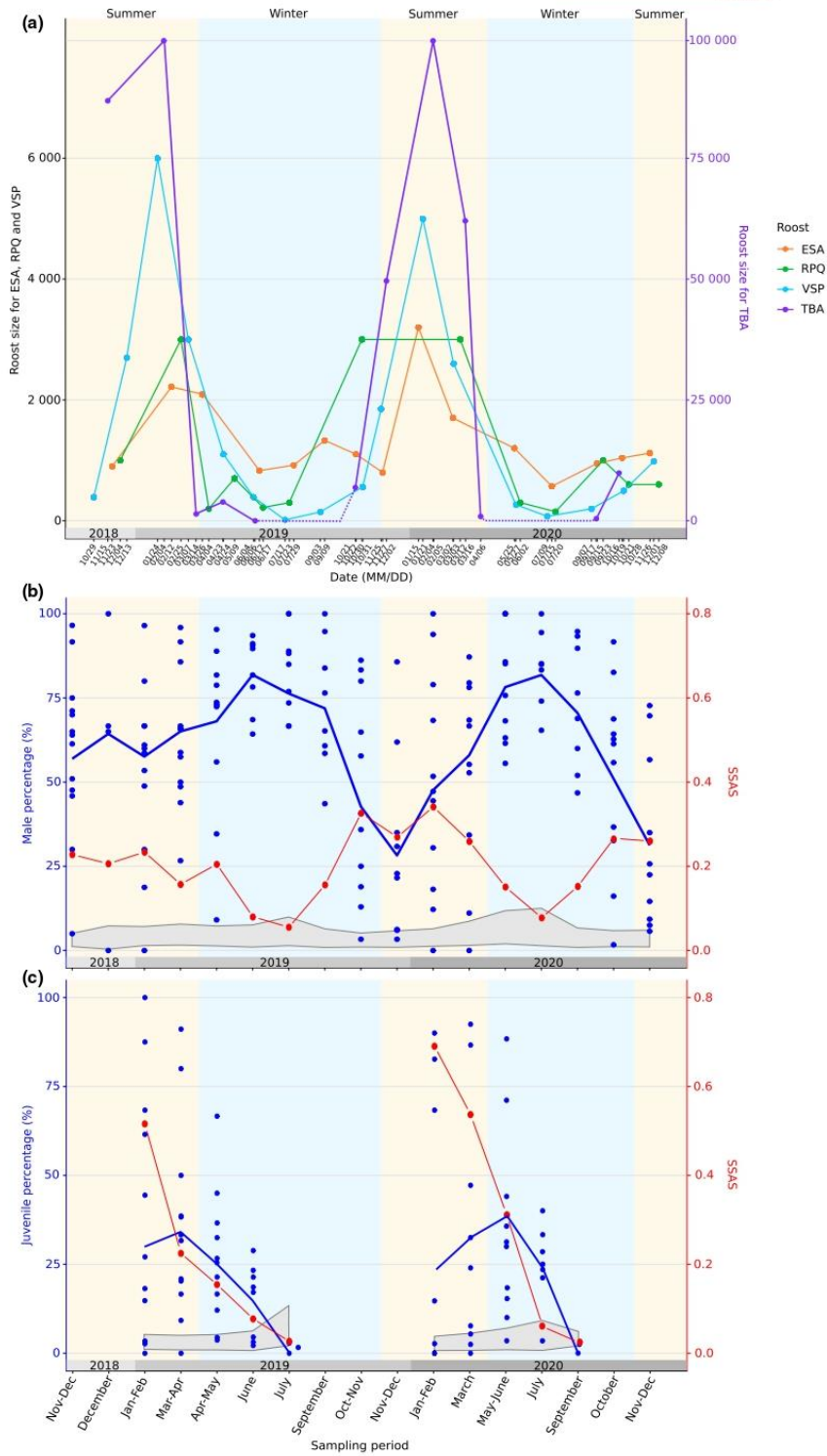
3.1 | Temporal evolution of roost size

Over a 27-month period, we gathered 200 estimates of roost size for 17 different roosts. Estimates of roost sizes were from 0 to 100,000 individuals (details in Table 1). A marked temporal variation of the roost size was found (GAMM₁: $p < .001$; Table S2). In particular, a synchronized increase in roost size during austral summer, starting in October and reaching a maximum around January and February, was observed (Figure 3a for biggest roosts and Figure S1 for the others). Increase in roost size during summer was particularly massive in the TBA and VSP roosts; in TBA, the number of bats went from 0 to 100,000, and in VSP the roost size increased by a factor of 400 (Figure 3a). During austral winter, low numbers of bats (sometimes none) were generally observed, except in TGI and MON roosts where larger numbers were present (Figure S1).

3.2 | Sex-ratio and sexual segregation

Over the 17 sampling periods, we captured 6721 bats in 19 roosts (Table 1). The global sex-ratio was male-biased ($56 \pm 1.2\%$ of males; $\chi^2_1 = 46.95$, $p < .001$), and varied among roosts (LMM₂: $\chi^2_{16} = 71.51$, $p < .001$) as well as over sampling periods (LMM₂: $\chi^2_{16} = 92.46$, $p < .001$; Figure 4 and Table S2). However, this male-biased sex-ratio was driven by adults ($57 \pm 1.3\%$ of males; $\chi^2_1 = 58.49$, $p < .05$) since sex-ratio was at equilibrium in juveniles ($49 \pm 3.2\%$ of males; $\chi^2_1 = 0.28$, $p > .05$). Most of the studied roosts harbored a majority of males all year long, although others have hosted either more males or more females, and even a balanced sex-ratio, depending on the time of the year (Figure 4). A female-biased sex-ratio was observed in a single roost (TBA), being the largest maternity roost (where parturition occurred) known on the island. Similarly, we found that larger roost size was significantly associated with a lower percentage of males within roosts ($r^2 = -0.70$, $p < .001$; Figure 5a).

FIGURE 3 Temporal variation of (a) roost size and (b, c) segregation patterns of the Reunion free-tailed bat. (a) For better visualization, roost size is only presented for the four largest roosts. Data for the other roosts can be found in Figure S1. Roost size scale is presented on both axes because of large differences for the TBA roost size. The scale for TBA roost is indicated on the right of the graph (in purple) and portion of dotted line correspond to the period when the cave is known to be empty based on earlier studies (Dietrich et al., 2015). (b, c) Sexual Segregation and Aggregation Statistic (SSAS) was calculated per sampling period, when the value is up to the gray zone it indicates that bats are segregated and when value is within the gray zone it indicates that bats are randomly associated in roosts. In (b) SSAS represents the segregation between sexes in adults. For each sampling period, male percentage in the multiple roosts (blue points), and its mean (continuous blue line) are also indicated. In (c) SSAS represents the segregation between age classes (adults vs. juveniles). Juveniles cannot be reasonably distinguished after June. For each sampling period, the percentage of juveniles is also represented for the multiple roosts and the continuous line represents the mean. Summer and winter periods correspond to rainy and dry seasons and are indicated by yellow and blue backgrounds.



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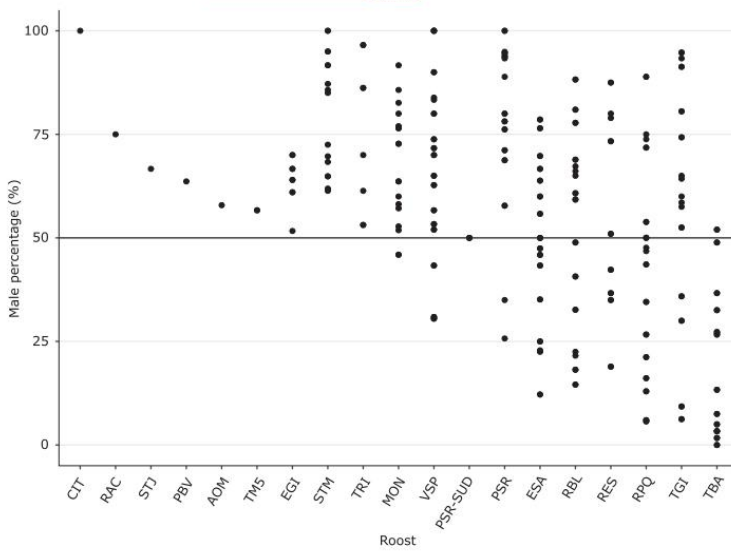


FIGURE 4 Sex-ratio variation between roosts. For each roost, multiple points represent the percentage of males at different sampling periods. For 7 roosts, they were sampled only once and only one point is represented.

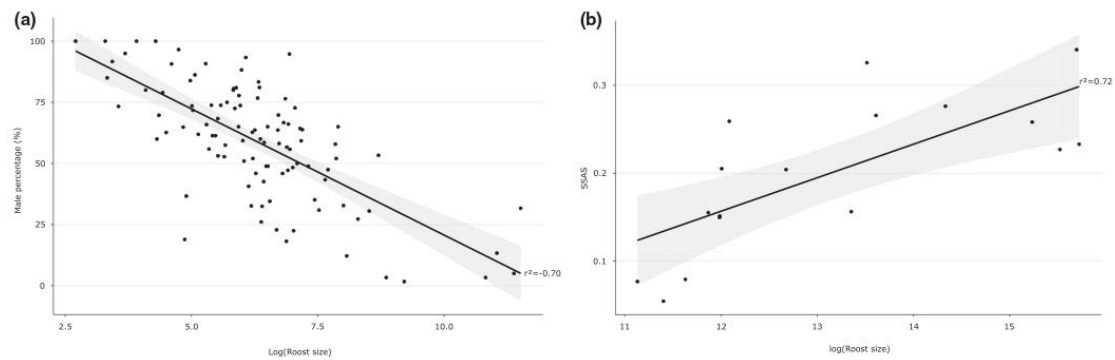


FIGURE 5 Correlation of the roost size (expressed as its logarithm) with (a) the sex-ratio and (b) the Sexual Segregation and Aggregation Statistic (SSAS) in adults.

We found that the sex-ratio was correlated to the segregation pattern between sexes as measured by the SSAS ($r^2 = -0.84, p < .001$; Figure S2). Indeed, the analysis of sex-ratio in adults over time revealed a significant female-biased sex-ratio in summer (from October to December; Figure 3b and Figure S3), which also correlated to a clear segregation pattern between sexes at this period, highlighted by the increase in the SSAS. In contrast, in winter (June and July), the percentage of males was particularly high, and males and females were more randomly aggregated within roosts. Consequently, the segregation pattern observed between sexes was also positively associated with larger roost sizes ($r^2 = 0.72, p < .001$; Figure 5b).

3.3 | Reproductive phenology

A total of 201 reproductively active males (with large testes) were captured. Most (95%) were synchronously observed from late January to April, both in 2019 and 2020 in 12 roosts, suggesting that the mating

period probably occurred during these months (Figure 6). A few reproductively active males (5%) were also observed in October both years. A high percentage of reproductively active males were observed in male-biased sex-ratio roosts (MON, PSR and STM).

Pregnant females represented 14% of total captured bats and were observed from October to December, in almost all roosts sampled at this period ($n = 11$). The three roosts with the highest percentage of pregnant females in the November–December sampling period were TBA, TGI, and RPQ, reaching 86% of captured individuals in TBA and TGI (in 2018 and 2020 respectively), and 92% in RPQ (in 2019). Despite the fact that pregnant females were spread across roosts, parturition occurred in just a few roosts: TBA, TGI, and PSR-SUD, based on the observation of newborns from early December to late January. After the parturition break for captures (December to mid-January), we caught lactating and postlactating females in 11 roosts until March.

Although parturition occurred only in a few roosts, flying juveniles were observed in almost all roosts from January to September

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FIGURE 6 Hypothesized reproductive cycle of *Mormopterus francoismoutoui* based on a fine-scale monitoring of 19 roosts in Reunion Island over a 27-month period.

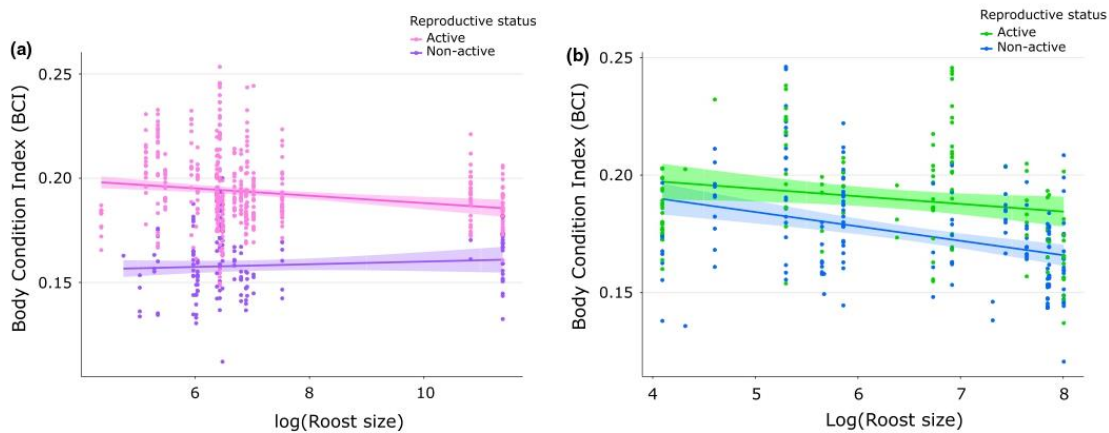
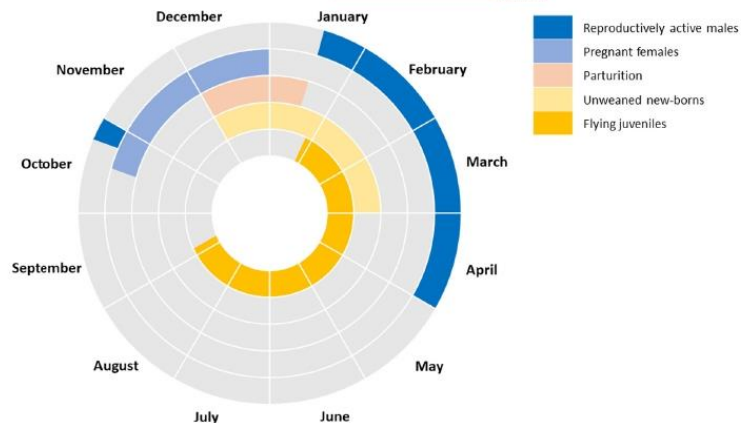


FIGURE 7 Correlation between the body condition (BCI) and the roost size (expressed as its logarithm) in (a) adult females according to their reproductive status during the pregnancy period (active status includes pregnant and a few lactating females), and (b) adult males according to their reproductive status during mating period (active status corresponds to males with large testes).

(Figure 6, Figure S4 and Table 1), with the highest numbers in TBA and TGI roosts (39% and 22% of juveniles captured over the study, respectively). Flying juveniles were captured 39 and 46 days after the first observation of parturition, in 2019, and 2020, respectively. The SSAS showed that juveniles and adults were particularly segregated in January and February, and then tended to be randomly distributed toward winter months (Figure 3c). However, this segregation pattern between ages was not correlated to the percentage of juveniles in the bat population ($r^2 = 0.58, p > .05$, Figure 3c). Finally, we can notice that TM5 (sampled mid-February) was a temporary roost, only composed of presumably recently weaned juveniles (Figure S4).

3.4 | Sexual dimorphism and body condition

Although the difference was subtle, adult males had a significant longer forearm (mean = 39.28 mm, SE = 0.013), as compared to adult females (mean = 39.19 mm, SE = 0.015) (LMM₃; $\chi^2_1 = 21.40, p < .001$; Figure S5 and Table S1). The analysis on juveniles suggested the

same pattern (LMM₄; $\chi^2_1 = 4.84, p = .03$) and also showed forearm length increasing with time (LMM₄; $\chi^2_1 = 18.13, p < .001$, Figure S6). Furthermore, the relation between the forearm length and the mass was significantly positive ($r^2 = 0.21, p < .001$; Figure S7).

The BCI value of individuals was strongly dependent on age and sex, with an interaction between both variables (LMM₅; $\chi^2_1 = 22.73, p < .001$; Table S2). As expected, adults had a higher BCI value than juveniles (LMM₅; $\chi^2_1 = 906.93, p < .001$). In adults, females showed a higher BCI value than males (Tukey's post hoc test: $p = .02$), while in juveniles, males were in better condition (Tukey's post hoc test: $p = .02$, Figure S8). Analysis over time revealed a strong variation of BCI for adults (Figure 2c,d): females showed an increase BCI during November–December months corresponding with pregnancy (LMM₆; $\chi^2_1 = 605.36, p < .001$), whereas in males, the increase occurred later around March (LMM₇; $\chi^2_1 = 445.27, p < .001$; Table S2). This increase was associated with a significant positive effect of the reproductive status for both females (LMM₆; $\chi^2_1 = 349.13, p < .001$) and males (LMM₇; $\chi^2_1 = 103.61, p < .001$; Table S2). For both sexes, a continuous decrease of the BCI was then observed during winter (Figure 2c,d).

Analysis throughout the pregnancy period showed a negative effect of roost size on the BCI, only for reproductively active females (LMM₈: $\chi^2_1 = 5.23$, $p = .02$; Table S2), which were thus in better condition in smaller roosts (Figure 7a). However, this result was no longer significant when the same analysis was performed excluding the large maternity roost of TBA (LMM₉: $\chi^2_1 = 0.001$, $p = .97$; Table S2). For males, analysis throughout the mating period showed no significant correlation between the roost size and the BCI of active or nonactive males (LMM₁₀: $\chi^2_1 = 1.09$, $p = .29$; Figure 7b and Table S2).

4 | DISCUSSION

Based on an intensive and fine-scale study of multiple roosts, we revealed a highly dynamic roosting behavior and phenology of the Reunion free-tailed bat. Longitudinal monitoring first confirmed the large population size of this bat species, probably far exceeding 120,000 individuals all over the island, and representing a mean density of bats of at least 48 bats/km². This relative large population size could result from the low number of indigenous predators, which is typically encountered in small oceanic islands (Lomolino et al., 2013). The Reunion free-tailed bat could also take advantage of the growing urbanization of the island, which creates attractive areas with a huge variety of suitable roosts in terms of volume and configuration (such as chambers within bridges) (Goodman et al., 2008). In addition, this bat species is likely experiencing low competition for resources, as the only another insectivorous bat present on the island, the Mauritian tomb bat, is far less abundant (O'Brien, 2011). Despite such a large population size, the Reunion free-tailed bat is facing displacement from its roosts, as its excrement and musky smell leads to considerable nuisance for humans (Augros et al., 2015). Significant effort has been devoted to roost translocation strategies, but because of mitigated results due to unsuccessful operations, such roost disturbance may represent a threat for the conservation of this bat species.

Over the 19 studied roosts, a strong heterogeneity in roost size and sex-ratio was observed, suggesting a complex social organization and distinct functions of the different roosts. The population is composed of a continuum of roosts, encompassing male, mixed, maternity (female-biased) roosts, and juvenile roosts, all probably interconnected by important seasonal movements. Although we cannot exclude a higher mortality in females, we rather think that the global male biased sex-ratio found in this study is linked to a sampling bias toward adult males because adult females seem spatially and temporally restricted to a few roosts (see below) and were difficult to capture during winter especially. The even sex-ratio observed in juveniles adds support to this hypothesis.

The strong heterogeneity in roost size and sex-ratio was characterized by marked seasonal variation. In the majority of roosts, cyclic and synchronized occupation peaks were recorded during austral summer, coinciding with a global female-biased sex-ratio during this period. This is coherent with a widespread aggregation of pregnant females within roosts, and segregation between males and

females. Such aggregation behavior is commonly observed in bats, for example, in the Brazilian free-tailed molossid *Tadarida brasiliensis*, which forms the largest maternity roosts in the mammal world (McCracken & Wilkinson, 2000). Seasonal sexual segregation has also been documented in many bat species, especially temperate bats (Encarnaçao, 2012; Katsis et al., 2021) but also tropical bats (Cheng & Lee, 2004; Llaven-Macias et al., 2021) such as the Madagascar sucker-footed bat *Myzopoda aurita* (Ralisata et al., 2010). Such roosting behavior has been associated with different thermoregulatory strategies and energetic requirements, or even competition for food (Encarnaçao, 2012; Katsis et al., 2021; Levin et al., 2013). Here, we found that the body condition of pregnant females decreased in larger roosts (although this relationship was driven by the big TBA maternity roost). Our results thus suggest some costs of aggregation for pregnant females, with for example a higher parasitism and infection risk (Lourenço & Palmeirim, 2007; Lučan, 2006; Reckardt & Kerth, 2009). Indeed, previous studies in the Reunion free-tailed bat showed an increased prevalence in bacterial and viral infections during the pregnancy period (Dietrich et al., 2015; Joffrin et al., 2022).

Surprisingly, while pregnant females were observed in almost all roosts, we only identified three maternities where parturition occurred synchronously (TBA, TGI, and PSR-SUD). These results suggest dispersal of late-pregnant females for parturition, as seen in other bat species, such as the Soprano pipistrelle *Pipistrellus pygmaeus*, which moves to the maternity roosts a few days before parturition (Bartonička & Gaisler, 2007). In several bat species, energy conservation is an important selective pressure for pregnant females which select warmer roosts, thus reducing costs of maintaining normothermy or forced torpor (López-Baucells et al., 2016; Lourenço & Palmeirim, 2004; Sedgely, 2001; Vonhof & Barclay, 1996). Interestingly, the three maternity roosts in our study were located in a huge natural cave where bat density is very high, in an electric building (releasing heat), and under a sheet metal roof (accumulating heat during the day), all of these conditions probably offering warm conditions. Deciphering the environmental conditions of these maternity roosts would help understanding microhabitat preferences in the Reunion free-tailed bat, critical for effective conservation.

We observed a segregation pattern between adults and juveniles, particularly high in January and February. This may be explained by the fact that parturition occurred only in a few roosts, and that lactating females likely moved to other day-roosts than the maternity roosts, at least when their young are already a few weeks old. Indeed, lactating females we caught, from mid-January until March, were observed in 11 roosts, and for 9 of them, no parturition occurred. It is important to notice that around mid-January, young were probably already a few weeks old. This suggests that, after a few weeks after birth, lactating females switched day-roosts and went back to maternity roosts at night to suckle their nonflying young until weaning. Roost switching behavior can result from an adaptation of female bats to avoid parasite infestation (Bartonička & Gaisler, 2007). Indeed, although not measured in this study, high levels of ectoparasites on bats were observed at the time of parturition, particularly on newborns. Our data also suggest that juveniles

of the Reunion free-tailed bat started dispersing rapidly from maternity roosts—about 6 weeks after birth, which is consistent with the decrease over time of the segregation pattern between adults and juveniles. In the molossid bat *T. brasiliensis*, juveniles begin to fly after 5–6 weeks and are weaned 2 weeks after the first flight (Boero et al., 2020). Radio-tracking of individuals, associated with the analysis of parasitism/infection levels, are necessary to better understand roost switching and dispersal behavior of the Reunion free-tailed bat.

The reproductive cycle of males of the Reunion free-tailed bat has never been documented. Surprisingly, our study revealed that males have large testes synchronously in all roosts around March. These males were in better condition than nonreproductively active males, which could be the consequence of an accumulation of energy reserve for mating to support the reproduction cost (Racey & Entwistle, 2000). Together, these results suggest that mating behavior likely occurred around March in the Reunion free-tailed bat, a considerable time from the parturition period, and thus implying a long interruption of the reproductive cycle (about 7 months between mating and the start of pregnancy). This phenomenon is commonly observed in temperate bats, where mating occurs in autumn before hibernation, while parturition is in summer, as shown in the greater mouse-eared bat *Myotis myotis* and Natterer's bat *Myotis nattereri* (Pfeiffer & Mayer, 2013). Several mechanisms can be involved in reproductive delay like sperm storage, delayed implantation or delayed development of the embryo (Crichton, 2000; Pfeiffer & Mayer, 2013), and can be linked to daily torpor in tropical bat species (Racey & Entwistle, 2000). The reproductive cycle of the Reunion free-tailed bat seems to be similar to the south-eastern free-tailed bat *Momopterus planiceps*, a molossid living in southeast Australia, having a single mating per year with sperm storage (Racey & Entwistle, 2000).

However, some males with large testes were also observed in October (coinciding with the beginning of the pregnancy period), and thus suggesting a second mating event, potentially in response to receptive females failing to fertilize in March. Such behavior has been documented for the European brown long-eared bat *Plecotus auritus* males that could fertilize females after hibernation by storing sperm until spring (Pfeiffer & Mayer, 2013). In temperate regions, autumn swarming in specific underground sites is thought to be linked to mating (Furmankiewicz & Altringham, 2007; Kerth et al., 2003). Here, we observed males with larger testes in all the studied roosts, but this does not mean that mating is widespread among all roosts. The use of infra-red cameras to study bat behavior within roosts may help identifying mating sites, which has crucial implications for conservation (Furmankiewicz, 2016; Neubaum & Siemers, 2021; Piksa & Bogdanowicz, 2011).

The end of austral summer was characterized by a decrease in roost size and a male biased sex-ratio, suggesting that individuals, mainly females, leave the studied roosts. In addition, during austral winter, the body condition for both males and females drastically decreased, which may be linked to some limitation in food resources and the use of daily torpor. This mechanism reduces metabolism and body temperature in response to a reduced food availability during winter (Racey & Entwistle, 2000). Even if occupancy in some roosts

remains important during winter, we hypothesize that bats, and especially females, live in small groups or even solitary during winter (Augros et al., 2015), especially because we have never observed large groups of females during winter. Reunion island is a small oceanic island, and thus straight distances possibly traveled by bats are probably less than 75 km (longest diagonal). However, the island is highly mountainous (highest peak standing at 3070 m), offering possibilities for short-distance movements. For example, one natural roost was observed at 2070 m in October (Sanchez & Probst, 2013), showing that this bat species is able to live in high-elevation roosts. Although altitudinal bat migration appears more common in bats in the temperate regions than in the tropics, few cases have been described in tropical species (McGuire & Boyle, 2013). For example, in the lesser long-nosed bat *Leptonycteris verbabuena*, after juveniles start flying, females move to higher elevations to search for more resources. These migrations are sex biased as males remain in the southern portion of the range until mid-summer, and join the reproductive females at the northern high-elevation sites only in the autumn (Cockrum, 1991). Whether the Reunion free-tailed bat uses the same migration strategy is unknown, but further telemetry and genetic studies of bats sampled at higher altitudes will help test this hypothesis and eventually identify wintering roosts (Johnson et al., 2015, 2017; Michaelsen et al., 2013; Moussy et al., 2013).

Our study is the first comprehensive description of the biology of the Reunion-free tailed bat based on extensive capture data. The fine-scale sampling scheme implemented here was necessary to reveal the complex social organization in this bat species. The Reunion free-tailed bat provides a relevant example of how dynamic are sex-specific behavioral strategies in bats, even for tropical species living in small oceanic islands. Information provided by our study will be critical to the delineation of effective conservation plans, especially in the context of growing urbanization and agriculture expansion in Reunion Island. Further investigations including bat tracking, roosting conditions, and genetic studies are warranted to better understand the seasonal and spatial movements of individuals within the island. Our study highlights that fine-scale monitoring of island bat populations is crucial to help conservation and management of these highly threatened mammal species.

AUTHOR CONTRIBUTIONS

Samantha Aguilon: Conceptualization (equal); formal analysis (lead); investigation (equal); visualization (lead); writing – original draft (lead); writing – review and editing (equal). **Gildas Le Minter:** Investigation (equal); writing – review and editing (equal). **Camille Lebarbenchon:** Funding acquisition (equal); investigation (equal); writing – review and editing (equal). **Axel O. G. Hoarau:** Investigation (equal); writing – review and editing (equal). **Céline Toty:** Investigation (equal); writing – review and editing (equal). **Léa Joffrin:** Investigation (equal); writing – review and editing (equal). **Riana V. Ramanantsalama:** Investigation (equal); writing – review and editing (equal). **Stéphane Augros:** Investigation (equal); writing – review and editing (equal). **Pablo Tortosa:** Funding acquisition (equal); investigation (equal); writing – review and editing (equal). **Patrick Mavingui:** Supervision

(equal); writing – review and editing (equal). **Muriel Dietrich:** Conceptualization (lead); formal analysis (equal); funding acquisition (equal); investigation (lead); supervision (equal); validation (lead); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

We have no competing interests to declare.

DATA AVAILABILITY STATEMENT

Data and analysis script are archived on Zenodo (with embargo until February 1st 2024) (<https://doi.org/10.5281/zenodo.7540372>).

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SUPPORTING INFORMATION

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II. Histoire évolutive et structure génétique du Petit Molosse

Suite à l'étude du cycle biologique, nous avons analysé 1136 individus de Petits Molosses provenant de 18 gîtes, en utilisant deux marqueurs génétiques en parallèle : un gène mitochondrial et 10 marqueurs microsatellites nucléaires. L'histoire évolutive du Petit Molosse a tout d'abord été analysée grâce à une approche phylogénétique à l'échelle régionale, en combinant des échantillons de chauves-souris *Mormopterus* des îles voisines (Madagascar et Maurice), afin de retracer et dater les événements de colonisation. Ensuite, en utilisant une approche de génétique des populations à une échelle locale, nous avons analysé la diversité et la structure génétique du Petit Molosse au sein de l'île de la Réunion. Nous avons notamment testé l'hypothèse d'une philopatrie importante chez les femelles, en comparaison des mâles, suite à nos résultats présentés dans l'article 1 où une agrégation annuelle et massive de femelles gestantes dans les gîtes avait été observée, et pouvait mener à une structure spatiale chez les femelles à cette période. Les variations importantes d'effectifs que nous avons observés dans les gîtes suggéraient également des mouvements saisonniers, pouvant mener à une structure génétique entre gîtes plus ou moins forte au cours du cycle biologique. Nous avons donc testé cette hypothèse en analysant si la structure génétique variait entre trois périodes du cycle biologique (en été pendant la gestation puis l'accouplement, et en hiver), et vérifié en particulier l'hypothèse d'un brassage génétique pendant la période d'accouplement. Enfin, le Petit Molosse étant une espèce synanthropique, nous avons testé l'hypothèse d'une expansion récente de sa population liée à l'urbanisation de l'île. Les analyses suggèrent un seul événement de colonisation du Petit Molosse, suivi d'une diversification *in situ* et de l'évolution de lignées ancestrales, vivant aujourd'hui en sympatrie au sein d'une grande métapopulation sur l'île. En effet, nous n'avons pas détecté de structure spatiale, ni de changement de cette structure au cours du cycle biologique, et seulement un faible signal de philopatrie chez les femelles. Cette étude montre l'importance d'utiliser des marqueurs aux propriétés différentes afin de mieux comprendre les processus évolutifs passés et actuels impliqués dans les processus de diversification. Ces résultats font l'objet d'un article en révision dans le journal *Global Ecology and Conservation* (**Article 2** et Annexe 2).

Article 2

En révision dans Global Ecology and Conservation.

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1 **Stuck on a small tropical island: wide *in-situ* diversification of an urban-**
2 **dwelling bat**

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20 **Short title:** Diversification of an island endemic bat

21 **Abstract**

22 Island endemic bats are a considerable cause of conservation concerns, as islands are
23 vulnerable ecosystems facing natural and anthropogenic threats such as growing
24 urbanization. Here, we studied the Reunion free-tailed bat (*Mormopterus francoismoutoui*),
25 an endemic species to Reunion Island that has adapted to urban settings. We investigated the
26 evolutionary history of *Mormopterus* at a regional scale, as well as on Reunion Island sex-
27 specific and seasonal patterns of genetic structure. We used an extensive spatio-temporal
28 sampling including 1,136 individuals from 18 roosts and three biological seasons (non-
29 reproductive/winter, pregnancy/summer, and mating), with additional samples of
30 *Mormopterus* species from neighbouring islands (*M. jugularis* from Madagascar and *M.*
31 *acetabulosus* from Mauritius). Complementary information gathered from both microsatellite
32 and mitochondrial markers revealed high genetic diversity but no signal of spatial genetic
33 structure and weak evidence of female philopatry. Regional analysis suggests a single
34 colonization event for *M. francoismoutoui*, dated around 175,000 years ago, and followed by
35 *in-situ* diversification and the evolution of divergent ancestral lineages, which today form a
36 large metapopulation. Population expansion was relatively ancient (55,000 years ago) and
37 thus not linked to human colonization and the availability of human-constructed day-roost
38 sites. Discordant structure between mitochondrial and microsatellite markers suggests the
39 presence of yet-unknown mating sites, or the recent evolution of putative ecological
40 adaptations. Our study illustrates the challenge of detailed genetic studies to provide critical
41 insights to insular ecology and evolutionary history, and the importance of both mitochondrial
42 and nuclear DNA in exploring *in-situ* diversification of an urban-dwelling bat, endemic to a
43 small island.

44

45 **Keywords**

46 Chiroptera; Molossidae; Reunion Island; population genetic structure; demographic history

47

48 **1. Introduction**49 Bats are the most widely distributed terrestrial mammals on Earth but many species are facing
50 population declines in response to human activities and global change (Frick et al., 2020).51 Given their ability to fly long distances, bats are often the only mammals naturally colonizing
52 islands. Indeed, more than half of known bat species live on islands, and 25% of them are even
53 endemic to islands (Jones et al., 2009). Island endemic bats represent 50% of the world's
54 threatened bats and are thus a major priority for conservation (Conenna et al., 2017, Jones et
55 al., 2009). However, the ecology and evolution of island bats is often understudied, even
56 though this information is critical to assess their conservation status and design management
57 plans (Frick et al., 2020).58 Once established on islands, bats face a diversity of selective pressures that influence
59 their evolution and diversification. Indeed, due to reduced vertebrate species richness on
60 islands with small surface areas, such insular ecosystems may lack predators or competitors
61 and offer vacant ecological niches, which can favour the expansion of bat populations (Salinas-
62 Ramos et al., 2020). Further, insular ecosystems often have limited food resources and can
63 be subject to extreme events, such as volcanic activity, hurricanes or droughts, which can
64 reduce bat populations (Calderón-Acevedo et al., 2021; Jones et al., 2001). Islands are
65 vulnerable ecosystems that are highly susceptible to recent human-associated global changes,
66 such as sea level rise and invasion by non-native species (Bellard et al., 2014). Due to their
67 geographic isolation and limited dispersal opportunities, island endemic bat species may be
68 particularly exposed to the adverse effects of climate change (Festa et al., 2023). For example,

69 a recent study on a Mediterranean island, the endemic Sardinian long-eared bat (*Plecotus*
70 *sardus*, family Vespertilionidae) underwent a dramatic crash in population size, potentially
71 due to recurrent wildfires and extreme temperatures (Ancillotto et al., 2021). Also, recent
72 urbanization of island ecosystems could negatively affect the ecology of bat populations,
73 although tolerance to anthropogenic activities has been described in some bat species (Jung
74 & Threlfall, 2018; Russo & Ancillotto, 2015). Altogether, both historical and more
75 contemporary factors can have significant implications for the long-term survival and
76 conservation of island endemic bats.

77 Genetic analyses have become an essential tool for studying the ecology and evolution
78 of island bats. However, because of complex histories including allopatric divergence,
79 colonization, and hybridization on islands, studies have highlighted the need for the rigorous
80 use of both mitochondrial and nuclear microsatellite markers (Kuo et al., 2015). Indeed, these
81 markers have different evolutionary timescales, permitting the assessment of historical and
82 contemporary population structure, as well as different inheriting modes, widely used to
83 assess sex-specific life-history traits (Pinzari et al., 2023; Taki et al., 2021). Maternally-
84 inherited mitochondrial DNA (mtDNA) can trace colonization histories and past divergence,
85 and provide estimates of female site fidelity and dispersal, while polymorphic nuclear
86 microsatellite DNA are good candidates to infer recent gene flow and can provide information
87 for both sexes (Zhang & Hewitt, 2003).

88 By examining the genetic diversity and structure of island bat populations, we can infer
89 drivers of gene flow, estimate population sizes, and understand demographic history. For
90 example, genetic analysis of Miller's mastiff bat (*Molossus milleri*, family Molossidae)
91 spanning Jamaica, Cuba, and the Cayman Islands suggested populations underwent
92 bottlenecks, likely due to climate change in the early Pleistocene (Loureiro et al., 2020).

93 Moreover, several studies have shown stronger genetic structure in the philopatric sex (mostly
94 female), resulting from sex-biased dispersal behaviours in bat populations (Halczok et al.,
95 2018; Jang et al., 2021; Moussy et al., 2013; Naidoo et al., 2016). In addition, bats often exhibit
96 seasonal behaviours, in relation to change in food availability, habitat use, or reproductive
97 cycle, and these factors may play critical roles in shaping genetic diversity patterns (Moussy
98 et al., 2013). For example, in the little brown bat (*Myotis lucifugus*, family Vespertilionidae)
99 and the northern long-eared bat (*M. septentrionalis*), individuals at swarming sites in autumn
100 displayed a greater mtDNA genetic diversity than those at summering sites suggesting that
101 swarming sites gather individuals from several summering populations (Johnson et al., 2015).
102 Studies of genetic structure of island endemic bat species have mainly been carried out at the
103 scale of multiple neighbouring islands (archipelago), but studies at a single-island scale,
104 investigating sex or seasonal variation, are still limited (Ratrimomanarivo et al., 2009).
105 Obtaining a comprehensive picture of the local genetic structure of island endemic bats
106 requires a fine-scale sampling scheme, including material from multiple sites and from
107 different seasonal periods, thus allowing the detection of subtle diversity patterns, especially
108 on small islands.

109 The Reunion free-tailed bat (*Mormopterus francoismoutoui*, family Molossidae) is a
110 tropical insectivorous bat endemic to Reunion Island. This volcanic *in-situ* formed island is
111 located in the southwestern Indian Ocean (Mascarene Archipelago) and emerged from the
112 sea about 3 million years ago (Cadet, 1980). Reunion is located 950 km east of Madagascar,
113 which is home to the Peter's wrinkle lipped bat (*M. jugularis*), and 175 km southwest of
114 Mauritius Island, which is home to the Natal free-tailed bat (*M. acetabulosus*). Reunion Island
115 is shaped by a very mountainous landscape with the highest point at 3,070 m (Piton des
116 Neiges) and a still active volcano (Piton de La Fournaise at 2,632m). Although small in size

117 (2,512 km²), this very rugged landscape could represent several topographical barriers to bat
118 dispersal.

119 *Mormopterus francoismoutoui* is broadly distributed on the island and roosts in natural
120 settings, such as caves and cliff crevices. This bat species has adapted to anthropogenic
121 settings and thrives in the lowland urbanized areas where numerous roost sites occur in
122 buildings and under bridges (Augros et al., 2015; Goodman et al., 2008). However, little is
123 known about how urbanization might modify life-history traits, population size, and genetic
124 structure in this species. A recent longitudinal monitoring study of several roosts revealed
125 highly dynamic roosting behaviours (Aguillon et al., 2023). Specifically, large female
126 aggregations (up to 50,000 pregnant individuals) within a limited number of maternity roosts
127 were observed synchronously during austral summer, which coincided with a female-biased
128 sex-ratio at roost sites (Aguillon et al., 2023; Dietrich et al., 2015), and suggested female
129 philopatry in this species. Moreover, towards the end of the austral summer, there was a
130 decrease in roost size and a shift in sex-ratio (from female to male-biased), suggesting
131 important seasonal sex-specific movements on the island. These details support the results of
132 initial genetic work on this species, based on a limited number of samples ($n = 31$), that
133 suggested little genetic structure and no isolation by distance on the island (Goodman et al.,
134 2008).

135 In order to investigate the evolutionary history and genetic structure of *M.*
136 *francoismoutoui*, we used an extensive spatio-temporal field sampling and the
137 complementary information of microsatellite and D-loop mtDNA markers. We first analysed
138 the evolutionary history of this species, by examining its relationship to other regional
139 *Mormopterus* bats on neighbouring islands (Madagascar and Mauritius) in order to trace and
140 date colonization events. We also explored the demographic history of *M. francoismoutoui* by

141 testing the hypothesis of a population expansion linked to the recent urbanization of Reunion
142 Island. We then analysed spatio-temporal patterns of genetic diversity and population
143 structure and specifically tested female philopatry and seasonal changes in the genetic
144 structure linked to the dynamic roosting behaviour of this species. We expected that genetic
145 structure to be more prominent in females during summer due to important female
146 aggregation observed during this period, and lower during the mating season because of
147 mixing of individuals within roosts.

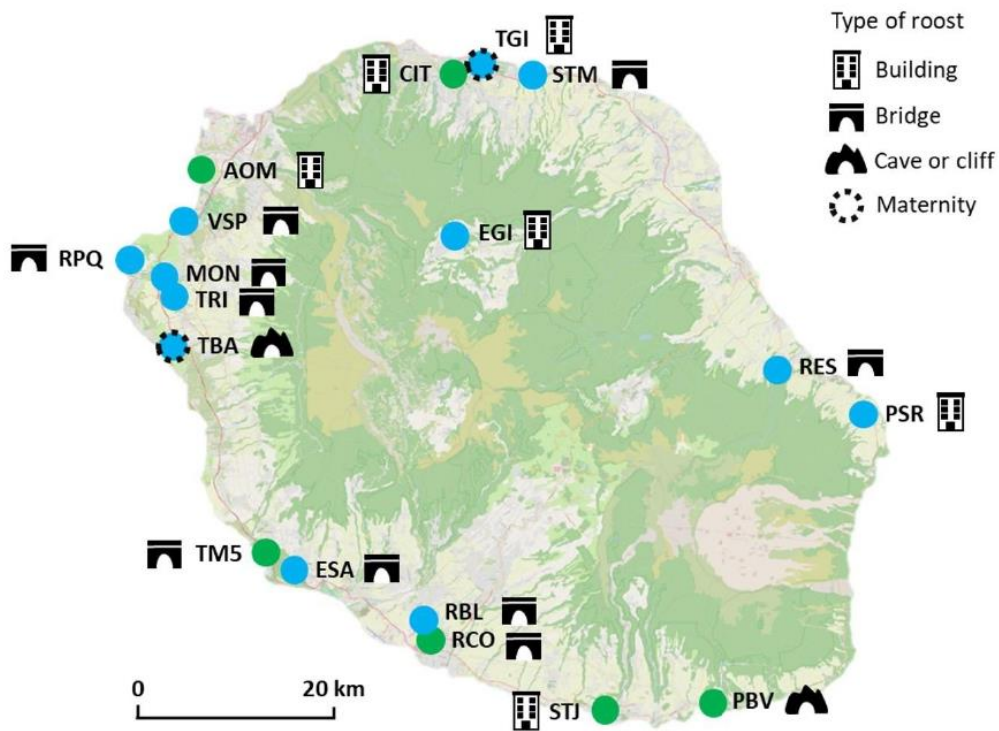
148

149 **2. Material and Methods**

150 **2.1. Field sampling**

151 Samples were collected during different seasons at 18 roosts (coded with a 3-letter code)
152 across Reunion Island (Fig. 1). Details of the longitudinal sampling of roosts can be found in
153 Table 1. . Six of these roosts (AOM, CIT, PBV, RAC, STJ, and TM5) were sampled once during
154 the study, because of opportunistic sampling. The remaining roosts were sampled multiple
155 times between October 2018 and March 2020. We collected repeat samples from eight roosts
156 (ESA, MON, PSR, RBL, RPQ, STM, TGI, and VSP) during three biological seasons: (i) the
157 pregnancy period (austral summer) from late October to early December 2018), (ii) the non-
158 reproductive period (austral winter) in June and July 2019, and (iii) the putative mating period
159 in March 2019 and 2020 (Aguillon et al., 2023, Table 1). Three roosts (EGI, RES, and TBA) were
160 quasi-empty during the non-reproductive/winter period, explaining the lack of data during
161 this season and one roost (TRI), was not sampled in March because of logistic constraints
162 (Table 1).

163



164

165 **Fig. 1. Sampling sites of *Mormopterus francoismoutoui* on Reunion Island. Details on roost**
 166 **sites are presented in Table 1.** The 12 roosts in blue were monitored regularly over two years
 167 while the six in green were sampled only once. The green colour indicating forested areas and
 168 the pale blue indicating urbanized areas. Modified from Aguillon et al. (2023).

169

170 **Table 1. Summary of mitochondrial and microsatellite data used in this study for**
 171 ***Mormopterus francoismoutoui*.** Maternity roosts are indicated with an asterisk (*) next to the
 172 roost name. The first number indicates the total number of sequences, with females and
 173 males in parentheses and the sites are indicated in Figure 1.

Roost	Habitat	Pregnancy	Non-reproductive	Mating	Opportunistic sampling		Total
		2018	2019	2019 – 2020	February	October	
		(summer)	(winter)	(March)	2019	2018	
AOM	Building			14 (8/6)			14
				30 (15/15)			30
CIT	Building	15 (0/15)					15
		29 (0/29)					29
EGI	Building	26 (8/18)		15 (8/7)			41
		34 (11/23)		30 (15/15)			64
ESA	Bridge	15 (9/6)	16 (9/7)	15 (8/7)			46
		36 (19/16)	34 (18/16)	26 (19/7)			96
MON	Bridge	18 (3/15)	15 (7/8)	15 (8/7)			48
		33 (3/30)	30 (15/15)	25 (16/9)			88
PBV	Cliff				11 (4/7)		11
					11 (4/7)		11
PSR	Building	17 (6/11)	15 (2/13)	15 (7/8)			47
		34 (12/22)	34 (2/32)	28 (7/21)			96
RAC	Bridge					16 (4/12)	16
						14 (4/10)	14
RBL	Bridge	16 (8/8)	15 (7/8)	15 (7/8)			46
		31 (13/18)	34 (11/23)	28 (20/8)			93
RES	Bridge	15 (7/8)		15 (7/8)			30
		32 (15/17)		30 (7/23)			62
RPQ	Bridge	15 (6/9)	15 (8/7)	15 (8/7)			45
		37 (19/18)	33 (11/22)	30 (15/15)			100
STJ	Building		15 (8/7)				15

			30 (13/17)			30	
STM	Bridge	15 (7/8)	13 (3/10)	15 (3/12)		43	
		34 (17/17)	13 (3/10)	23 (3/20)		70	
TBA*	Cave	31 (29/2)		15 (7/8)		46	
		34 (31/3)		29 (22/7)		63	
TGI*	Building	19 (12/7)	15 (7/8)	15 (7/8)		49	
		38 (20/18)	36 (13/23)	29 (15/14)		103	
TM5	Bridge			15 (7/8)		15	
				28 (13/15)		28	
TRI	Bridge	15 (1/14)	15 (8/7)			30	
		29 (1/28)	29 (9/20)			58	
VSP	Bridge	16 (7/9)	15 (7/8)	15 (7/8)		46	
		38 (18/20)	35 (7/28)	28 (15/13)		101	
Total		233	149	179	26	16	603
		439	308	336	39	14	1136

174

175 We captured bats during dusk emergence, mainly using harp traps (Faunatech Ausbat)
 176 and Japanese monofilament mist nets (Ecotone) set close to the roost exit as described in
 177 Aguillon et al. (2023). Because of difficulties in installing harp traps or mist nets at the exit of
 178 some roosts, we sometimes employed a butterfly net on an elongated pole to catch bats, by
 179 carefully approaching resting individuals during the day. After capture, bats were immediately
 180 hydrated with water using a sterile syringe and placed in a clean individual bag close to a
 181 source of warmth (hot water bottle), and processed at the capture site. We visually
 182 ascertained the sex and age of each individual. Age was determined by examining the
 183 epiphysis fusion in finger articulations that are not ossified for juveniles. Wing punch samples

184 (~ 2 mm) were taken on each wing, and stored dried in a cool box in the field before being
185 transferred at -80°C at the laboratory. Finally, each bat was tattooed on the right propatagium
186 with an individual alphanumeric code and released at the capture site.

187 Handling of bats was performed using personal protective equipment and gloves were
188 disinfected between each individual bat and changed regularly, and all the equipment was
189 disinfected between sites (see protocol in Aguillon et al., 2023 for more details). Bat capture
190 and manipulation techniques were evaluated by the ethic committee of Reunion Island,
191 approved by the Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation
192 (APAFIS#10140-2017030119531267), and conducted under a permit (DEAL/SEB/UBIO/2018-
193 09) delivered by the Direction de l'Environnement, de l'Aménagement et du Logement (DEAL)
194 of Reunion Island.

195 Samples (organ pool: spleen, lung, kidney) of *M. acetabulosus* from Mauritius (2012)
196 and *M. jugularis* from Madagascar (2012-2013) were obtained from vouchered individual bats
197 collected associated with zoonotic disease studies (Gomard et al., 2016; Joffrin et al., 2020;
198 Mélade et al., 2016), and from three different roost sites on each island. Mauritius samples
199 were collected under a memorandum of agreement for the supply of biological material by
200 Government of Mauritius (delivered by the National Park and Conservation Service for
201 authorization of Mauritius), signed on 17 December, 2010. Madagascar samples were
202 collected under the permits delivered by the Direction du Système des Aires Protégées and
203 Direction Générale de l'Environnement et des Forêts: no. 350/10/MEF/SG/DGF/DCB.SAP/SCB,
204 no. 032/12/MEF/SG/DGF/DCB.SAP/SCBSE, no. 067/12/MEF/SG/DGF/DCB.SAP/SCBSE, no.
205 194/12/MEF/SG/DGF/DCB.SAP/SCB, no. 283/11/MEF/SG/DGF/DCB.SAP/SCB, no.
206 077/12/MEF/SG/DGF/DCB.SAP/SCBSE, no. 238/14/MEEF/SG/DGF/DCB.SAP/SCB, and no.
207 268/14/MEEF/SG/DGF/DCB.SAP/SCB.

208 2.2. DNA extraction, PCR, sequencing, and genotyping

209 Wing punch samples of Reunion free-tailed bats were processed with the Cador Pathogen 96
210 Qiacube HT kit (Qiagen, Hilden, Germany). Samples were lysed before DNA extraction, in 180
211 μL of ATL buffer and 20 μL of Proteinase K at 56°C during 1h30. Then, the buffer VXL mixture
212 was prepared replacing Proteinase K by sterile water. Total nucleic acids were extracted in an
213 automated extractor Qiacube with slight modifications of the Q Protocol, including 350 μL of
214 ACB, 100 μL of AVE, and 30 μL of TopElute. Nucleid acids from Mauritius and Madagascar
215 samples were already available (protocols of extraction described in Gomard et al., 2016;
216 Joffrin et al., 2020; Mélade et al., 2016).

217 Subsequently, a fragment of the hypervariable domains I and II of the D-loop region
218 (with no repeats) was amplified by PCR (expected: 896 bp) in a 20 μL reaction mixture
219 containing 2 μL of DNA, 10 μL of GoTaq® Green Master Mix 2X (Promega, Madison, Wisconsin,
220 United States), 1 μL of each newly designed primers at 10 μM D-loop-F (5'-
221 CAAGACTTCAGGAAGAAGCTAACA-3') and D-loop-R-Lg (5'-TATTCGTATGTATGTCCTGTAACCA-
222 3'). PCR program included an initial denaturation step (95°C for 2 min), followed by 35 cycles
223 of denaturation (95°C for 30 sec), annealing (50°C for 30 sec), elongation (72°C for 1 min 30
224 sec), and a final elongation step (72°C for 7 min). PCR products were Sanger-sequenced by the
225 GENOSCREEN platform (Lille, France). The D-loop chromatograms were visually checked using
226 Geneious 9.1.8 (Biomatters Ltd, Auckland, New Zealand) and sequences were aligned using
227 CLC Sequence Viewer 7.6.1 (Qiagen Aarhus A/S, Aarhus, Denmark). DNA extracted were
228 genotyped by GENOSCREEN, using a panel of 12 previously described microsatellite markers,
229 according to the protocol and primers of Dietrich et al. (2019).

230

231

232 2.3. Genetic relationships among regional *Mormopterus*

233 In order to investigate genetic relationships among the three regional *Mormopterus* species,
234 we reconstructed a Bayesian tree based on Yule model (Yule, 1925), based on a dataset
235 including 30 D-loop sequences (alignment of 811 bp) for each island, and using the island as a
236 trait to resolve the spatial origin of the nodes using BEAST v.2.6.4 (Bouckaert et al., 2019). We
237 used a HKY model (Hasegawa et al., 1985) with invariant and gamma distribution according to
238 the best substitution model on AIC criterion using JModelTest (Darriba & Posada, 2016). We
239 used an uncorrelated lognormal relaxed molecular clock (Drummond et al., 2006) of 0.2
240 substitutions/site/million years (Petit et al., 1999), with a 100 million chain length and
241 sampling every 10^4 steps, and a burning of 10%. We ran three analyses and combined log
242 outputs (removing 10% of burning for each output) using LogCombiner v2.6.4 (Rambaut &
243 Drummond, 2015). Traces of Markov Chain Monte Carlo (MCMC) were checked for
244 convergence of the posterior estimates of the effective sample size (ESS) to the likelihood
245 using Tracer v1.7.1 (Rambaut et al., 2018). We combined tree outputs (removing 10% of
246 burning for each output) to obtain a consensus tree using LogCombiner v2.6.4 (Rambaut &
247 Drummond, 2015) and then TreeAnnotator v2.6.4 (Rambaut & Drummond, 2019). We also
248 investigated the haplotype number and genetic distances between sequences from each
249 island using DnaSP v6.12.03 (Rozas et al., 2017).

250 Microsatellite analysis of regional samples was performed using STRUCTURE v2.3
251 (Pritchard et al., 2010), with LocPrior (Hubisz et al., 2009) and no admixture model, using
252 uncorrelated allele frequencies among group. We ran 10 replicates with a burn-in of 10^6 steps,
253 10^6 recorded steps for the MCMC, and K from 1 to 5 groups (corresponding to the number of
254 species, plus two). We apply the Evanno method (Evanno et al., 2005) to estimate the best K.

255 We also calculated the global number of genotypes for each island using GenAlex 6.503
256 (Smouse & Peakall, 2012).

257

258 **2.4. Analyses of *M. francoismoutoui***

259 Using mitochondrial D-loop data, genetic diversity indices, including haplotype number,
260 haplotype diversity (Hd), and nucleotide diversity (π), were measured at the roost-level using
261 DnaSP v6.12.03 (Rozas et al., 2017), for the entire Reunion Island dataset and then separately
262 for each sex and season. Differences among roosts, sexes, and seasons were tested using
263 analyses of variance (ANOVA) in RStudio 1.4.1106 (RStudioTeam, 2021). Spatial structure was
264 assessed by calculating genetic distances (Φ_{st}) among roosts for the entire Reunion Island
265 dataset and then separately for each sex and season using Arlequin 3.5.2.2 (Excoffier &
266 Lischer, 2015). The significance of multiple tests was corrected with the Holm method (Holm,
267 1979) using RStudio. To test for temporal differences in the spatial structure, Φ_{st} values were
268 compared among seasons using ANOVAs and Tukey's post-hoc tests. Subsequently, to test for
269 the presence of isolation by distance (IBD), we performed a Mantel test with 1,000
270 permutations using Arlequin and calculated the correlation between genetic and geographic
271 distances. IBD was first tested for the complete Reunion Island dataset, and then separately
272 for each sex and season. Finally, we also used an AMOVA test (analysis of molecular variance)
273 in Arlequin and defined "population" as individuals from a single roost to test for a roost-
274 associated genetic structure in the Reunion population. The significance of this test was
275 assessed by 1,000 permutations of individuals among roosts.

276 We determined the most appropriate nucleotide substitution model of Reunion Island
277 D-loop sequences based on AIC criterion (Akaike, 1974) using JModelTest v2.1.10 (Darriba &
278 Posada, 2016). Then, using all Reunion D-loop sequences (alignment of 985 bp), we

279 constructed a Bayesian tree using BEAST v.2.6.4 (Bouckaert et al., 2019) with TN93 site model
280 with invariant and gamma distribution (Tamura & Nei, 1993) including roost location as a trait.
281 We used the same parameters in BEAST as described above for the regional analysis.

282 To investigate the demographic history of *M. francoismoutoui*, we calculated the
283 expected frequency distributions of pairwise differences between D-loop sequences
284 (mismatch distribution) in DnaSP v6.12.03 (Rozas et al., 2017). We also used a neutrality test
285 with 1,000 simulated samples using Arlequin v.3.5.2.2 (Excoffier & Lischer, 2015) based on
286 Harpending's raggedness index (r , Harpending, 1994), Fu's F_s (Fu, 1997), and the sum of
287 squared deviations (SSD) between observed and expected mismatch indices. We then
288 calculated the expansion time using the formula $t = \frac{\tau}{2\mu k}$ from Rogers & Harpending (1992)
289 where τ is the expansion date calculated with the mismatch distribution, μ is the mutation
290 rate, and k is the average number of nucleotide sites per haplotype. Global population size
291 change through time was reconstructed using a coalescent Bayesian skyline model (CBS,
292 Drummond et al., 2005) with the same parameters as described above in BEAST v.2.6.4
293 (Bouckaert et al., 2019). We performed BEAST analyses changing the dimension group
294 parameter from 3 to 10 groups, according to the results of the Yule Bayesian tree. We ran
295 three analyses for each group and followed the same method described for Yule Bayesian tree
296 and choose the best k according to the higher ESS.

297 For the microsatellite data, genotype determination was performed using
298 GeneMapper 6 (ThermoFisher). We tested the dataset for scoring errors, out of range allele,
299 and null alleles for each roost using Microchecker v.2.2.3 (Van Oosterhout et al., 2004). Using
300 GenAlex 6.503 (Smouse & Peakall, 2012), the global number of genotypes was calculated, and
301 for each roost, deviations from Hardy-Weinberg equilibrium were tested for each locus. We
302 then assessed the number of alleles per locus (N_a), the observed (H_o) and expected (H_e)

303 heterozygosity, and the fixation index (F_{is}) per locus using GenAlex 6.503 (Smouse & Peakall,
304 2012). A permutation test with 1,000 permutations was used to perform linkage
305 disequilibrium analysis between each pair of loci using Genetix v.4.05.2 (Belkhir et al., 2004).
306 We used Fstat v.2.9.4 (Goudet, 2003) to calculate the inbreeding coefficient (F_{IS}) within each
307 roost (Weir & Cockerham, 1984) and tested the significance by randomizing alleles among
308 individuals within roosts (5,000 permutations). We estimated the observed (H_o) and expected
309 (H_e) heterozygosity in each roost, first using the whole dataset, and subsequently estimated
310 these two parameters for each sex and season, testing for sex and temporal differences using
311 an ANOVA in RStudio. Effective population size (N_e) was estimated for the global population
312 because of a lack of genetic structure (see results) with the linkage-disequilibrium model and
313 assuming random mating using NeEstimator v2.1 (Do et al., 2013). For this, we used a
314 minimum allele frequency of 0.05 and 0.02 to calculate upper and lower limits of N_e .

315 Genetic distances (F_{st}) between roosts were calculated globally, and then separately
316 for each sex and season, using Arlequin 3.5.2.2 (Excoffier & Lischer, 2015). The significance of
317 multiple tests was corrected with Holm method using RStudio. To test for temporal
318 differences in the spatial structure, F_{st} values were compared among seasons using ANOVAs
319 and Tukey's post-hoc tests. To check for IBD, we performed Mantel tests (1,000 permutations)
320 using Arlequin 3.5.2.2 (Excoffier & Lischer, 2015), as for mitochondrial data.

321 To test for a roost-associated genetic structure in the population, we used an AMOVA
322 in Arlequin, as described for mtDNA. Then, we employed STRUCTURE v2.3 (Pritchard et al.,
323 2010), and performed two analyses with and without the LocPrior model, which uses location
324 to test for a weak signal of population structure (Hubisz et al., 2009). We used the admixture
325 model with correlated allele frequencies among groups, and 10 replicate runs were performed
326 with a burn-in of 10^6 steps and 10^6 recorded steps for the Monte Carlo Markov Chain (MCMC).

327 We ran K from 1 to 19 groups (corresponding to the number of studied roosts, plus one) and
328 applied the Evanno method (Evanno et al., 2005) to estimate the best K. To determine the
329 optimal number of genetic clusters, we performed a k-means clustering analysis, tested K from
330 2 to 19 over 26 indices according to the “majority rule”, using the *NbClust* package (Charrad
331 et al., 2014) in Rstudio. We also performed a principal coordinate analysis (PCoA) using
332 GenAlex 6.503 to visualize possible genetic clusters.

333 Finally, to compare mitochondrial and nuclear results, we overlaid the genetic clusters
334 identified using microsatellite markers and clades depicted by the BEAST phylogeny obtained
335 with the D-loop sequences. Based on the dimension group parameter estimated by the best
336 skyline converging model in BEAST, we defined five mtDNA clusters according to posterior
337 probabilities > 0.99 (see results). We used a generalized linear model (GLM) in Rstudio,
338 including the nuclear clusters as the numeric response variable (value of PC1 from PCoA) and
339 the mtDNA genetic clusters as the explanatory response.

340

341 **3. Results**

342 **3.1. Data quality**

343 Altogether, we obtained good quality D-loop sequences for 603 *M. francoismoutoui* and 30
344 sequences for each additional *Mormopterus* species (Mauritius and Madagascar). We
345 genotyped 1,136 individuals of *M. francoismoutoui* using the 12 microsatellite loci, and 30
346 individuals from Mauritius (*M. acetabulosus*) and Madagascar (*M. jugularis*). One locus
347 (MF_loc11) was removed because of a high percentage of uninterpretable weak signals in the
348 Reunion Island dataset (~ 41% of individuals). Also, we removed individuals from Reunion for
349 which at least six loci were not genotyped ($n = 22$), leading to a final microsatellite data set

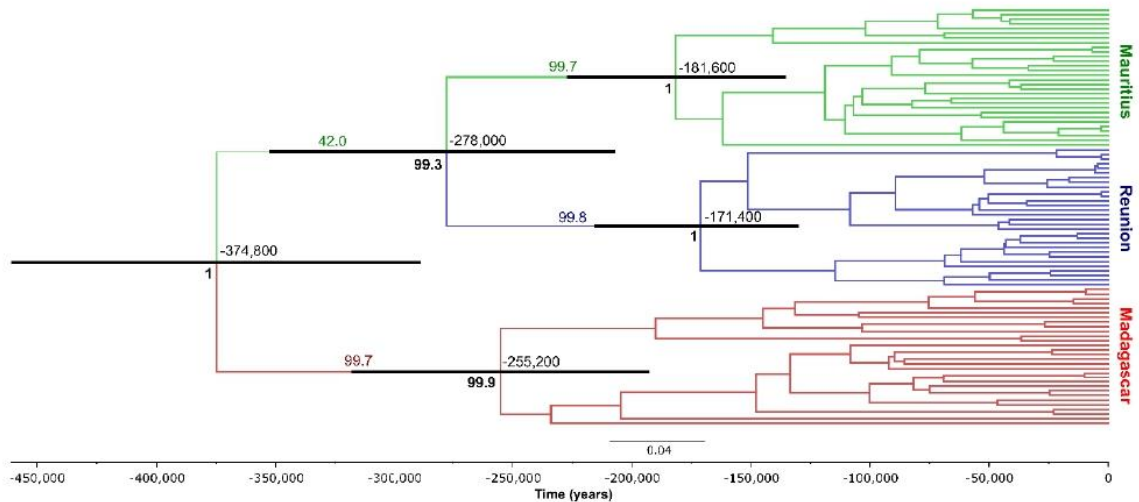
350 containing 3.7% of missing alleles. In the Reunion dataset, a majority of loci significantly
351 deviated from Hardy-Weinberg equilibrium, especially MF_Loc03, MF_Loc04, MF_Loc05, and
352 MF_Loc15. Null alleles were detected in several loci, especially in MF_Loc04 and MF_Loc015.
353 Three loci were implicated in several linkage disequilibria: MF_Loc03, MF_Loc05, and
354 MF_Loc28. Global Fis was low (mean: 0.022), and the expected heterozygosity (H_e) per locus
355 ranged from 0.481 to 0.881, and the observed heterozygosity (H_o) ranged from 0.479 to 0.874
356 (Table S1). We also found important values of allelic richness, with an average of 14.8 alleles
357 per locus.

358

359 **3.2. Genetic relationship among regional *Mormopterus***

360 Mitochondrial DNA revealed a high number of haplotypes on each island: 30 for Madagascar,
361 29 for Mauritius, and 26 for Reunion Island (out of 30 sequences per island). Using the
362 microsatellite markers, all genotypes were different. The time calibrated Bayesian phylogeny
363 strongly supported three genetic clades corresponding to each bat species, thus confirming
364 their monophyly (Fig. 2). The species from Mauritius and Reunion were separated by a genetic
365 distance of 5.0%. Species from Mauritius and Madagascar, and then Reunion and Madagascar
366 were separated by a divergence of 6.1% and 6.4%, respectively. Interestingly, STRUCTURE
367 analyses identified two genetic clusters, separating the Malagasy species in one cluster, and
368 the two species on Reunion and Mauritius in a second cluster (Fig. S1). Surprisingly, when $K =$
369 3, only 20% of the runs assigned each bat species to a different cluster. Indeed, most (80%) of
370 the runs for $K = 3$ grouped Reunion and Mauritius bats in a single genetic cluster, while
371 Malagasy bats were composed of two clusters with a few individuals having mixed genotypes.
372 The BEAST analysis showed that the inferred TMRCA for the three *Mormopterus* species was
373 374,800 years ago (HPD: 287,500 – 467,000) and the divergence of *M. francoismoutoui* on

374 Reunion from *M. acetabulosus* on Mauritius was dated at 278,000 years ago (HPD: 209,100 –
 375 355,100). The diversification of *M. jugularis* on Madagascar was dated at 255,200 years ago
 376 (HPD: 192,800 – 319,200) and occurred later for the species on Mauritius (181,600; HPD:
 377 135,800 – 232,500) and on Reunion (171,400; HPD: 129,600 – 218,000).
 378



379

380 **Fig. 2. Bayesian chronogram inferred from mitochondrial data (D-loop) for three**
 381 ***Mormopterus* species occurring on southwestern Indian Ocean islands, using BEAST**
 382 **software.** The Yule model was used with HKY (I+G) substitution model and relaxed molecular
 383 clock of 0.2 substitution/site/million years. Scale bar at the bottom represents divergence
 384 time in years from past (left) to recent (right) time. Colours of the branches depict sample
 385 locality: Mauritius (*M. acetabulosus*) in green, Reunion (*M. francoismoutoui*) in blue, and
 386 Madagascar (*M. jugularis*) in red. Posterior probabilities values of main nodes are indicated
 387 below branches in bold black. Black horizontal bars represent the 95% HPD of node ages with
 388 main age values indicated above the bar. Posterior probabilities associated with island origin
 389 trait are indicated above branches and coloured accordingly.

390

391 **3.3. Genetic diversity of *Mormopterus francoismoutoui***

392 Mitochondrial DNA and microsatellite markers revealed a high genetic diversity within the
393 population of *M. francoismoutoui*. For mtDNA, 410 haplotypes (out of 603 sequences) were
394 identified with an average haplotype diversity (Hd) of 0.998 (Table 2) and a global nucleotide
395 diversity (π) of 0.0284. Using the microsatellite markers, we identified 1,135 genotypes (in
396 1,136 individuals), and only two individuals (both captured in the TGI roost) shared the same
397 genotype. The average observed (Ho) and expected (He) heterozygosities were high (Ho =
398 0.778 ± 0.009 , He = 0.797 ± 0.008) and there was no evidence of inbreeding between
399 individuals occupying the same roosts, as none of the F_{IS} values were significantly different
400 from zero (Table 2). For both the mtDNA (Hd and π) and nuclear (Ho and He) data, there was
401 no significant differences in the level of genetic diversity between roosts, nor between sexes
402 and seasons (ANOVA, all $p > 0.05$, Table S2 and S3 for D-loop, Table S and S5 for
403 microsatellites).

404

405 **Table 2. Global genetic diversity indices of *Mormopterus francoismoutoui*, calculated with**
406 **mitochondrial DNA (D-loop) and 11 microsatellite markers. For roosts details see Figure 1**
407 **and Table 1.** N: number of individuals, Hd: haplotype diversity, π : nucleotide diversity, Ho:
408 observed heterozygosity (\pm standard error), He: expected heterozygosity (\pm standard error),
409 and F_{IS} : inbreeding coefficient (none are significantly different from zero).

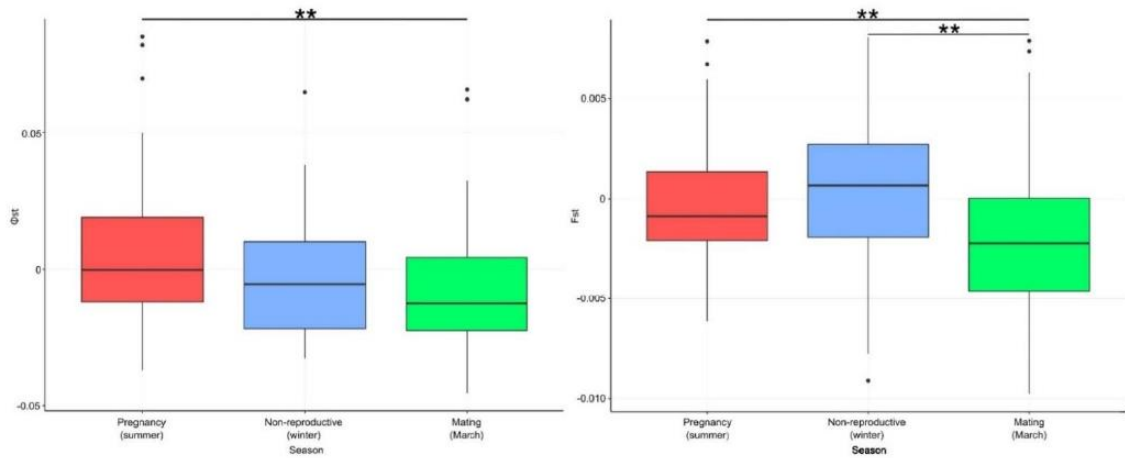
Roost	Mitochondrial data			Microsatellite data			
	N	Hd	π	N	Ho	He	Fis
AOM	14	0.989	0.0264	30	0.762 ± 0.035	0.793 ± 0.037	0.058
CIT	15	1.000	0.0304	29	0.787 ± 0.035	0.791 ± 0.029	0.022
EGI	41	0.999	0.0287	64	0.766 ± 0.040	0.807 ± 0.030	0.060
ESA	46	0.998	0.0272	96	0.776 ± 0.033	0.806 ± 0.033	0.044
MON	48	1.000	0.0291	88	0.797 ± 0.031	0.800 ± 0.035	0.009
PBV	11	1.000	0.0342	11	0.759 ± 0.032	0.762 ± 0.030	0.053
PSR	47	0.997	0.0297	96	0.786 ± 0.037	0.797 ± 0.036	0.018
RAC	16	1.000	0.0309	14	0.836 ± 0.070	0.772 ± 0.036	-0.045
RBL	46	0.999	0.0282	93	0.763 ± 0.028	0.802 ± 0.035	0.055
RES	30	1.000	0.0284	62	0.744 ± 0.040	0.802 ± 0.033	0.081
RPQ	45	0.997	0.0297	100	0.788 ± 0.031	0.805 ± 0.034	0.026
STJ	15	1.000	0.0278	30	0.782 ± 0.029	0.796 ± 0.034	0.036
STM	43	1.000	0.0289	70	0.797 ± 0.037	0.809 ± 0.032	0.022
TBA	46	0.998	0.0290	63	0.783 ± 0.040	0.801 ± 0.034	0.032
TGI	49	0.997	0.0308	103	0.766 ± 0.044	0.806 ± 0.035	0.054
TM5	15	1.000	0.0305	28	0.752 ± 0.044	0.791 ± 0.035	0.070
TRI	30	1.000	0.0299	58	0.781 ± 0.038	0.801 ± 0.031	0.034
VSP	46	0.999	0.0290	101	0.779 ± 0.035	0.804 ± 0.034	0.036
TOTAL	603	0.998	0.0284	1136	0.778 ± 0.009	0.797 ± 0.008	0.034

410

411 **3.4. Genetic structure within the Reunion Island population**

412 Globally, for both mitochondrial and nuclear markers, no isolation by distance ($r_{\text{mtDNA}} = 0.006$,
413 $p = 0.46$; $r_{\text{nuclear}} = 0.11$, $p = 0.14$) was detected, nor significant pairwise differentiation among
414 roosts (F_{st} and Φ_{st} , Table S6). The same results were found when analyses were performed
415 separately for each sex and season (Table S7 for IBD results). However, we identified
416 differences in Φ_{st} and F_{st} values among seasons (ANOVA, $p = 0.003$ and $p = 0.001$,
417 respectively). The mitochondrial marker showed higher Φ_{st} values in the pregnancy period
418 compared to those of the mating period ($p = 0.002$), while for microsatellites, F_{st} values during
419 both the pregnancy ($p = 0.008$) and the non-reproductive period ($p = 0.003$) were higher
420 compared to those during the mating period (Fig. 3). AMOVA results with both markers
421 showed that genetic variation (100% for mtDNA and 99.99% for microsatellites) was largely
422 due to differences among individuals within roosts. Results from the STRUCTURE analyses
423 revealed no genetic clustering (no conclusive Evanno result, Fig. S2), while the k-means
424 clustering analysis evaluated the optimal number to three genetic clusters (Fig. S3). This result
425 was corroborated by the PCoA but with only a small genetic variation explained by the two
426 first axes (PC1: 4.73% and PC2: 3.31%, Fig. 4). Interestingly, these three clusters included a
427 mixture of bats from the different roost sites.

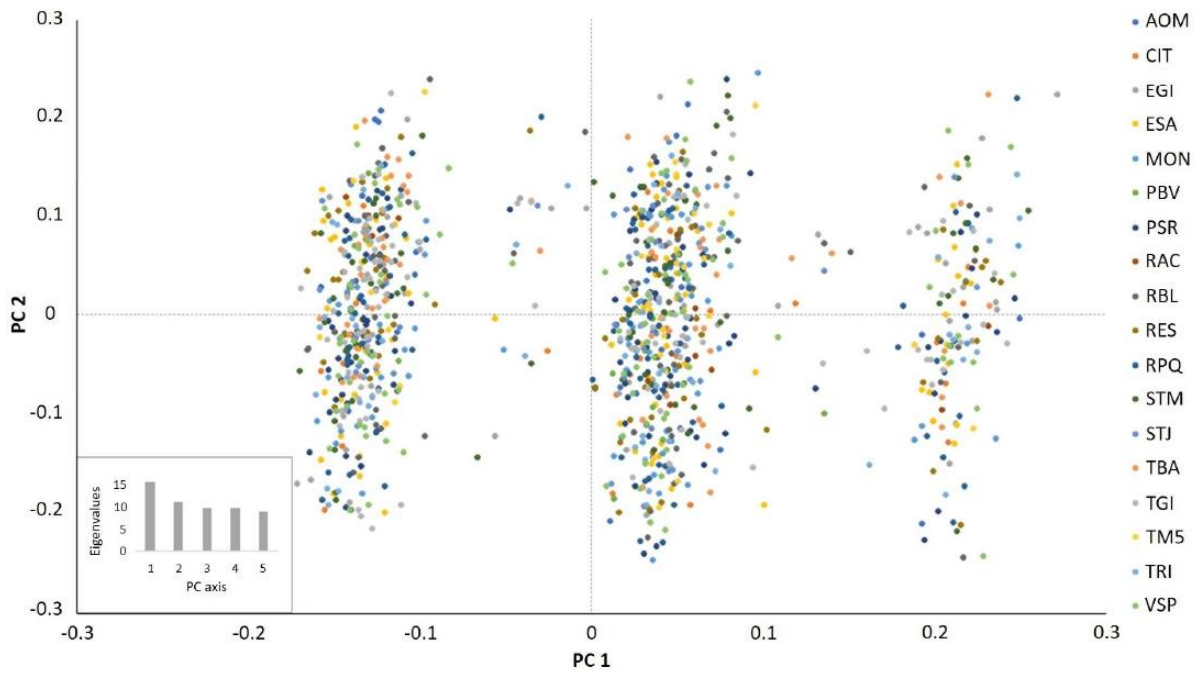
428



429 **Fig. 3. Temporal differences in Φ_{st} (mtDNA) and F_{st} (microsatellites) values between seasons**

430 **in *Mormopterus francoismoutoui*. ** $p < 0.01$ (Tuckey's post-hoc tests).**

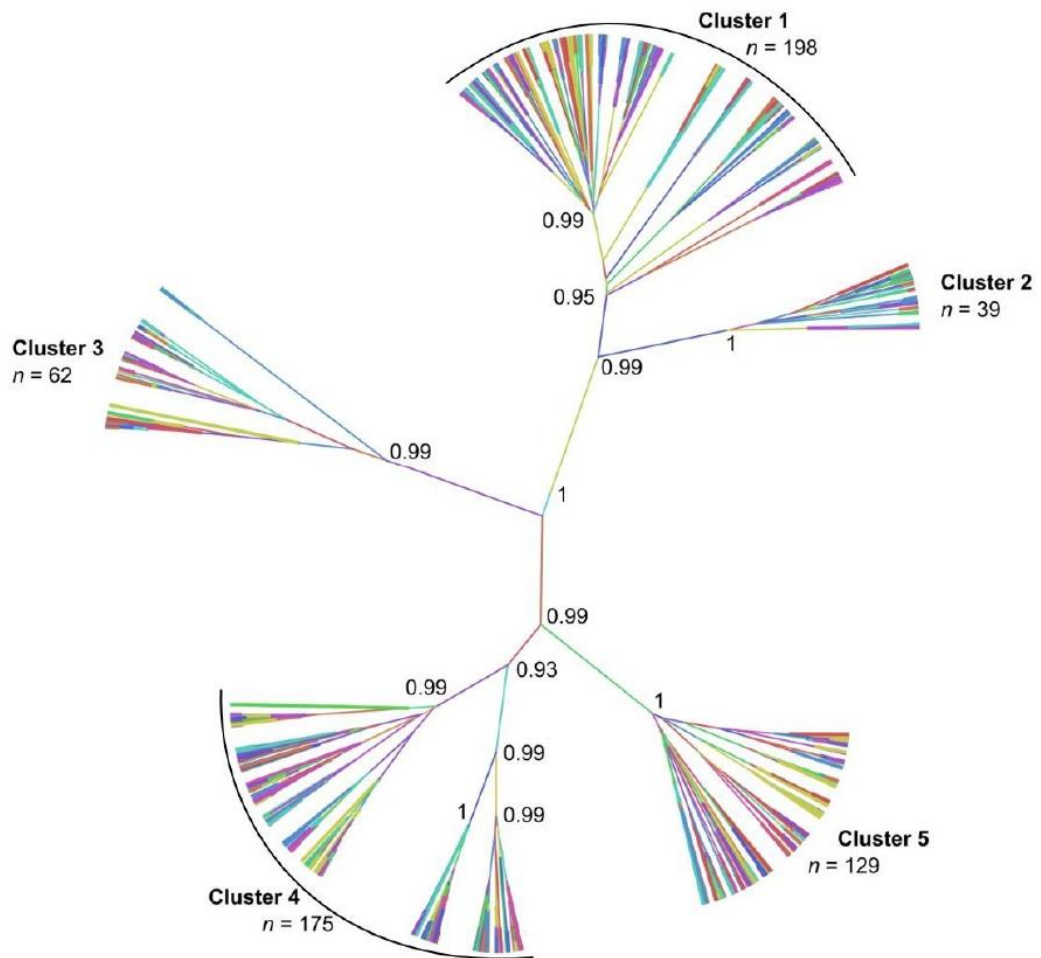
431



432 **Fig. 4. Principal Coordinates Analysis (PCoA) of microsatellite markers for *Mormopterus***
433 ***francoismoutoui*.** Roosts are indicated by different colours and the eigenvalues of the first five
434 principal component (PC) are shown.

435 The Bayesian tree built with the Yule model of speciation, revealed several well-
436 supported genetic clusters (posterior probability > 0.95, Fig. 5). These clusters included bats
437 from all roosts, but the best reconstruction of ancestral nodes failed to predict the roost origin
438 of individuals. In addition, the best skyline converging model gave the same tree topology and
439 indicated the occurrence of five genetic groups (highlighted in bold in Fig. 5), followed by
440 models with six and 10 groups with a close likelihood ESS (Table S8). These five clusters were
441 separated by a maximum of 3.3% of divergence. When overlaying the three genetic clusters
442 identified with microsatellite markers on the five clusters identified with the skyline BEAST
443 phylogeny, no significant results were found revealing that genetic clusters from both markers
444 are different (GLM, $\chi^2_4 = 0.03$, $p = 0.72$).

445



446 **Fig. 5. Bayesian tree topology inferred from mitochondrial data (D-loop) for the 18 roosts of**
 447 ***Mormopterus francoismoutoui*.** The Yule speciation model was used with TN93 (I+G)
 448 substitution model and a relaxed molecular clock of 0.2 substitution/site/million years.
 449 Colours correspond to different roost sites and line weigh represents the probability of roost
 450 location. Posterior probability values higher than 0.95 are indicated next to the node of main
 451 genetic clusters. The five clusters identified with the best skyline converging model are
 452 indicated in bold and correspond to posterior probabilities > 0.99.

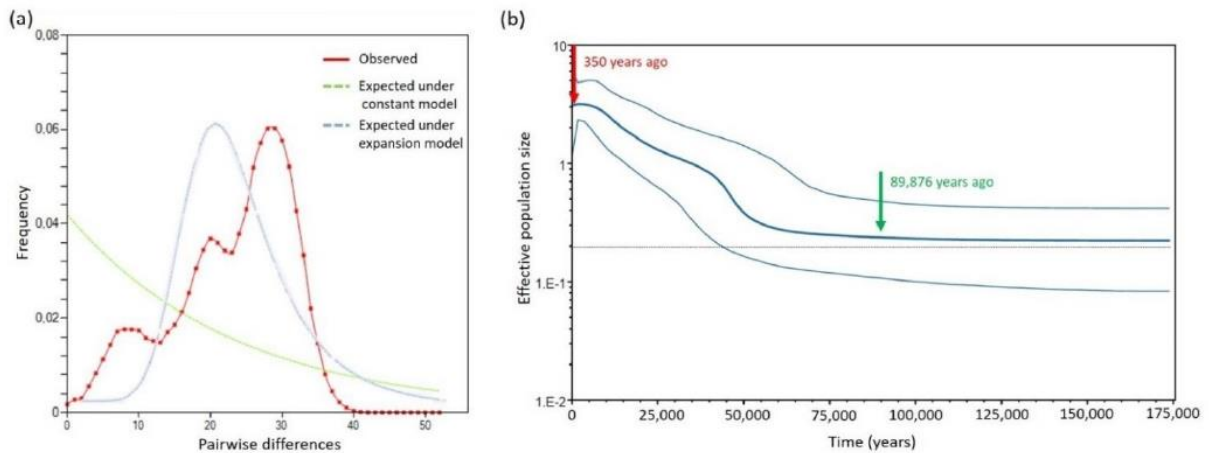
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454

455 **3.5. Population demographic history of *Mormopterus francoismoutoui***

456 Estimations of effective genetic population size with the lowest allele frequency at 0.05 and
457 0.02 led to infinite estimate of N_e (95% $CI_{0.05}$: 7783.5 - Infinite and 95% $CI_{0.02}$: 22347.5 –
458 Infinite). Moreover, the mismatch distribution under the expansion model showed a clear
459 signal of demographic expansion with a multimodal distribution with three peaks (Fig. 6a).
460 Raggedness index and Sum of Square Deviation had non-significant values under the model of
461 demographic expansion ($r = 0.0005$, $p = 1$; $SSD = 0.002$, $p = 0.71$). The result of F_u 's F_s showed
462 significant negative values indicating an excess of rare haplotypes compared to expected
463 values under neutral model ($F_s = -23.33$, $p = 0.04$). All these results suggested an ancient
464 demographic expansion in the population. Based on information calculated from the
465 mismatch distribution test, we determined the expansion time $t = \frac{\tau}{2\mu k}$ with $\tau = 29.156$, $\mu =$
466 0.2 substitutions/site/million years (Petit et al., 1999) and $k = 811$ bp. We found an expansion
467 time $t = 89,876$ years. This was coherent with the Bayesian skyline plot showing a stable
468 population size starting from 175,000 years and up to 90,000 years. Then, a slight increase in
469 population size began and was followed by a drastic expansion around 55,000 years, lasting
470 about 10,000 years. In the last 45,000 years, the population size still increased but at a slower
471 rate. The end of the curve suggested a recent stabilization or a decrease in population size
472 occurring about 500 years ago (Fig. 6b).

473



474 **Fig. 6. Demographic history of *Mormopterus francoismoutoui* inferred from mitochondrial**
 475 **data (D-loop).** (a) Frequency of pairwise differences distribution (mismatch distribution). The
 476 frequency observed is represented by the red dotted line, the expected frequency under the
 477 hypothesis of population constant model is indicated by the green line, and population
 478 expansion model by the blue line. (b). Coalescent Bayesian skyline plot (with dimension group
 479 parameter = 5). Time is indicated in the x-axis from recent (left) to past (right) and the estimate
 480 effective population size (N_e) is represented in the y-axis. The central blue line is the median
 481 surrounded by the upper and lower estimates of 95% credibility interval. The Reunion free-
 482 tailed bat estimated time of expansion is indicated with the green arrow around 90,000 years
 483 before present and the red arrow represent the colonization of the island by humans since
 484 350 years.

485

486 4. Discussion

487 Despite living on the small oceanic island of Reunion (2,512 km²), our study revealed an
 488 extreme high genetic diversity in *Mormopterus francoismoutoui*, with 68% of unique D-loop
 489 haplotypes and 99.9% of unique microsatellite genotypes. Only one microsatellite genotype

490 was found to be shared by two individuals, which were both captured in the same roost (TGI),
491 suggesting a kinship link between them. Our results support those of Goodman et al. (2008)
492 and are similar to previous studies reported in much bigger islands, such as in *M. jugularis* on
493 Madagascar (587,041 km², Ratrimomanarivo et al., 2009) and *Myotis punicus* on Corsica
494 (8,722 km²) and Sardinia (24,090 km², Biollaz et al., 2010). High levels of genetic diversity in
495 island endemic bats can be explained by large population size (Frankham, 1996), which is
496 supported by our results providing infinite large effective population size estimates for
497 *Mormopterus francoismoutoui*. Such results are coherent with the fact that Molossidae bats
498 form large and dense colonies (e.g. *Tadarida brasiliensis* of the family Molossidae, McCracken
499 & Wilkinson, 2000) and corroborate our field observations of numerous roosts across Reunion
500 Island, with an estimated current population size probably far over 120,000 individuals
501 (Aguillon et al., 2023).

502 Our Bayesian phylogenetic analysis indicates that *M. francoismoutoui* form a distinct
503 monophyletic lineage that diverged about 278,000 years ago from *M. acetabulosus*. The
504 monophyly of the Reunion species supports the hypothesis of a single colonization event by
505 overwater dispersal, although the geographic origin of its ancestor could not be determined
506 in our study. Previous taxonomic studies on *Mormopterus* bats from the Mascarene Islands
507 showed morphological similarities with *M. norfolkensis* from Australia, while *M. jugularis* from
508 Madagascar was reported closer to *M. doriae* of Sumatra (Goodman et al., 2008; Peterson,
509 1985). The different patterns of grouping among southwestern Indian Ocean island members
510 of the genus obtained with STRUCTURE (Fig. S1) may indeed suggest different geographic
511 origins for Mascarene and Madagascar *Mormopterus*, respectively. However, because of
512 mixed genotypes in a few Malagasy bats, we cannot rule out migrant ancestry with occasional
513 dispersal of bats from Mascarene Islands to Madagascar. Further, there is evidence of

514 dispersal of the Mascarene populations to the African continent (Goodman et al., 2008).
515 Detailed genetic studies are available for certain *Mormopterus* species, such as here for the
516 southwestern Indian Ocean islands and Reardon et al. (2014) for the Australian species. No
517 global analysis is available across the broad geographic range of members of the genus and
518 this will be needed to better define the origin of the southwestern Indian Ocean island species.

519 Once established on Reunion Island, the population of *M. francoismoutoui* remained
520 stable and started increasing slowly 90,000 years ago, with a notable expansion around 55,000
521 years ago (Upper Pleistocene). This timing coincides with the estimated period when both
522 volcanos (Piton des Neiges and Piton de la Fournaise) were active: from 65,000 years to 20,000
523 years (Nehlig & Marie, 2005). Such volcanic activity could have created new suitable habitats
524 (like cliff crevices and caves), and thus increased possible roost sites and enhanced range
525 expansion of this species. Indeed, it has been previously suggested that certain bat species
526 may benefit from volcano activity. For example, the New Zealand short tailed bat (*Mystacina*
527 *tuberculata*, family Mystacinidae) underwent a range expansion possibly following rapid
528 forest recolonization after a volcanic eruption (Lloyd, 2003).

529 Reunion Island was colonized by humans about 350 years ago, with a dramatic human
530 population expansion during the 20th century (Sandron, 2007). Contrary to our predictions
531 that recent urbanization of Reunion Island, specifically construction of permanent structures
532 (*i. e.* buildings, bridges) might have enhanced bat population size, our results suggest that the
533 population expansion of *M. francoismoutoui* ceased or slowed down about 500 years ago. This
534 result could be due to model error and based on a single locus (Ho & Shapiro, 2011). However,
535 such a pattern of human intervention and increased population size has already been
536 described for several Amazonian bats of the family Phyllostomidae associated with 17th
537 century deforestation (Silva et al., 2020). After human colonization of Reunion Island, the

538 natural ecosystems were profoundly and rapidly modified associated with a growing
539 urbanization of the island (Lagabrielle et al., 2009). *Mormopterus francoismoutoui*, which is
540 not a forest-dwelling species, uses natural large caves and crevices along cliff faces as roosting
541 sites, which can be disturbed by anthropogenic activities, such as human visitation and guano
542 collection. This bat species also occupies day-roost sites in urban areas, but given the odour
543 associated with such roosts, they are discouraged and a by-product might be higher rates of
544 bat mortality (Augros et al., 2015). Moreover, extensive landscape modifications and human
545 activities may have changed behaviour and physiology of this species, and may negatively
546 affect the fitness of individuals and population size (suggested by the Bayesian skyline
547 analyses), as has been shown for other bat taxa (Russo & Ancillotto, 2015). For example,
548 previous work found that the molossid bat, *Tadarida brasiliensis*, roosting at human-
549 constructed bridges in Texas, experienced reduced immune system functioning, which could
550 be linked to physiological stress (Allen et al., 2009), and which in turn could lead to negative
551 impacts on bat populations. The evolution of *M. francoismoutoui* population size over recent
552 decades has not been monitored, at least because the large and dense populations at roost
553 sites make direct counts of individual bats difficult. Occupancy modelling based on acoustic
554 survey data, together with data from recaptured bats and mark-recapture models, should
555 provide an alternative method to precisely assess trends in population size in relation to
556 human activities (Oyler-McCance et al., 2017; Rivers et al., 2006; Rodhouse et al., 2019).

557 Our results suggest that the large current population of *M. francoismoutoui*
558 experiences important levels of gene flow, as no significant genetic differentiation among
559 roosts was found, as well as, low levels of inbreeding and no isolation by distance across
560 sampled populations. Further, the high genetic diversity linked to the large population size
561 may counter-balance a weak signal of spatial genetic structure (Gauffre et al., 2008).

562 Interestingly, we found stronger Φ_{st} values during the summer, which might be associated
563 with some degree of female philopatry during the pregnancy and parturition periods and
564 supported with the massive aggregations of pregnant females observed during this period,
565 specifically at the TBA roost (Table 1, Aguillon et al., 2023; Dietrich et al., 2015). Subsequently,
566 in March, both Φ_{st} and F_{st} values were the lowest, suggesting that bats dispersed and mixed
567 within roosts, coherent with the mating period in a completely panmictic population (Moussy
568 et al., 2013). Interestingly, the F_{st} values calculated with the microsatellites were still high
569 during the non-reproductive winter months. This discordance between markers may be the
570 consequence of behavioural aspects of males, which are probably more sedentary during non-
571 reproductive period, and fits with observations that bats, particularly adult females, leave the
572 studied roost sites during winter and disperse to unknown wintering sites (Aguillon et al.,
573 2023). Our results thus suggest high levels of dispersal in this species across Reunion Island,
574 and its capacity to disperse over the island's mountainous landscape. However, it is important
575 to note a similar lack of genetic structure in *M. jugularis* on Madagascar and not related to sex
576 classes (Ratrimomanarivo et al., 2009). These results likely indicate a common evolutionary
577 trend among *Mormopterus* species on southwestern Indian Ocean Islands, and more broadly
578 in Molossidae species, such as the Mexican free-tailed bat *Tadarida brasiliensis* capable of
579 long-distance migration and high-altitude displacements (Glass, 1982; McCracken et al.,
580 2008), and yet showing genetic differentiation between islands in the Bahamas (Speer et al.,
581 2017). Interestingly, roosts occupied by *M. francoismoutoui* are often located in urban areas
582 which could have facilitated dispersal by increasing connectivity between populations as
583 molossid bats in general seem less affected by human-induced land changes (Richardson et
584 al., 2021; Russo & Ancillotto, 2015).

585 Despite the absence of a geographical pattern in the genetic structure of *M.*
586 *francoismoutoui*, our phylogenetic analyses showed that it has diversified into at least five
587 divergent mtDNA lineages that are not geographically separated, but rather found in sympatry
588 in roosts. Sympatric mtDNA lineages are not commonly described in animals, and especially
589 in bats (Andriollo et al., 2015; Sun et al., 2016) and their origin and maintenance are often
590 difficult to resolve (Hogner et al., 2012; Makhov et al., 2021; Webb et al., 2011). This may be
591 explained by stochastic lineage sorting processes that occur in panmictic populations with
592 large effective population size (Hogner et al., 2012; Webb et al., 2011). Also, we cannot
593 exclude the possibility of female philopatry that would increase population structure in the
594 mtDNA marker (Moussy et al., 2013), and this remained the same even when genetic analyses
595 were performed for each sex. The divergence of mtDNA lineages may also reflect long periods
596 of geographical isolation of small bat populations after the colonization of Reunion Island.
597 These initial bat populations could have limited dispersal because of the intense volcano
598 activity leading topographic separation of portions of the island. Our Bayesian phylogeny
599 dated the start of the *in-situ* diversification back to 175,000 years ago, and the sharp increase
600 in population size (about 55,000 years ago) coincides with the apparition of multiples lineages
601 within the population (Fig. 2). As well, we found a multimodal distribution under the
602 expansion model of the mismatch distribution, which could be linked to the expansion of the
603 different genetic clusters (Du et al., 2019). Interestingly, the mtDNA genetic structure was not
604 observed in the microsatellite analyses, which can be explained by increased allelic homoplasy
605 at microsatellite loci that may mask genetic differentiation over long periods of time in species
606 with large populations (Estoup et al., 2002). Our results may also suggest the recent admixture
607 of ancient lineages, that are no more reproductively isolated, which might have contributed
608 to the high nuclear polymorphism detected (Andriollo et al., 2015; Sun et al., 2016). Indeed,

609 such recent gene flow would erase genetic signatures at microsatellite loci more rapidly than
610 mtDNA loci, explaining the absence of a strong signal of nuclear structure.

611 However, in the case of recent gene flow in *M. francoismoutoui*, our nuclear data
612 suggest that it does not occur randomly within the population, as the clustering analysis and
613 PCoA on microsatellite markers indicated the presence of three distinct clusters (also in
614 sympatry within roosts), although the percentage of variance explained is low. More
615 interestingly, these nuclear clusters did not overlay mtDNA clusters and were not detected
616 with the STRUCTURE analysis. This discordance between both markers can be explained by
617 different evolutionary time processes and inheritance (Harrison, 1989; Toews & Brelsford,
618 2012). Such opposite patterns between markers have previously been described in different
619 bat species (Laine et al., 2023; Naidoo et al., 2016; Sun et al., 2016) and highlight the need to
620 use several markers for reconstructing complex evolutionary histories, especially when
621 population structure is weak (Kuo et al., 2015). The microsatellite structure may correspond
622 to a few isolated mating roosts on the island or could reflect putative adaptations like
623 morphological or acoustic differences, as described for the big-eared horseshoe bat
624 (*Rhinolophus macrotis*, family Rhinolophidae, Sun et al., 2016). Further bat tracking studies
625 would provide better understanding of spatial and temporal movements of individuals on
626 Reunion Island and potentially identify currently unknown mating sites (Conenna et al., 2019).
627

628 **5. Conclusion**

629 Our study illustrates how understanding the ecology and evolution of insular endemic bats
630 can be challenging and thus the importance of integrating past evolutionary processes and
631 contemporary gene flow. Here, we demonstrate that fine-scale sampling scheme and multi-

632 marker comparisons at regional and local scales are necessary, but do not guarantee, to obtain
633 a complete picture of the population structure and history of an island endemic bat species.
634 Such genetic approaches in combination with understanding a range of ecological parameters
635 are also crucial to reduce uncertainty in conservation decision making of vulnerable mammals
636 due of their endemism status, such as *Mormopterus francoismoutoui*. For example,
637 conservation actions for bats living in such a metapopulation can focus on the identification
638 of possible threats such as urbanization.

639

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653

654 **Conflict of interest**

655 We have no competing interests to declare.

656

657 **Data accessibility**

658 Mitochondrial sequences (D-loop) has been deposited in Genbank under ID's from OR081945
659 to OR082607 and microsatellites genotypes and metadata are available at Zenodo
660 (<https://doi.org/10.5281/zenodo.8069702>). D-loop sequences for the Reunion Island dataset
661 are coded with the roost name, the field identification number and the sex (F = female and M
662 = male). Regional sequences are coded with the island (MADA = Madagascar and MAU =
663 Mauritius) and field identification number. A pre-print is available on bioRxiv, doi:
664 <https://doi.org/10.1101/2023.06.22.546033>.

665

666 **Author contributions**

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678

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CHAPITRE 2

Dynamiques d'excrétion chez le Petit Molosse

Dans ce second chapitre, nous avons étudié les dynamiques d'excrétion chez le Petit Molosse en relation avec les données écologiques du chapitre 1. Nous avons analysé en parallèle trois agents infectieux aux caractéristiques différentes, nous permettant de caractériser les patrons spatio-temporels d'excrétion et de co-excrétion. Nous avons également travaillé à deux échelles différentes, l'une populationnelle en caractérisant les prévalences d'excrétion dans les gîtes, et l'autre individuelle en caractérisant le statut d'excrétion des individus et l'évolution de la diversité génétique virale au cours du temps grâce aux données de recaptures des individus sur le terrain.

I. Dynamiques spatio-temporelles d'excrétion et de co-excrétion

Dans une première étude, nous avons donc recherché la présence par PCR de paramyxovirus, bactéries leptospires et herpesvirus dans l'urine et la salive de 5949 Petits Molosses durant deux années consécutives. Bien qu'une étude précédente ait montré des pics d'excrétion pour les paramyxovirus et bactéries leptospires en été austral (Dietrich *et al.*, 2015b), les informations relatives aux individus et gîtes n'ont pas été récoltées et le rôle de ces facteurs dans les dynamiques d'excrétion restait donc méconnu. Ainsi, grâce à des modèles statistiques, nous avons testé s'il existe une synchronicité de l'excrétion entre gîtes, une influence de la taille des colonies, du sex-ratio, et des facteurs individuels (âge, sexe, statut reproducteur) sur la prévalence d'excrétion et de co-excrétion (deux ou trois agents infectieux simultanément). Les co-infections pouvant donner lieu à des interactions entre infections, nous avons donc également analysé l'interaction entre agents infectieux, en testant notamment si la présence d'un agent infectieux favorise l'excrétion des autres, et s'il y a un effet cumulatif lors de multiples (bi- et tri-) infections. A l'échelle individuelle, nous avons analysé la temporalité du statut infectieux pour 526 individus recapturés entre 25 et 719 jours d'intervalle, afin de tester l'hypothèse de persistance de l'infection dans l'organisme. Les résultats suggèrent une protection générale des juvéniles par des anticorps maternels, mais qui disparaissent plus ou moins rapidement selon l'agent infectieux. Nous démontrons un rôle majeur de la reproduction chez les adultes sur l'excrétion des paramyxovirus et bactéries leptospires, à la fois chez les femelles pendant la gestation, et chez les mâles pendant la période d'accouplement. Au contraire, pour les herpesvirus, des comportements ou des adaptations physiologiques chez les mâles favoriseraient leur excrétion. Nos résultats montrent que

l'excrétion d'un agent infectieux est positivement corrélée à l'excrétion des autres. En revanche, la probabilité d'en excréter un troisième s'avère être moins importante quand deux agents infectieux sont déjà excrétés, suggérant ainsi des interactions négatives quand des infections sont cumulées. Enfin, les données des recaptures suggèrent que les paramyxovirus, bactéries leptospires et herpesvirus établissent tous une infection persistante chez les chauves-souris. Ces données spatio-temporelles pourraient être mise en relation avec l'urbanisation croissante de l'île afin de comprendre comment les modifications de l'habitat induites par l'homme, influencent l'écologie des chauves-souris et de leurs agents infectieux. Ces résultats sont présentés dans un article en préparation (**Article 3** et Annexe 3).

Article 3

En préparation pour soumission dans la revue PNAS

Aguillon S., Turpin M., Le Minter G., Lebarbenchon C., Hoarau A.O.G., Toty C., Duchet A., Joffrin L., Ramanantsalama R.V., Tortosa P., Mavingui P. & Dietrich M. Fine-scale spatio-temporal monitoring of an island endemic bat reveals how individual and population heterogeneity shapes triple co-excretion dynamics.

1 **Fine-scale spatio-temporal monitoring of an island endemic bat reveals how**
2 **individual and population heterogeneity shape triple co-excretion dynamics**

3

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21 **Author Contributions:**

22 S.A. and M.D. designed research. S.A., M.T., G.L.M., C.L., A.O.H., C.T., A.D., L.J., R.V.R., P.T.,
23 and M.D. performed data samplings and laboratory works. S.A. and M.D. analyzed data.

24 S.A. and M.D. led the writing, with final approval from all co-authors.

25

26 **Competing Interest Statement:**

27 We have no competing interests to declare.

28

29 **Classification**

30 Biological Sciences – Population Biology

31 **Keywords**

32 bats; tropical island; co-excretion dynamics; ecological factors; persistent infection

33 **Short title**

34 Drivers of co-excretion dynamics in bats

35

36 **Abstract**

37 Understanding underlying processes that drive pathogen transmission in bats is crucial
38 for preventing spillover events. However, this requires fine-scale ecological
39 investigations, which are hardly comprehensively implemented because of the
40 complexity and heterogeneity of bat populations. We monitored, over a 24-month
41 period, the excretion of paramyxoviruses and *Leptospira* bacteria in urine as well as
42 herpesviruses in saliva, through the sampling of 5,949 Reunion free-tailed bats
43 (*Mormopterus francoismoutoui*) occupying 17 distinct roosts. We analyzed the influence
44 of within-host and roost-level factors on co-excretion dynamics. Presented data evidence
45 a continuous microbial excretion with high infection prevalence (37% - 87%), low levels
46 of temporal synchrony among roosts and no signal of density-dependent transmission for
47 any of the studied infections. Recapture data support persistent infections for all
48 infectious agents, alongside with clearance followed by reinfection for paramyxovirus and
49 *Leptospira* only. Our results also suggest that sex-specific social behavior or physiological
50 adaptations drive herpesvirus excretion, while paramyxovirus and *Leptospira* excretion
51 reactivate during reproduction. Finally, our data suggest the protection of juveniles by
52 maternal antibodies with different waning dynamics according to the considered
53 infection, which could reflect the important contribution of juveniles in the geographic
54 dispersal of infection. Interestingly, the excretion of one infectious agent was positively
55 correlated to the excretion another infectious agents. However, the probability of
56 excreting a third one was reduced for individuals already bi-excreting, suggesting negative
57 interactions for cumulative infection. Connecting such spatio-temporal data with the
58 growing urbanization on Reunion Island could further help understanding how human-
59 mediated changes in the ecology of bats can affect excretion dynamics and thus spillover
60 events.

61

62 **Significance Statement**

63 Understanding the dynamics of pathogens excretion in wild bat populations is pivotal in
64 addressing spillover risks, but is challenged by regulations issues as well as ecological
65 traits associated to these unique mammals. Here, we used an original island-endemic
66 model to build up a fine-scale spatio-temporal analysis of the excretion of two viruses
67 (paramyxovirus and herpesvirus) and a bacteria (*Leptospira*) at both population- (roost)
68 and individual- (through recaptured bats) levels. Excretion patterns appear specific to
69 each infection and driven by the age and the reproduction of both females and males.
70 Our work also suggests that a combination of viral persistence and clearance, as well as
71 interactions between cumulative infections, are important within-host processes that
72 contribute to the transmission of infections in bat populations. Lastly, it appears that
73 juveniles play an important role in the dispersal of some but not all of these infections,
74 likely depending on the dynamics of maternal immunity for each specific infection. This
75 information is crucial to better predict shedding pulses and prevent future spillovers.

76

77 **Main Text**

78 **Introduction**

79 Recent human outbreaks, such as those caused by the Ebola and SARS-CoV-2 viruses,
80 have highlighted the importance of understanding processes driving the transmission of
81 infectious agents within bat populations (Letko et al., 2020). Indeed, a direct link between
82 infection dynamics in reservoir bats and zoonotic spillovers has been pointed out for
83 several viral human diseases (Amman et al., 2012; Becker et al., 2023; Plowright et al.,
84 2015). Thus, identifying the ecological drivers of pathogen transmission within bat
85 populations, at both within- and between-host levels, is a key element for predicting
86 spillover events (Plowright et al., 2016). Unfortunately, ecological studies investigating
87 infection dynamics in bats often lack of robust spatio-temporal datasets (Becker et al.,
88 2019). This gap results from regulatory (most bat species are protected) as well as
89 logistical constraints due to the elusive and roaming nature of bats that make these
90 animals difficult to study in long-term periods. Moreover, as gregarious mammals, bats
91 often display spatially discrete populations within a metapopulation (Calisher et al.,
92 2006). Such spatio-temporal heterogeneity is characterized by periodically interacting
93 spatial discrete patches, with various sex and age-structure, roost size and function
94 (Aguillon et al., 2023a; Delpietro et al., 2017), which adds complexity to disentangle
95 individual and populational drivers of infection dynamics in bats (Epstein et al., 2020;
96 Griffiths et al., 2020; Meza et al., 2022).

97 With molecular tools becoming increasingly accessible for the study of infectious
98 agents, it is now recognized that co-infections are common in bats (Anthony et al., 2013;
99 Baker et al., 2012; Davy et al., 2018; Hoarau et al., 2020; Wang et al., 2023). However,
100 most of the previous longitudinal studies on bats have focused on a single infectious
101 agent, or different variants within the same viral family (e.g. Joffrin et al., 2022; Mortlock
102 et al., 2019; Peel et al., 2019). Thus, our knowledge on spatio-temporal co-infection
103 dynamics with different groups of infectious agents (DNA/RNA viruses, bacteria, fungi)
104 remains limited (Hoarau et al., 2023; Lane et al., 2022; Selmann et al., 2017). Theory

105 predicts that co-infections can have major consequences on within-host infection
106 dynamics because of potential interactions that might be antagonistic or mutualistic
107 (Hoarau et al., 2020). For example, the infection of field voles with the bacterium
108 *Anaplasma phagocytophilum* increased by five times the susceptibility to cowpox virus
109 (Telfer et al., 2010). Examining the spatio-temporal patterns of simultaneous excretion of
110 infectious agents within bat roosts (co-circulation) and individual hosts (co-infection) is
111 likely to provide much-needed further insights into underlying processes driving infection
112 dynamics (Peel et al., 2019).

113 Island biotas are widely considered as relevant model systems in ecology and
114 evolutionary biology studies (Warren et al., 2015), including those focusing on emerging
115 diseases (Tortosa et al., 2012). Islands are well-known ecosystems hosting limited species
116 number and high levels of endemism (Kier et al., 2009). Due to their ability to over water
117 dispersal, remote oceanic islands favor allopatric speciation of bats: among the 1,456
118 currently recognized species, 25% are endemic to islands (Conenna et al., 2017). The clear
119 boundaries and often reduced surface of islands can help sample island endemic bat
120 populations at a fine resolution, which is crucial to make reliable conjectures about spatial
121 and temporal infection dynamics (Plowright et al., 2019). Moreover, the reduced number
122 of bat species on islands may also simplify ecological interactions, by minimizing
123 interspecies contacts, and by sampling monospecific bat roosts, which can contribute to
124 a more in-depth understanding of infection dynamics at the population level. Due to the
125 reduced species richness typical of small territories, insular ecosystems may also lack
126 predators or competitors and offer empty ecological niches, which can favor the spread
127 of bat colonists and the expansion of their population size (Aguillon et al., 2023a). This is
128 especially true for bat families with highly gregarious behavior such as free-tailed bats
129 (Molossidae), for which roosts can host hundreds of thousands of individuals (Betke et
130 al., 2008; Mcfarlane et al., 2015). Having bats with high bat densities in an area, allow
131 sampling large numbers of individuals, which is essential for a reliable estimation of
132 infectious disease prevalence within roosts. Island endemic bats, with such an abundant,
133 simplified, and well-defined system, represent a rare opportunity to collect extensive

134 spatio-temporal data, and a unique natural laboratory to capture a comprehensive
135 picture of subtle mechanisms underlying infection dynamics in bats (Peel et al., 2018).

136 The Reunion free-tailed bat (*Mormopterus francoismoutoui*; Goodman et al.,
137 2008) is a small tropical insectivorous bat endemic to Reunion Island. This volcanic
138 territory is located in the southwestern Indian Ocean and has emerged about 3 million
139 years ago (Cadet, 1980). Although small in size (2,512 km²), Reunion Island is shaped by a
140 mountainous landscape and has undergone significant landscape changes following 350
141 years of human colonization associated with intensive urbanization and agriculture
142 (Lagabrielle et al., 2009). Reunion free-tailed bats are broadly distributed on the island,
143 and monospecific roosts occur in a diversity of natural habitats, such as caves and cliffs.
144 These molossid bats have also adapted to urbanization and use numerous anthropogenic
145 roosts such as buildings and bridges, hosting from a few hundred to more than 100,000
146 individuals (Aguillon et al., 2023a; Goodman et al., 2008). A recent longitudinal
147 monitoring of several roosts revealed highly dynamic roosting behaviors, with roosts of
148 various sizes, sex-ratio, and functions (female-biased maternities, mixed colonies or male-
149 biased roosts) (Aguillon et al., 2023a). Specifically, large female aggregations (up to
150 50,000 pregnant females) are observed synchronously every year before parturition in
151 austral summer (December), which coincides with a female-biased sex-ratio and a spatial
152 sexual segregation during this period of the year (Aguillon et al., 2023a; Dietrich et al.,
153 2015). Moreover, the end of austral summer (May) is characterized by a decrease in roost
154 size and a change in sex-ratio (from female- to male-biased), suggesting important
155 seasonal sex-specific movements within the island, which is corroborated by the absence
156 of spatial genetic structure in this metapopulation (Aguillon et al., 2023b).

157 Reunion free-tailed bats are natural hosts for several infectious agents (Hoarau et
158 al., 2023; Joffrin et al., 2021, 2022), including paramyxovirus and *Leptospira* bacteria
159 (Dietrich et al., 2015). Previous non-invasive sampling of bat faeces and urine underneath
160 roosts showed shedding pulses occurring in austral summer (Dietrich et al., 2015),
161 followed by a sustained transmission during winter (Hoarau et al., 2023). However, these
162 non invasive studies were based on a reduced number of samples, and no information on

163 bat individuals was recorded as specimens were not hand-manipulated, precluding direct
164 insights into the role of individual factors (e.g. sex, age, reproductive status, body
165 condition) in shaping these infection dynamics. Moreover, investigations were
166 respectively performed on a single (Dietrich et al., 2015) or on two maternity roosts
167 (Hoarau et al., 2023), and the role of population-level factors (e.g. roost size, sex-ratio)
168 remained unexplored. Here, we used data from fine-scale spatio-temporal monitoring
169 and sampling of *M. francoismoutoui*, based on the study of 17 roosts located throughout
170 Reunion Island and sampled during two consecutive years. We measured the
171 simultaneous excretion (through nucleic acid detection) of three infectious agents in each
172 bat, focusing on paramyxovirus (viral RNA) and *Leptospira* (bacteria DNA) both shed in
173 urine, and herpesvirus (viral DNA) in the saliva. Specifically, we first aimed at analyzing
174 spatio-temporal trends in excretion prevalence and testing for roost synchronicity in
175 pathogen shedding. Then, we identified which variables at the individual (sex, age,
176 reproductive status) or roost (roost size, sex-ratio, and level of sexual spatial segregation)
177 level did drive the prevalence of single or multiple infections, and the probability of
178 individuals to switch their excretion status (within-host dynamics).

179

180 **Results**

181 **Spatio-temporal dynamics of excretion prevalence.**

182 Between January 2019 and December 2020 (24 months), we captured 5,949 Reunion
183 free-tailed bats in 17 roosts all over Reunion Island (Fig. 1), including 526 recaptures (up
184 to four recaptures were recorded for the same individual). Excretion data were obtained
185 from 5,518 urine samples for paramyxoviruses (PMV) and *Leptospira* bacteria (LEPTO) and
186 from 3,981 saliva samples for herpesviruses (HSV), including 3,784 individuals for which
187 all three infectious agents were tested. Over the entire studied period, we measured a
188 high global prevalence with $36.63\% \pm 1.27\%$ of bats excreting PMV, $46.48\% \pm 1.32\%$
189 excreting LEPTO and $87.14\% \pm 1.04\%$ excreting HSV (Table 1). We used generalized
190 additive mixed models (GAMM, *SI Appendix*, Tables S1 and S2) to characterize changes in
191 prevalence over space and time. Results revealed no difference in prevalence between

192 the two consecutive years, except for HSV (M3: $\chi^2_{0.8} = 4.64$, $P < 0.01$). Globally, we found
 193 statistical evidence of intra-annual variations for PMV and HSV prevalence only (M1: $\chi^2_{6.7}$
 194 = 16.30, $P = 0.04$; M3: $\chi^2_{8.2} = 40.06$, $P < 0.001$), but when investigating data in adults only,
 195 similar temporal patterns were observed for all three infections, with a higher prevalence
 196 in October-December and a decrease from March to June (Fig. 2A).

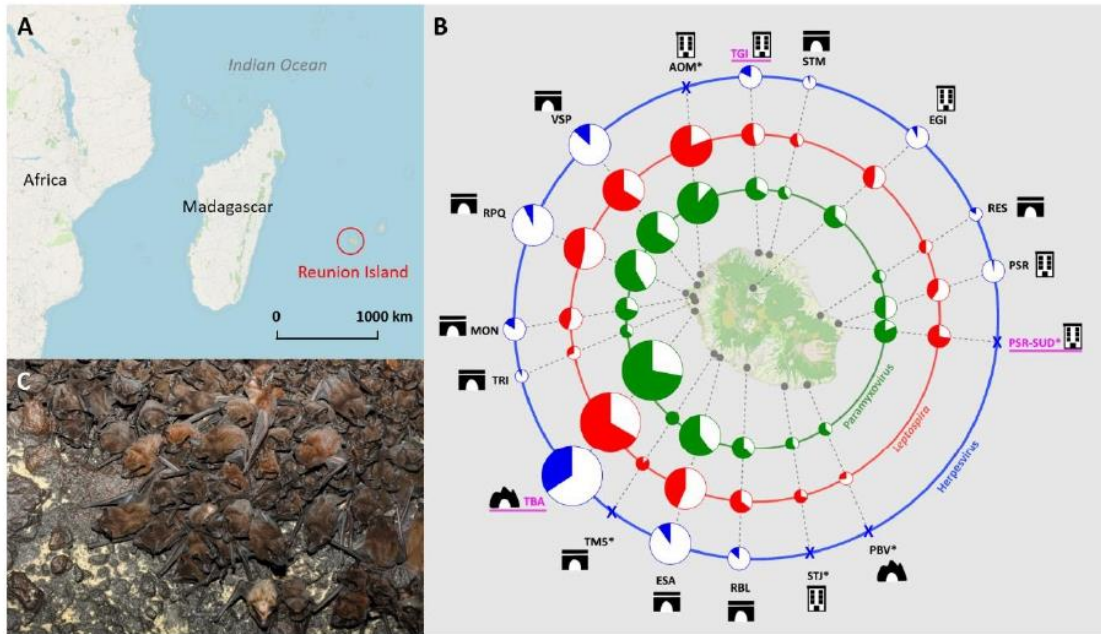
197 Excretion of the three infectious agents was detected in all tested roosts, except
 198 PMV in the TM5 roost (temporary roost exclusively composed of juveniles; see Aguillon
 199 et al., 2023a). Globally, no variation of PMV prevalence was observed among roosts,
 200 although we did detect a significant difference for HSV and LEPTO prevalence (M2: $\chi^2_{8.2} =$
 201 96.73, $P < 0.001$; M3: $\chi^2_{4.4} = 9.61$, $P = 0.02$). A low level of temporal synchrony was
 202 detected among roosts over the studied period, as only 11.1 % of pairwise comparisons
 203 were significant (*SI Appendix*, Fig. S1), and synchrony did not increase among nearby
 204 roosts (PMV: $r^2 = -0.11$; LEPTO: $r^2 = 0.42$; HSV: $r^2 = -0.06$; all $P > 0.05$).

205

206 **Table 1. Details on the sampled roosts of *Mormopterus francoismoutoui* and the**
 207 **number of tested and positive samples.**

Roost	Habitat	No. of sampling periods	Roost size estimation (min and max when available)	No. of urine tested	No. of PMV positive (%)	No. of LEPTO positive (%)	No. of saliva tested	No. of HSV positive (%)
AOM	Building	1	2560	52	6 (11.5)	10 (19.2)	0	no data
EGI	Building	3	no data	149	56 (37.6)	79 (53.0)	89	82 (92.1)
ESA	Bridge	15	570 - 3200	634 (PMV) 635 (LEPTO)	254 (40.0)	360 (56.7)	452	406 (89.9)
MON	Bridge	15	210 - 1150	488	144 (28.4)	268 (54.9)	419	355 (84.5)
PBV	Cliff	1	300	11	4 (36.4)	8 (72.7)	0	no data
PSR	Building	15	no data	512 (PMV) 510 (LEPTO)	255 (49.8)	305 (59.8)	396	387 (97.8)
PSR-SUD	Building	1	no data	51	9 (17.6)	14 (27.4)	0	no data
RBL	Bridge	15	350 - 1300	658	238 (36.2)	235 (35.7)	425	369 (86.8)
RES	Bridge	8	35 - 410	223	91 (41.0)	111 (49.8)	212	179 (84.4)
RPQ	Bridge	15	150 - 3000	535 (PMV) 536 (LEPTO)	229 (41.9)	287 (53.4)	416	386 (92.8)
STJ	Building	1	200	34	14 (41.2)	10 (29.4)	0	no data
STM	Bridge	12	27 - 350	364	145 (39.9)	166 (45.6)	281	267 (95.0)
TBA*	Cave	11	0 - 100,000	500	140 (28.0)	168 (33.6)	330	217 (65.8)
TGI*	Building	15	75 - 1270	496	167 (33.7)	229 (46.1)	432	356 (82.4)
TM5	Bridge	1	200	28	0	4 (14.3)	0	no data
TRI	Bridge	4	153 - 251	125	45 (36.0)	86 (68.8)	112	104 (92.9)
VSP	Bridge	15	15 - 6000	658	224 (34.0)	225 (34.2)	417	361 (86.6)
Total	/	/	/	5,518	2,021 (36.6)	2,565 (46.5)	3,981	3,469 (87.1)

208



209

210 **Figure 1. Widespread triple excretion in *Mormopterus francoismoutoui*.** (A) Location of
 211 Reunion Island and (B) the studied roosts. (C) A picture of bats aggregated in the
 212 maternity roost (TBA site) before parturition. In (B) studied roosts are represented by grey
 213 circles on Reunion Island map and prevalence is indicated in white in pie charts for
 214 paramyxovirus (in green), *Leptospira* bacteria (in red) and herpesvirus (in blue). A cross
 215 denotes the absence of prevalence data (no tested samples). Pie diameter corresponds
 216 to four categories of roost size: small < 500 individuals, medium: 500-2000 individuals,
 217 big: 2000-6000 individuals, and huge > 100,000 individuals. Accurate roost size estimation
 218 was not being performed for EGI and PSR roosts, but they are represented as medium
 219 roosts because several hundred/thousands are expected. Maternity roosts are
 220 underlined in pink. Roosts that were monitored only once are depicted by an asterisk.
 221 Maps were created using Qgis (QGIS Development Team, 2022) with a background of
 222 OpenStreetMap under License CC- BY- S. For better visualization, this figure was inspired
 223 by Streicker et al. (2012).

224

225 Roost-level drivers of excretion.

226 Roost size varied from 15 to 100,000 individuals but was not correlated with the
227 prevalence of PMV and LEPTO (M1: $z = 0.62$; M2: $z = 1.41$; all $P > 0.05$), while HSV
228 prevalence slightly decreased in larger roosts (M3: $z = -2.92$, $P < 0.01$). However, this
229 negative correlation was driven by the low HSV prevalence in juveniles (the period when
230 roost sizes were highest), and disappeared when juveniles were removed from the model
231 (M3 without juveniles: $z = -0.99$, $P = 0.32$). Finally, prevalence was not correlated with the
232 sex-ratio within roosts (M1: $z = 0.01$; M2: $z = -1.05$; M3: $z = -1.81$; all $P > 0.05$) nor with
233 sexual segregation levels of adults among roosts (PMV: $r^2 = 0.39$; LEPTO: $r^2 = 0.43$; HSV: r^2
234 $= 0.51$, all $P > 0.05$).

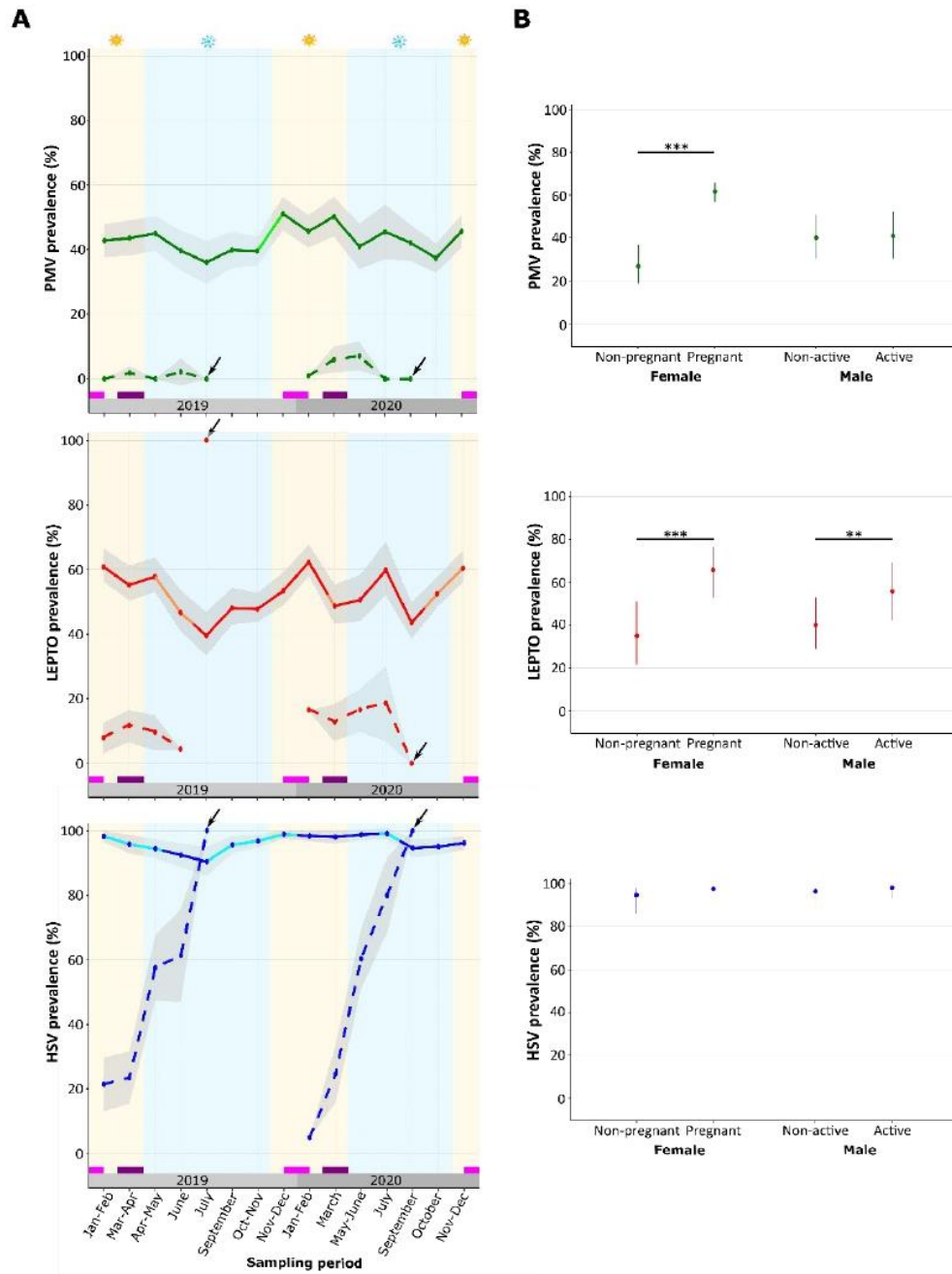
235

236 Individual drivers of excretion.

237 Models revealed that adults were systematically more frequently excretors than juveniles
238 (M1: $z = -5.82$; M2: $z = -6.13$; M3: $z = -11.56$, all $P < 0.001$). Mean prevalence of excretion
239 in adults was $43.00 \pm 1.42\%$ for PMV, $52.85 \pm 1.43\%$ for LEPTO and $96.20 \pm 0.65\%$ for HSV.
240 At the time when juveniles started to fly (January-February; Aguillon et al., 2023a),
241 excretion prevalence in juveniles was either null (PMV) or low (LEPTO: $12.03 \pm 4.11\%$ and
242 HSV: $15.03 \pm 5.66\%$). Then, over six months, HSV prevalence increased drastically in
243 juveniles to reach $60.67 \pm 7.82\%$, while it remained low or only slightly increased for PMV
244 and LEPTO (Fig. 2A). Excretion prevalence also varied according to sex, but only in adult
245 bats, and differently according to the targeted infectious agent. Indeed, adult males were
246 more frequently HSV excretors than females (Tukey's post hoc test: $t = -4.10$, $P < 0.001$),
247 although the opposite pattern was observed for PMV (Tukey's post hoc test, $t = 4.57$, $P <$
248 0.001).

249 To test the influence of reproduction on the excretion status in adults, we focused
250 our analysis on adults during two reproductive periods: pregnancy (November-
251 December) and mating (March-April). During pregnancy, the detection of the two urine-
252 excreted agents, PMV and LEPTO, was higher in pregnant than in non-pregnant females
253 (M4: $\chi_1^2 = 37.49$; M5: $\chi_1^2 = 24.99$, all $P < 0.001$, Fig. 2B). Similarly, during the mating period,

254 reproductively active males were more frequently found excreting than non-reproductive
 255 males, but only for LEPTO (M8: $\chi_1 = 6.76$, $P < 0.01$, Fig. 2B). Higher HSV prevalence was
 256 also observed in both reproductively active females and males, but the difference was
 257 not significant, probably because of a high variability within the non-reproductive groups.



258

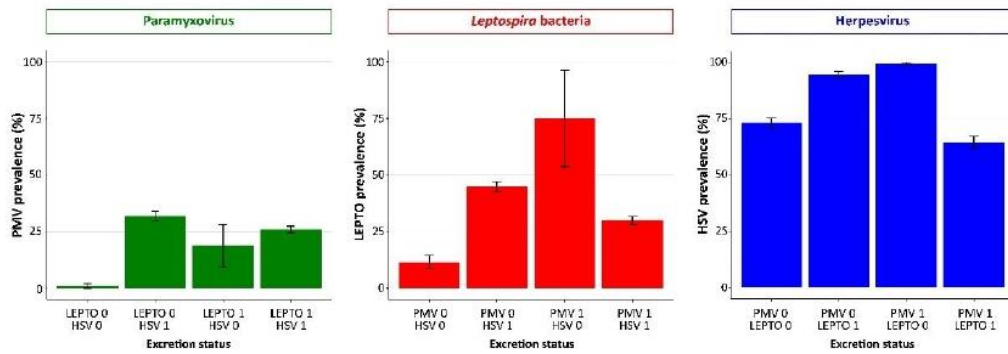
259 **Figure 2. Age and reproduction are the main drivers of paramyxovirus, *Leptospira***
260 **bacteria and herpesvirus excretion dynamics in *Mormopterus francoismoutoui*.**

261 (A) Observed prevalence in adults (solid line) and juveniles (dotted line), with grey areas
262 representing 95% confidence intervals. Periods of significant change (where GAM
263 derivative 95% does not overlap zero) are highlighted by light segments. Arrows indicate
264 periods where only one individual was tested. Pink bars indicate the parturition period
265 and purple indicate the mating period. Austral summer and winter are indicated in yellow
266 and blue, respectively. (B) GAMM-predicted prevalence in adults according to their
267 reproductive status (active or not). Significant effect of GAMMs are indicated: *** $P <$
268 0.005 and ** $P <$ 0.01.

269

270 **Co-excretion dynamics.**

271 Among the 3,784 bats with paired samples (both urine and saliva collected), 36.89% ($n =$
272 1,396) were bi-excretors and 22.46% ($n = 850$) were tri-excretors (*SI Appendix*, Fig. S2).
273 Only 399 bats (10.54%) did not excrete any of the three screened infectious agents, with
274 79.2% of them being noteworthy juveniles. The excretion of one infectious agent was
275 strongly positively correlated with the excretion of any other infection (for all
276 combinations in M1, M2, M3, all $P <$ 0.001). Indeed, when considering bats excreting one
277 infectious agent, the probability of excreting a second infectious agent was multiplied
278 from 1.29) to 31.9 folds, for HSV when excreting LEPTO and for PMV when excreting HSV,
279 respectively (Fig. 3). By contrast, individuals already excreting two infectious agents were
280 less frequently excreting the third one (M1: $z = -3.12$, $P <$ 0.01; M2: $z = -2.42$, $P = 0.02$;
281 M3: $z = -2.42$, $P = 0.02$). For example, *Leptospira* prevalence increased by 3.89 times when
282 bats were already excreting HSV, and by 6.50 times when already excreting PMV, but only
283 by 2.60 times when excreting both HSV and PMV (Fig. 3). In other words, among the
284 infected animals, the occurrence of bi-infection was higher than that of mono- or tri-
285 infection.



286

287 **Figure 3. The probability of excretion increases with bi- and tri-infections.**

288 Infectious status is coded with 0 for non-excreting bats and 1 for excreting bats. The 95%
 289 confidence interval is indicated by a black bar.

290

291 Models showed no significant intra-annual variations for bi- and tri-excretions,
 292 except for PMV-HSV excretion (M12: $\chi_{6.7} = 19.03$, $P = 0.02$), and no effect either of sex-
 293 ratio, sexual segregation or roost size. By contrast, we detected a strong influence of age
 294 and sex, with bi- and tri-excretions being more frequent in adults (M10: $z = -4.74$; M11: z
 295 $= -10.70$; M12: $z = -7.37$; M13: $z = -4.71$; all $P < 0.001$), especially in adult females (Tuckey's
 296 post hoc test, M10: $t = 4.68$, $P < 0.001$; M12: $t = 3.68$, $P < 0.01$, M13: $t = 4.30$, $P < 0.001$).
 297 Finally, pregnancy increased the probability of bi- and tri-excretions in females (M14: $\chi_1 =$
 298 19.85 ; M15: $\chi_1 = 23.04$; M16: $\chi_1 = 28.81$; M17: $\chi_1 = 17.39$; all $P < 0.001$) and reproductively
 299 active males had a higher probability of excreting simultaneously LEPTO and HSV
 300 compared to non-reproductive males (M19: $\chi_1 = 8.36$; $P < 0.01$).

301

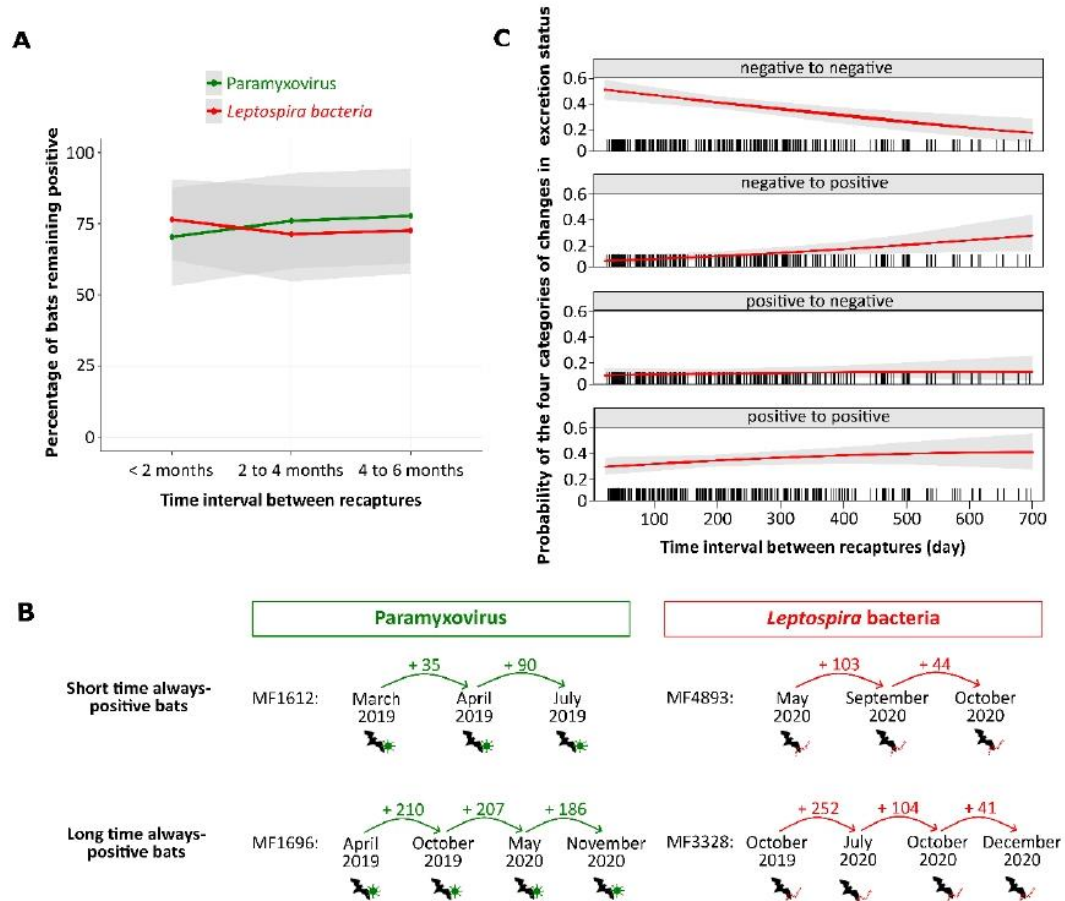
302 **Within-host dynamics of excretion.**

303 All captured bats were marked (by tattooing on the wing) allowing to collect excretion
 304 data at the individual level. Using samples from 526 recaptured specimens, 388 urine
 305 samples were tested for PMV and 389 for LEPTO, leading to a final dataset composed of
 306 471 and 457 samples for PMV and LEPTO, respectively, when initial capture samples were
 307 including to recapture samples. We also tested 55 saliva samples from 31 recaptured bats

308 for the presence of HSV. All bats initially testing positive for HSV ($n = 29$) remained positive
309 over time (between 25 and 405 days between recapture events), while the only two
310 initially-negative bats were testing positive at their first recapture. Thus, within-host
311 analyses using multinomial logistic regressions of excretion status changes were only
312 performed on PMV and LEPTO data. Recapture intervals ranged from 25 to 719 days (*SI*
313 *Appendix*, Fig. S3A), during which we defined four conversion classes: negative to negative
314 (no conversion), negative to positive, positive to negative, and positive to positive (no
315 conversion). For many recaptured animals, bats kept the same excretion status over time.
316 Indeed, between 50.74% (PMV) and 40.04% (LEPTO) of recaptured bats remained
317 negative or, to a lesser extent (PMV: 25.69%, LEPTO: 34.79%) positive. Interestingly, when
318 looking at short windows of time (from 1 to 6 month-intervals), a major proportion (about
319 75%) of PMV or LEPTO-positive bats remained positive (Fig. 4A). Some bat individuals
320 remained positive across all their recapture events (Fig 4B).

321 The probability of the four excretion status changes was dependent on the time
322 interval for LEPTO (M23: $\chi^2_3 = 17.60$, $P < 0.001$, Fig. 4C and *SI Appendix*, Fig. S3B), but not
323 for PMV (M22, $\chi^2_3 = 5.11$, $P = 0.16$, *SI Appendix*, Fig. S3C). Indeed, Figure 4 showed that
324 LEPTO-negative bats had a higher probability of remaining negative over short periods of
325 time, although the probability of becoming positive increased with time. However, when
326 individuals were excreting LEPTO at the time of their first capture, the probability of
327 becoming negative was not dependent on the time. In contrast, the probability of being
328 still excreting LEPTO did increase with time. We also found a significant effect of changing
329 reproductive status on the probability of changing both PMV and LEPTO excretion status
330 (M22: $\chi^2_9 = 32.45$, $p < 0.001$; M23: $\chi^2_9 = 20.11$, $P = 0.02$, *SI Appendix*, Fig. S4). Indeed, bats
331 that remained non-reproductively active over recapture events (reproduction: 0/0), also
332 mainly remained LEPTO- and PMV-negative (conversion: 0/0). Similarly, most bats
333 successively captured when reproductively active (reproduction: 1/1) remained LEPTO-
334 and PMV-positive (conversion: 1/1). Most bats becoming positive across recaptures also

335 became reproductively active, especially for PMV (20.97% for PMV and 11.67% for
 336 LEPTO).



337
 338 **Figure 4. Temporal changes of excretion status in recaptured *M. francoismoutoui* bats.**
 339 (A) Probability of remaining LEPTO and PMV positive through time. (B) Examples of bats
 340 with always-positive status for PMV and LEPTO over recapture events. The time interval
 341 between two recaptures is indicated above the arrow. (C) Probability of the four
 342 categories of LEPTO excretion status change, with a 95% confidence interval in grey.

343

344 Discussion

345 Using an original island-endemic bat model, we demonstrated the widespread and all-
 346 year-long co-circulation of three viral and bacterial infections in Reunion free-tailed bat

347 populations, which confirms the circulation of PMV and LEPTO (Dietrich et al., 2015;
348 Hoarau et al., 2023) and describes for the first time HSV infection in Reunion free-tailed
349 bats. We revealed high global prevalence of PMV (37%) and LEPTO (46%), and extremely
350 high prevalence of HSV (87%). This is coherent with the high prevalence of HSV generally
351 found in other bat species, as recently reported in the Mexican free-tailed bat *Tadarida*
352 *brasiliensis* (Moreira-Marrero et al., 2021) and in the common vampire bat *Desmodus*
353 *rotundus* (Griffiths et al., 2020, 2022). High infection prevalence in Reunion free-tailed
354 bats could be explained by a strong gregarious behavior of these bats, which can
355 aggregate up to 900 individuals per m² (Héré et al., 2009). The proximity of individuals
356 within high-density roosts can facilitate transmission through bat fluids (saliva, urine), in
357 which case, roost size is often assumed to positively correlate with prevalence (Plowright
358 et al., 2015; Serra-Cobo et al., 2013; Streicker et al., 2012). However, we did not find such
359 a correlation, suggesting that density-dependent transmission may not play a major role
360 in the circulation of paramyxovirus, *Leptospira*, and herpesvirus in Reunion free-tailed
361 bats. However, we cannot exclude that if prevalence is biased towards certain groups of
362 individuals that aggregate in high density within area, then transmission is likely to be
363 related to the density of these high-risk bat groups, rather than the overall roost size
364 measured in our study (Begon et al., 2002). Interestingly, we did not find evidence of
365 spatial synchrony between roosts, despite probably frequent intra and inter-seasonal
366 movements within this bat metapopulation (Aguillon et al., 2023a, 2023b). Thus, transient
367 epidemics, passing as waves of bat-to-bat transmission between roosts, are probably not
368 the main mechanism involved in maintenance (Epstein et al., 2020; Laughlin et al., 2019;
369 Plowright et al., 2015) and the probability of excretion seems therefore more related to
370 individual factors than to population level characteristics.

371 Chronic or episodic shedding from persistently infected bats can explain high
372 prevalence across roosts and time. Indeed, our work shows that nearly all (96%) of
373 recaptured bats, initially HSV-positive, were still excreting HSV in saliva over recaptures
374 supporting lifelong HSV infection in Reunion free-tailed bats. HSV are known to establish
375 latent infection in humans (Cohen, 2020) and rodents (Erazo et al., 2021), and the same

376 mechanism is also suspected in bats (Gerow et al., 2018; Griffiths et al., 2023).
377 Interestingly, in *D. rotundus*, the length of the active periods of betaherpesvirus (when
378 shedding occurs) appears to be longer than the latency state (when shedding resumes)
379 (Griffiths et al., 2023), which is coherent with our recapture data showing almost always
380 HSV-positive bats over recaptures. For PMV and LEPTO, we showed that viral RNA and
381 bacterial DNA were present in urine for up to 3 months after their initial detection in a
382 large proportion of recaptured bats (75%). Together with examples of bats remaining
383 positive over multiple recapture events, this supports that persistent infection can also
384 explain PMV and LEPTO temporal dynamics. However, we also evidenced positive to
385 negative excretion switches for PMV and LEPTO, suggesting that cycles of clearance
386 followed by reinfection also occur in the bat population. Persistent LEPTO infection in bats
387 unsurprising as this bacterial infection is often considered chronic in other reservoir hosts,
388 bacteria forming protective biofilms in renal tubules (Almeida et al., 2019). Experimental
389 infection studies have shown that *Leptospira interrogans* can stably colonize the kidneys
390 of rats and mice over their lifespan (Cordonin et al., 2020; Ratet et al., 2014). Although
391 chronic infection is typical in reservoir hosts, intermittent shedding is also commonly
392 reported, suggesting that planctonic bacteria are not permanently shed from stable
393 biofilms colonizing renal tubules (Cordonin et al., 2020; Monti et al., 2023). Presented
394 data show that when individuals are excreting LEPTO at the first capture, the probability
395 of becoming negative is not dependent on the time, which is in keeping with an
396 intermittent excretion. On contrary, mechanisms underlying within-host PMV dynamics
397 are still debated (Glennon et al., 2019; Plowright et al., 2016). Several field and lab
398 evidence support that henipavirus, for example, can persist within bat individuals with
399 long-term continuous or intermittent excretion (Epstein et al., 2020; Gibson et al., 2021;
400 Peel et al., 2012, 2018; Rahman et al., 2010; Sohayati et al., 2011). A modeling study also
401 suggests that latent henipavirus infections can occur alongside with occasional viral
402 clearance, followed by temporary immunity and reinfection (Glennon et al., 2019). This
403 same study supports that bats are much more likely to develop a latent infection than
404 clearing it. Finally, it has been hypothesized that a small portion of super-long-shedder

405 individuals with a long infectious period could maintain Hendra virus in bat populations
406 (Plowright et al., 2011). Distinguishing between these different infection processes is
407 complex. Finally, since because we only measured the presence of viral and bacterial
408 nucleic acids in our study, an important step will be to assess the infectiousness of
409 excreted samples further to finely validate the role of long-term continuous and
410 intermittent excretion in transmission (Plowright et al., 2015).

411 In persistent infections, the reactivation process is often linked to several stressors
412 that may affect host immunity (Becker et al., 2020; Grinde, 2013). Reproduction, by
413 imposing immunological challenges, is recognized as a major stressor that could lead to
414 the reactivation of viral and bacterial infections (Negrey et al., 2022). Such processes are
415 expected to be particularly pronounced in female mammals, for whom reproduction
416 requires large and prolonged energetic investments (Butte et al., 2004; Speakman,
417 2008). We showed that pregnancy increased the probability of PMV and LEPTO (but not
418 HSV) excretions, as well as bi- and tri-excretions in Reunion free-tailed bats. Variable
419 patterns between infectious agents are coherent with the increased seroprevalence
420 observed in reproductively active females of *P. scapulatus* infected with Hendra virus
421 (Plowright et al., 2008), and the absence of such correlation for HSV-infected *Rousettus*
422 *aegyptiacus* and *Miniopterus natalensis* (Dietrich et al., 2018). Moreover, recapture data
423 showed that most bats becoming PMV and LEPTO positive across recaptures were also
424 those becoming reproductively active. We thus hypothesize that both PMV and LEPTO
425 excretions are reactivated during female's pregnancy in Reunion free-tailed bats. In
426 humans, such process has been described for the VZV (Varicella Zoster Virus), for which
427 reactivation occurs commonly during the third trimester of pregnancy (Mourad et al.,
428 2022). Reactivation during bat pregnancy could explain the enhanced prevalence
429 observed during the pregnancy period (October to December), a pattern reported in
430 other field studies (Dietrich et al., 2015, 2018; Drexler et al., 2011; Hoarau et al., 2023;
431 Joffrin et al., 2022; Mortlock et al., 2019). Such reactivation could also lead to horizontal
432 transmission to non-immune bats, including juveniles. However, we cannot exclude that
433 immigration of infected bats also has a role in transmission during late pregnancy, as

434 massive aggregations of bats occur at this time of the year (Aguillon et al., 2023a). Our
435 study therefore supports the role of a trade-off between pregnancy and immunity. Some
436 studies have hypothesized that such process, beside conferring protection to newborns
437 through the transfer of protective maternal antibodies, could benefit to the mothers
438 through immunity boost (Mourad et al., 2022).

439 Although less documented, male reproduction trade-offs have been suggested in
440 bats, with higher Hendra seroprevalence observed in *Pteropus scapulatus* males during
441 the mating season (Plowright et al., 2008). Our study revealed that during the mating
442 period, reproductively active males had a higher probability of excreting LEPTO and
443 LEPTO-HSV, as compared to non-reproductive males. By contrast, no difference was
444 observed for PMV excretion. This suggests that trade-offs between reproduction and
445 immunity in males could drive excretion dynamics, but that differences exist depending
446 on the considered infectious agent. Such pattern could be mediated by reduced immune
447 function during spermatogenesis, a period of high energy demand and elevated
448 testosterone (Encarnaç o et al., 2004; Martin & Bernard, 2000). As well, reproductively
449 active males of *Myotis daubentonii* have lower cellular immunity than non-reproductive
450 individuals (Ruoss et al., 2019). Our results mirrors patterns commonly reported in human
451 leptospirosis, where sexually mature males are disproportionately more infected than
452 women and children (Puca et al., 2018).

453 Previous studies have shown that environmental stressors also can impact HSV
454 transmission in wildlife (Knowles et al., 2012). HSV was, for example, detected in the
455 blood of big brown bats (*Eptesicus fuscus*) arousing from hibernation, but not in non-
456 hibernating individuals, suggesting a reactivation of the virus resulting from the stress
457 associated with arousal (Gerow et al., 2018). Accordingly, our results showed a slight
458 increase of HSV prevalence in adult bats at the end of winter (July to November).
459 However, Reunion free-tailed bats are tropical, and we thus suspect no strict hibernation
460 occurs (Geiser, 2013), supported by observations of bat activity all year (Aguillon et al.,
461 2023a). The HSV increase in winter might alternatively result from a misclassification of
462 juveniles in adults, because differentiating those age classes based on the epiphysis fusion

463 is challenging at this period. Since juveniles are less infected than adults, we might have
464 artificially lowered the prevalence in adults.

465 Behavioral and physiological traits are two mechanisms that can produce sex-
466 biased prevalence in several wildlife species (Guerra-Silveira & Abad-Franch, 2013; Zuk,
467 2009). Here, males were more frequently excreting HSV than females, as previously
468 reported in rodents (Erazo et al., 2021) or for other DNA bat viruses such as adenoviruses
469 (Rossetto et al., 2020). Studies on HSV prevalence in bats are limited and report
470 contrasting results, with no sex difference in *D. rotundus* (Griffiths et al., 2020), while
471 Sjodin et al. (2019) suggest that either males or females can be more infected, depending
472 of the herpesviral strain. Male-biased infection may arise from sex-specific social behavior
473 like dominance or higher aggressiveness (physical injuries, i.e. bite) (Ancillotto & Russo,
474 2014; Ortega et al., 2008), that could enhance transmission in males (Becker et al., 2018;
475 Erazo et al., 2021). However, this hypothesis is not well-supported in Reunion free-tailed
476 bat populations, since increased transmission among aggressive males should lead to a
477 higher prevalence during the mating season, which was not observed. Sex hormones can
478 also strongly affect host susceptibility, through the modulation of immunity (Foo et al.,
479 2017). Testosterone may suppress immune responses in males, increasing susceptibility
480 to pathogen infection (Klein, 2004). The inclusion of hormone and immunity measures
481 should thus be considered in future infection studies to test this hypothesis in bats.

482 Maternal transmission of antibodies conferring specific protection to juveniles is
483 a well-known process in mammals, and has often been suggested and demonstrated in
484 bats (Amman et al., 2012; Baker et al., 2014; Breed et al., 2011; Drexler et al., 2011;
485 Hayman et al., 2018; Peel et al., 2012; Plowright et al., 2008). The level of maternally
486 transferred antibodies to juveniles is thought to reflect the amount of circulating
487 antibodies in females, and could thus positively correlate with infection prevalence
488 (Hasselquist & Nilsson, 2009). Here, although the prevalence in adult females was much
489 higher for HSV than for PMV and LEPTO, up to 20% (in 2019) of juveniles were HSV-
490 positives at the start of the juvenile period (January), while almost all juveniles were non-
491 infected by PMV (with an intermediate pattern observed for LEPTO). This suggests that

492 despite a wider excretion of HSV in the bat population, initial levels of maternal antibodies
493 could be lower for HSV, compared to PMV and LEPTO. If the waning of maternal
494 antibodies in juveniles depends on the initial levels provided by mothers, this can explain
495 the distinct excretion dynamics later observed in juveniles. Indeed, although most
496 juveniles remained PMV and LEPTO negative during the first six months of their life, HSV
497 excretion rapidly increased to reach 80% in July 2020. Contrary to HSV, the protection
498 against PMV and LEPTO seems therefore quasi-total (although lower for LEPTO) and
499 longer (> six months) for these two infections, suggesting different strategies of maternal
500 immunity in bats. Apparent short-term maternal immunity has been proposed in Egyptian
501 fruit bats (*Rousettus aegyptiacus*) infected with Marburg virus, for which juveniles'
502 infection increases by 5-fold within 3 months (Amman et al., 2012), while protection of
503 about 6-7 months has been reported from field and modelling studies on Lagos bat and
504 henipa viruses (Baker et al., 2014; Epstein et al., 2013; Peel et al., 2018). Finally, the rapid
505 and steady HSV infection in juveniles coincides with their initial dispersal all over the
506 island (Aguillon et al., 2023a) and could thus play a major role in the spatial spread of this
507 virus (Amman et al., 2012; Drexler et al., 2011; Hoarau et al., 2023; Plowright et al., 2008).
508 By contrast, the spread of PMV and LEPTO by juveniles is probably lower (or occurs later
509 in the year), as juveniles remain poorly infected at least during their first six months of
510 life, highlighting that the role of juveniles in infection dynamics is pathogen-dependent
511 (Plowright et al., 2011).

512 Our investigation targeting multiple simultaneous infections revealed a high co-
513 circulation of viruses and bacteria within the bat population, with 59% of bats excreting
514 at least two infectious agents. Our results also showed that bats excreting one virus or
515 bacteria had significantly more chance of excreting another one. This corroborates
516 previous results suggesting positive interactions that could facilitate the excretion of
517 paramyxo- (Dietrich et al., 2015; Peel et al., 2019) and corona-viruses (Davy et al., 2018;
518 Seltmann et al., 2017). In the study of Davy et al. (2018), the stress of fungal infection by
519 *Pseudogymnoascus destructans* led to a reactivation of coronavirus replication in the little
520 brown bat *Myotis lucifugus*, and co-infection even increased the severity of White Nose

521 Syndrome. Interestingly, in our study, when bats were already excreting two infectious
522 agents, they had less probability of excreting the third one, suggesting that negative
523 interactions can also occur when accumulating infections. Interactions between
524 infectious agents could modulate host susceptibility with contrasting effects (antagonistic
525 and mutualistic) depending on the community of infectious agents considered (Telfer et
526 al., 2010). Our results illustrate how considering simultaneously multiples infections,
527 although complex, is important to better understand drivers of infection in bats (Alizon,
528 2013; Habig et al., 2021; Hoarau et al., 2020).

529

530 **Conclusion**

531 Our study sheds light on the processes driving the spatio-temporal excretion dynamics of
532 RNA and DNA viruses as well as bacteria in bats. In addition to suggested latent
533 herpesvirus infections, our study suggests that bats commonly host persistent
534 paramyxovirus and *Leptospira* infections, with reproduction-related episodic excretion, in
535 both females and males. Surprisingly, transient epidemics and density-dependent
536 transmission do not appear as major drivers of infection. By contrast, juveniles play an
537 important role in the dispersal of these infections, with distinct waning immunity
538 dynamics likely modulating this role according to the considered infection. Testing these
539 hypotheses requires serological data and spatially explicit and mechanistic modeling
540 studies (Lunn et al., 2021). Disruption of balanced host-pathogen relationships due to
541 stressors, such as habitat loss or co-infections, may increase pathogen excretion, and
542 enhance the likelihood of spillovers (Becker et al., 2023). Further studies connecting
543 multi-infection dynamics to the growing urbanization process that faces Reunion free-
544 tailed bats will help in understanding how human-mediated changes in the ecology of
545 bats may affect natural excretion dynamics and in turn the probability of spillovers (Eby
546 et al., 2022).

547

548 Materials and Methods**549 Study sites and field sampling**

550 We monitored and sampled 17 roosts (coded with a 3-letter code) from January 2019 to
551 December 2020 (24 months), all over Reunion Island (Fig. 1). Among the 17 studied roosts,
552 12 were monitored across a maximum of 15 successive “sampling periods” occurring
553 every 3–8 weeks, each sampling period lasting for 3–4 weeks to be able to sample
554 multiple roosts (Table 1 and Table S3). An opportunistic sampling was performed for five
555 additional roosts, which were thus monitored only once during the studied period. To
556 investigate within-host dynamics, we used data from recaptured bats, and thus also
557 included previously collected samples from 2018 (referred as the first capture samples).

558 Bat monitoring includes bat capture, biological sampling and roost size estimation
559 and is fully described in Aguillon et al. (2023a). Captures were carried out using harp traps
560 (Faunatech Ausbat) and Japanese monofilament mist nets (Ecotone) during dusk
561 emergence, with a maximum of 60 individuals captured per night. Bats were hydrated
562 immediately after the capture and kept individually in a clean individual cotton bag hang
563 close to a warm water bottle. For each bat, we measured the forearm length and mass,
564 and determined the sex, age, and reproductive status. Age was established by examining
565 the epiphysis fusion in finger articulations that are not welded for juveniles which was
566 easily identifiable until July, while some juveniles might have been classified as adults in
567 the following months. Active reproductive status was recorded when females were
568 pregnant and lactating, and in males when they had large testes (see Aguillon et al., 2023a
569 for more details). A sterile swab (Puritan Medical Products, USA) was carefully inserted in
570 the corner of the lips to sample saliva and then placed in 250uL of MEM (Minimum
571 Essential Medium Eagle). Using a pipette and a sterile tip, urine droplets were collected
572 in the waterproof bag or directly at the urethral opening when handling bats, and then
573 placed in 50 μ L of MEM. All samples were stored in a cooled box in the field before being
574 transferred at -80°C at the laboratory the same night. Finally, each bat was tattooed on

575 the right proptagium with an individual alphanumeric code and released at the capture
576 site.

577 Bats were handled using personal protective equipment regularly changed and all
578 the equipment was disinfected between sites (more details in Aguillon et al., 2023a). Bat
579 capture and manipulation protocols were evaluated by the ethic committee of Reunion
580 Island, approved by the French Ministry of Research (APAFIS#10140-
581 2017030119531267), and conducted under a permit delivered by the Direction de
582 l'Environnement, de l'Aménagement et du Logement (DEAL) of Reunion Island
583 (DEAL/SEB/UBIO/2018-09).

584

585 **Molecular detection of infectious agents**

586 Urine and saliva samples were processed using the Cador Pathogen 96 Qiacube HT kit
587 (Qiagen, Hilden, Germany), using 25 µL and 100 µL of samples, respectively, mixed with
588 175 µL and 100 µL of VXL buffer for the lysis step. Total nucleic acids were extracted using
589 an automated extractor Qiacube with small modifications of the Q Protocol using 350 µL
590 of ACB, 100 µL of AVE, and 30 µL of TopElute. A reverse transcription was then performed
591 on urine extracts (Lebarbenchon et al., 2017), followed by the screening of paramyxovirus
592 and *Leptospira* bacteria, using a semi-nested PCR targeting the RNA polymerase gene and
593 a real-time PCR amplifying a fragment of the 16S rDNA gene, respectively (Dietrich et al.,
594 2015; Smythe et al., 2002; Tong et al., 2008). For paramyxovirus, amplicons of the first
595 PCR were diluted to 1:10, and 2 µL was then used in the semi-nested PCR.

596 For saliva extracts, a fragment of herpesvirus DNA polymerase was amplified using
597 a nested PCR. During the first PCR, 2 µL of DNA was amplified in a 25 µL mixture containing
598 12.5 µL of GoTaq® Green Master Mix 2X (Promega, Madison, Wisconsin, United States),
599 2.5 µL of each primer at 10 µM DFA (5'-GAYTTYGCNAGYYTNTAYCC-3') and KG1 (5'-
600 GTCTTGCTCACCAGNTCNACNCCYTT-3') (VanDevanter et al., 1996). We used the following
601 thermal conditions: 95°C for 2 min, and 45 cycles at 94°C for 30 sec, 46°C for 1 min and
602 72°C for 1 min, and a final step at 72°C for 7 min. During the nested PCR, 5 µL of amplicon
603 was amplified in a 25 µL mixture containing 12.5 µL of GoTaq® Green Master Mix 2X

604 (Promega, Madison, Wisconsin, United States), 2.5 μ L of each primer at 10 μ M TGV (5'-
605 TGTAACCTCGGTGTAYGGNTTYACNGGNGT-3') and IYG (5'-
606 CACAGAGTCCGTRTCNCCRTADAT-3') (VanDevanter et al., 1996). We used the following
607 thermal conditions: 95°C for 2 min, and 35 cycles at 94°C for 30 sec, 46°C for 30 sec and
608 72°C for 30 sec, and a final step at 72°C for 7 min. PCR products were revealed using
609 electrophoresis with 2% agarose gel stained with 2% GelRed (Biotium, Fremont, USA).

610

611 **Statistical analyses**

612 To evaluate spatio-temporal dynamics of excretion, we fit binomial generalized additive
613 mixed models (GAMM; Wood, 2017) via restricted maximum likelihood (REML) using the
614 *mgcv* package (Table S1). We used penalized smoother for the temporal trend variable
615 and controlled that the effective degrees of freedom (EDF) were higher than 1 (non-linear
616 effect). The number of basis functions (*k*) and smooth parameters (α) were adjusted
617 in each model using *gam.check* from the *mgcv* package to avoid overfitting and check for
618 model convergence. Temporal trend was implemented specifying a number for each
619 sampling period starting from 1 (January-February) to 16 (November-December, Table
620 S3). Due to the COVID-19 crisis and the resulting lockdown in France, we did not monitor
621 roosts in April 2020, and do not present data at that time. To properly account for the
622 temporal effect, we decided to code the May-June 2020 sampling period as the middle of
623 March (coded as 10) and July 2020 (coded as 13, Table S3). Final models for mono-, bi-
624 and tri- excretions included sampling period as smooth function and as linear function:
625 the roost size, sex-ratio, age and sex (and their interaction), including the roost, Julian day
626 and year as random effects. The significance of each modality of age/sex interactions was
627 tested using a Tukey's post hoc test. For mono-excretion models, we included the two
628 other infectious agents and the corresponding bi-excretion as a linear function (details in
629 Tables S2 and S3). Moreover, to determine periods of significant increase or decrease
630 prevalence of mono-excretions in adults, we used simplified models that only included
631 sampling period as a smooth function, and identified periods where the 95% confidence

632 interval (CI) of the GAM prediction's derivative did not overlap zero, using the R function
633 *derivSimulCI* (www.gist.github.com/gavinsimpson).

634 To assess the effect of reproductive status on excretion, we focused on adult bats
635 at two specific periods of the year: firstly in adult females during the pregnancy period
636 (November-December) and then in adult males during the mating period (March-April;
637 Aguillon et al., 2023a). We thus used a separate GLMM on a subset of data, for each sex
638 and included reproductive status as a linear effect, keeping roost, Julian day and year as
639 random effects (Table S1 and S2).

640 The influence of sexual spatial segregation patterns in adults was tested on the
641 prevalence of mono-, bi-, and tri-excretions, using the Sexual Segregation and
642 Aggregation Statistic (SSAS; Bonenfant et al., 2007) that compares observed aggregation
643 and segregation patterns to a random association of males and females among roosts.
644 We calculated SSAS values for each sampling period, and tested if the SSAS was correlated
645 to the global monthly prevalence of excretion using a Pearson correlation test.

646 Then, we analyzed the spatial (between roosts) synchrony in prevalence using the
647 same approach as in Meza et al. (2022). We selected the seven roosts monitored during
648 every sampling period (ESA, MON, PSR, RBL, RPQ, TGI and VSP). We used Mantel tests
649 (from *vegan* package) to investigate geographical distances between roosts and
650 calculated pairwise synchrony of prevalence by sampling period for each infectious agent
651 separately, using Spearman correlations tests (Fig. S1).

652 To investigate within-host excretion dynamics, we tested the probability of
653 changing infectious agent excretion status through time, by fitting a multinomial logistic
654 regression using the *multinom* function in the *nnet* package (R script of Streicker et al.,
655 2019) (Table S4). Because not enough saliva samples were available to analyze the
656 conversion of HSV excretion status, we only modeled conversion status associated with
657 PMV and LEPTO, separately. Conversion status was used as a nominal dependent variable
658 with four levels (negative to negative, negative to positive, positive to negative, and
659 positive to positive), with the following covariates: recapture interval (in days), sex, and
660 reproductive status conversion (active to active, non-active to active, active to non-active

661 and non-active to non-active). Age classes were not included in the models because of
662 the limited number of juveniles compared to adults ($n_{\text{JUV}} = 29$, $n_{\text{AD}} = 497$). This analysis
663 used the non-changing infection, negative to negative, as a baseline outcome against
664 which to compare other infectious status changes. To better visualize the probable
665 continuous excretion over short time windows (<6 months), we calculated and plotted
666 the percentage of bats remaining positive for PMV and LEPTO across different time
667 intervals: less than 2 months, between 2 and 4 months, between 4 and 6 months.

668 All analyses were performed in Rstudio 1.4.1106 (RStudio, 2020) using packages
669 *dplyr*, *effects*, *emmeans*, *lme4*, *ggeffects*, *ggplot2*, *mgcv*, *nnet*, and *vegan*.

670

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682

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1082

II. Diversité génétique et dynamique intra-hôte des herpesvirus

Notre précédente étude (Article 3) a révélé que le Petit Molosse de La Réunion excrète des herpesvirus avec une très forte prévalence dans la population (87%), et que ces virus établissent probablement une infection persistante chez les individus. Pour tester cette hypothèse, et mieux comprendre la dynamique d'infection à l'échelle individuelle (intra-hôte), nous avons, dans cette dernière partie, utilisé le séquençage haut-débit d'échantillons de salive de Petits Molosses positifs en herpesvirus ($n = 121$), en incluant des individus recapturés jusqu'à quatre fois ($n = 11$). Cette approche nous a permis de discriminer les différentes souches d'herpesvirus qui co-infectent les Petits Molosses. Nous avons ainsi pu analyser la structure génétique des souches d'herpesvirus à l'échelle populationnelle et individuelle. Nous avons tout d'abord testé l'influence des facteurs individuels (âge, sexe, statut reproducteur) sur la diversité virale, à l'échelle des souches et des sous-familles (alpha-, beta-, gamma-), en incluant les interactions potentielles entre sous-familles virales chez les individus co-infectés. Ensuite, nous avons analysé les dynamiques intra-individuelle d'excrétion, en estimant la probabilité de changer de sous-famille et en mesurant la diversification temporelle des souches au sein de chaque sous-famille. Nos résultats révèlent une diversité importante de souches d'herpesvirus ($n = 20$) chez le Petit Molosse, qui est souvent infectée par les trois sous-familles (44%). De plus, les patrons de co-infections sont presque aléatoires à l'exception des alpha- et gamma- qui semblent antagonistes. Nous montrons une diversité de souches réduite chez les juvéniles en comparaison des adultes, appuyant l'hypothèse d'une transmission d'anticorps maternels. Les données issues des recaptures d'individus montrent que la probabilité d'être infecté par une nouvelle sous-famille d'herpesvirus augmente avec le temps, et suggèrent donc l'accumulation de souches au cours de la vie, qui peuvent se mettre en latence. Pour chaque sous-famille d'herpesvirus, la majorité des chauves-souris gagne et perd en même temps des souches entre les captures, ce qui suggère l'existence de mécanismes de latence et de réactivation des herpesvirus. Pour finir, nos résultats montrent que les femelles gestantes excrètent une diversité plus importante d'herpesvirus par rapport aux non gestantes, suggérant que la gestation peut réactiver l'excrétion des herpesvirus et mener à la diversification de la communauté virale au sein des chauves-souris. Les résultats sont présentés dans un article en préparation (**Article 4** et Annexe 4).

Article 4

En préparation pour soumission dans la revue Biology Letters

Aguillon S., Turpin M., Le Minter G., Lebarbenchon C., Hoarau A.O.G., Mavingui P. & Dietrich M. Bat things come in threes: within-host dynamics of herpesvirus triple infection in bats.

1 **Bat things come in threes: within-host dynamics of herpesvirus triple infection**
2 **in bats**

3

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25 **Short title:** Herpesvirus co-infections in bats

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28

29 **Abstract**

30 Deciphering viral community ecology in bats is crucial to understand patterns of shedding and
31 the consequences for zoonotic spillover risk. Here, we investigate the genetic diversity and
32 within-host dynamics of herpesviruses (HSV) in a tropical insectivorous bat (*Mormopterus*
33 *francoismoutoui*) endemic to Reunion island. Saliva samples were collected over three years
34 in seven roosts and from recaptured bats. Illumina sequencing of HSV-positive samples
35 revealed a high diversity of strains (n=20), which belong to alpha, beta and gamma-HSV
36 subfamilies. We showed that triple infection is common (44%), and that patterns of co-
37 infection with different subfamilies were mostly random, except between alpha- and gamma-
38 HSV which seem antagonistic. We also demonstrated a lower HSV diversity in juveniles
39 compared to adult bats, which supports their protection by maternal antibodies. Recapture of
40 individuals suggested an accumulation of multiple HSV latent-infections over time, as the
41 probability to be infected with a new subfamily increased with time. Within each subfamily,
42 the genetic diversification of strains was highly dynamic, with 79% of bats both gaining and
43 losing strains between capture, which supports mechanisms of latency and reactivation. As
44 pregnant females were excreting a higher HSV diversity compared to non-pregnant ones, we
45 suggest that pregnancy may reactivate HSV and lead to a diversification of the within-host
46 viral community.

47

48 **Keywords**

49 Chiroptera; tropical island; herpesvirus, co-infection, interaction

50

51

52 1. Introduction

53 Multiple infections with distinct virus populations within a single host are common in wildlife
54 (Hoarau et al., 2020) and can occur either simultaneously (co-infection) or sequentially (super-
55 infection) (Alizon, 2013). Simultaneous viral infections can lead to within-host interactions
56 that might be beneficial for viruses, by facilitating genetic exchanges, providing essential viral
57 proteins and enhancing host immune suppression (Shartouny et al., 2022). Conversely,
58 interactions can be antagonistic, by interfering with replication or transmission, via resource
59 competition or interferon-mediated immunity (Delima et al., 2023; Essaidi-Laziosi et al., 2020).
60 In theory, the intensity of within-host interactions is expected to be stronger between closely
61 related parasites due, for example, to overlap in resource use and similarity in the elicited
62 immune recognition profiles (Alizon, 2013; Choisy & de Roode, 2010). Bats constitute a good
63 model to study co-infections as they are hosts of diverse zoonotic viruses and large
64 proportions of co-infected individuals have already been reported in natural populations (*e.g.*
65 42%; Jones et al., 2023; Wang et al., 2023). By altering key epidemiologic factors such as host
66 susceptibility and infection duration (Vaumourin et al., 2015), co-infections can have
67 important consequences for zoonotic spillovers and disease emergence (Hoarau et al., 2020).
68 Understanding how bat viruses may form interactive communities has thus both significant
69 evolutionary and epidemiological implications (Jones et al., 2023).

70 Herpesviruses (HSV) serve as a valuable model for studying within-host viral
71 interactions due to their high prevalence in diverse host species and their capacity to establish
72 latent infection shaped by latency phases and reactivation mechanisms (Cohen, 2020).
73 Herpesvirus family includes three subfamilies with different properties. In humans, alpha-
74 herpesviruses (alpha-HSV) are known for their rapid lytic replication and latency in non-
75 divided cells (neurons), while beta- and gamma-herpesviruses (beta-HSV and gamma-HSV)
76 establish latency in divided cells and have slower replication cycle, which can lead to persistent
77 or chronic infections, especially in individuals with compromised immune systems (Weidner-
78 Glunde et al., 2020). Moreover, the lytic cycle is the principal mechanism for alpha- and beta-
79 HSV, while for gamma-HSV, it is the latency that predominates (Weidner-Glunde et al., 2020).
80 The particularity of herpesvirus to establish chronic infection, combine with important viral
81 population sizes and cycles of reactivation, can lead to significant genetic diversification
82 (Lauring, 2020).

83 In bats, frequent co-infections have already been reported, with multiple HSV strains
84 and between different subfamilies (beta-HSV and gamma-HSV) (Griffiths et al., 2020; Harima
85 et al., 2023; James et al., 2020; Moreira Marrero et al., 2021). In contrary, infection with alpha-
86 HSV was only described in a few bat species and only reported as single infection (Inagaki et
87 al., 2020; Razafindratsimandresy et al., 2009; Sasaki et al., 2014). Moreover, recent work
88 suggests potential maternal protection in juveniles and higher prevalence in male adult bats
89 shaped by sex-specific behaviour or physiology (Aguillon et al., *in prep.*). However, these
90 studies were focused on prevalence data and only limited research on viral diversity has
91 investigated how host factors could shape multi-infection patterns and within-host dynamics
92 (Griffiths et al., 2022, 2023; Sjodin et al., 2019). In vampire bats (*Desmodus rotundus*)
93 infected with multiple beta-HSV strains, it is suggested that non-competitive strains and latent
94 infections coexist at the population level (Griffiths et al., 2022).

95 In this study, we investigate the genetic diversity and within-host dynamics of HSV in a
96 tropical insectivorous bat, *Mormopterus francoismoutoui*, endemic to Reunion Island. A
97 recent spatio-temporal study revealed that Reunion free-tailed bats are highly infected by HSV
98 (prevalence of 87%, n = 3,981 bats) with probable latency, explaining long-term viral excretion
99 in saliva (Aguillon et al., *in prep.*). We first tested the influence of individual factors (age, sex
100 and reproductive status) on viral diversity, and potential interactions between the co-infecting
101 HSV subfamilies. Within-host dynamics was assessed, through the recapture of bats, by
102 estimating the probability of changing excretion status (at the subfamily level), and measuring
103 intra-subfamily diversification of HSV strains over time.

104

105 **2. Materials and methods**

106 **2.1. Field sampling**

107 Longitudinal monitoring of *M. francoismoutoui* in Reunion Island has been ongoing since 2018
108 (Aguillon et al., 2023a). Saliva samples used in this study were collected in 2018, 2019 and
109 2020 in 7 roosts and included data from recaptured bats (totalizing between 4 and 5 capture
110 events over the 3 years investigated here).

111 Briefly, bat capture was performed during the dusk emergence as fully described in
112 (Aguillon et al., 2023a), by mainly using harp traps (Faunatech Ausbat) and Japanese

113 monofilament mist nets (Ecotone). For each individual, we determined the sex visually and
114 age was assessed by examining the epiphysis fusion in finger articulations that are not welded
115 for juveniles. Reproductively active status was recorded when females were pregnant and
116 lactating and in males when they had large testes (see Aguillon et al., 2023a for more details).
117 A sterile swab (Puritan Medical Products, USA) was carefully introduced in the corner of the
118 lips to sample saliva and then placed in 250 μ L of MEM (Minimum Essential Medium Eagle).
119 Samples were stored in a cool box in the field before to be transferred the same night at -80°C
120 at the laboratory. Finally, we tattooed bats on the right propatagium with an individual
121 alphanumeric code before releasing them in the capture site. Handling of bats was performed
122 using personal protective equipment and gloves were disinfected between each individual bat
123 and changed regularly, and all the equipment was disinfected between sites as well (see
124 protocol in Aguillon et al., 2023a).

125

126 2.2. Molecular analysis and bioinformatics

127 DNA extraction from saliva samples and amplification of a fragment of herpesvirus DNA with
128 a nested PCR were mainly performed as part of Aguillon et al. 's study (*in prep.*), and new
129 samples were processed with the same protocol. We took a subset of these data by selecting
130 PCR-positive bats from both sexes, adults and juveniles, and in active or not -active
131 reproductive stage, thus including three biological periods: putative mating (from April to
132 May, majority of males sampled), pregnancy (from October to December, majority of females
133 sampled) and juvenile weaning (from January to March, majority of juveniles sampled). To
134 prepare samples for Illumina sequencing, the second PCR of the nested protocol was repeated
135 with Illumina adapters. PCR amplicons were then processed following an Illumina MiSeq
136 250bp paired-end sequencing method at MacroGen Europe (The Netherlands, Amsterdam),
137 using the Herculase II Fusion DNA polymerase Nextera XT Index V2 library kit. Sequences have
138 been deposited in NCBI under the SRA accession number XXX and metadata are available in
139 Zenodo (XXX) under licence XXX.

140 Raw sequence reads were filtered, cleaned and trimmed, removing primers and low
141 quality reads (unexpected length, missing base) using the FROGS 4.0 pipeline (Bernard et al.,
142 2021; Escudié et al., 2018). Clustering of reads into operational taxonomic units (OTUs) was
143 performed using the SWARM algorithm (Mahé et al., 2014) with an aggregation distance of

144 5%, following by the removing of chimeras and the filtering of low proportion OTUs (frequency
145 below 0.005%). Resulting OTUs were considered as distinct HSV strains and were checked in
146 GenBank using the Basic Local Alignment Search Tool (BLAST, Altschul et al., 1990) to verify
147 their herpesvirus identity. We checked for appropriate sequencing depth per sample by
148 verifying that the percentage of estimated strain diversity covered in each sample ranged
149 between 95% and 100%, using the function *depth.cov* from the R package *hilldiv* (Alberdi et
150 al., 2019). To assess the completeness of our sampling, we also created accumulation curves
151 of strain diversity in each roost, with 95% confidence levels based on 1000 bootstraps, using
152 the R package *iNEXT* (Hsieh et al., 2016) (Fig. S1). Strain diversity was measured based on Hill
153 numbers and $q = 1$, which considers both richness and evenness of taxa.

154

155 2.3. Phylogenetics analysis

156 Taxonomic affiliations of HSV-strains into the three subfamilies (alpha-, beta-, gamma-HSV)
157 was performed using a Bayesian tree in BEAST v.2.6.4 (Bouckaert et al., 2019) based on a Yule
158 model and HKY site model with invariant and gamma distribution (Hasegawa et al., 1985). We
159 used a reference sequence dataset including previously identified bat alpha-, beta- and
160 gamma-HSV sequences retrieved from GenBank, ensuring to select a wide range of host bat
161 families. Sequence alignment was constructed using ClustalW (Thompson et al., 2003) and
162 MUSCLE (Edgar, 2004), and then visually checked in CLC sequence Viewer 7.6.1 (Qiagen
163 Aarhus A/S, Aarhus, Denmark). The most appropriate nucleotide substitution model of
164 sequences was determined using BIC criterions in JModelTest v2.1.10 (Darriba et al., 2012).
165 We used a strict molecular clock with a 100 million chain length and sampling every 10^3 steps,
166 and a burning of 10%. We ran three analyses and combined log outputs (removing 10% of
167 burning for each output) using LogCombiner v2.6.4 (Rambaut & Drummond, 2015). Traces of
168 Markov Chain Monte Carlo (MCMC) were checked for convergence of the posterior using
169 Tracer v1.7.1 (Rambaut et al., 2018). We combined tree outputs (removing 10% of burning for
170 each output) to obtain a consensus tree using LogCombiner v2.6.4 (Rambaut & Drummond,
171 2015) and then TreeAnnotator v2.6.4 (Rambaut & Drummond, 2019), and visualized the
172 consensus tree in FigTree v1.4.4. (Rambaut, 2018).

173

174 2.4. Statistical analyses

175 To assess the effect of age (juveniles vs. adults) on HSV diversity, we performed generalized
176 linear mixed models (GLMM) including age and sex (and their interaction) as fixed effects, and
177 the roost, the sampling period (mating, pregnancy, juvenile weaning), Julian day and the bat's
178 ID as random effects. HSV diversity was measured at the strain level, using both the number
179 of strains (modelled as a Poisson, M1 in Table S1) and Hill numbers ($q = 1$, modelled as a
180 Gaussian with log link, M2 in Table S1). In addition, to assess strain composition differences
181 between both age classes, we performed a permutational multivariate analysis of variance
182 (PERMANOVA) with 10^3 permutations, and visualized results with non-metric
183 multidimensional scaling (NMDS) plot using Bray-Curtis dissimilarity index in FROGS 4.1
184 (Bernard et al., 2021; Escudié et al., 2018).

185 In order to evaluate the effect of reproductive status (active vs. non active) of HSV
186 diversity (Hill numbers, $q = 1$), we used a gaussian GLMM (with log link) on a subset of data
187 focusing on adult bats during the mating and pregnancy periods. Reproductive status and sex
188 (and their interaction) were included in the model as fixed effects, and the roost, the sampling
189 period (mating, pregnancy), Julian day and bat's ID as random effects (M3 in Table S1). Finally,
190 to investigate potential interactions between HSV subfamilies, we used binomial GLMMs on
191 the same data subset, modelling the presence/absence of each subfamily using binomial
192 distribution (see M4, M5 and M6 in Table S1). Models included reproductive status and sex
193 (and their interaction), as well as the two other subfamilies as fixed effects, and the same
194 random effects as above (with exception for M6 where bat's ID has been removed because of
195 model failure to converge).

196 We investigated within-host dynamics of excretion on a subset of data including only
197 recaptured bats. Specifically, we estimated the probability of gaining or losing HSV subfamilies
198 through time, by fitting a multinomial logistic regression using the *multinom* function in the
199 *nnet* package using the R script from Streicker et al. (2019). Conversion status was used as a
200 nominal dependent variable with four levels (no changes, gain, loss, gain and loss) and we
201 defined the non-changing level as baseline outcome against which to compare other excretion
202 status changes. Finally, we analysed the evolution of HSV genetic diversity at the individual

203 level, by plotting the intra-subfamily strain diversity (as Hill number $q=1$) over time for each
 204 recaptured bat.

205

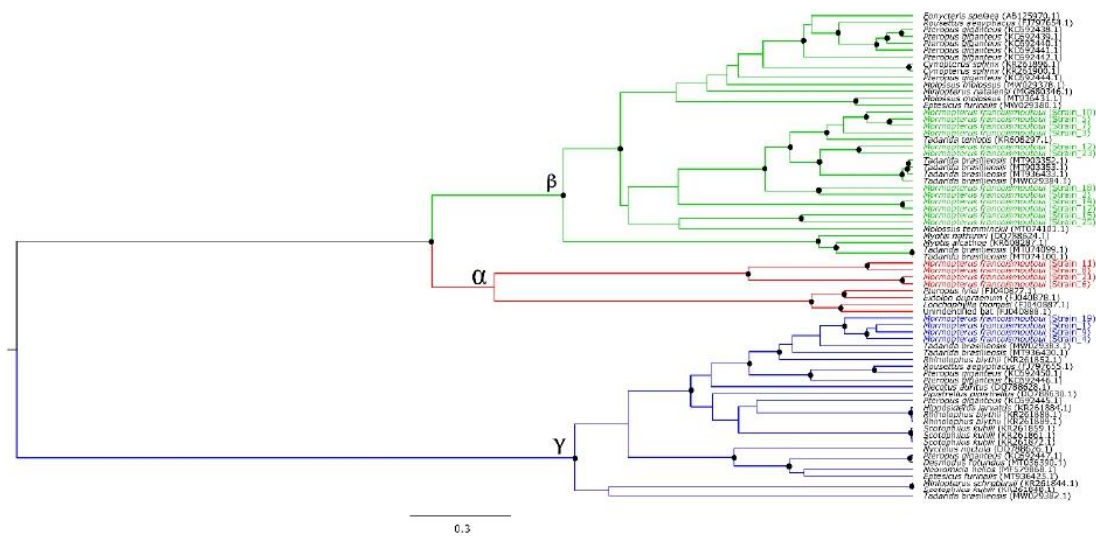
206 All models were constructed and analysed in Rstudio 1.4.1106 (RStudio, 2020) using packages
 207 *dplyr*, *effects*, *lme4*, *ggeffects*, *ggplot2* and *nnet*.

208

209 3. Results and Discussion

210 3.1. Diversity and abundance of HSV-strains and subfamilies

211 Based on the analysis of 121 saliva samples, we revealed a high diversity of herpesviruses in
 212 *M. francoismoutoui*, including 20 strains, which belong to the three subfamilies (Fig. 1). Most
 213 of bats were co-infected with two (40.50%) and three (43.80%) subfamilies, with up to 15
 214 strains, and 50% of individuals carried between 5 and 8 strains (Fig. S2), which confirms that
 215 HSV co-infections are common in bats (Jones et al., 2023). To our knowledge, we report here
 216 the first alpha-HSV in an insectivorous bat (Gonzalez & Banerjee, 2022), and the occurrence
 217 of triple HSV infection within a bat species.



218

219 **Figure 1. Reunion free-tailed bats are co-infected by three herpesvirus subfamilies.**

220 Sequences of *M. francoismoutoui* are colored according to the subfamilies they clustered.

221 Posterior probabilities > 0.75 are represented with a black point at nodes.

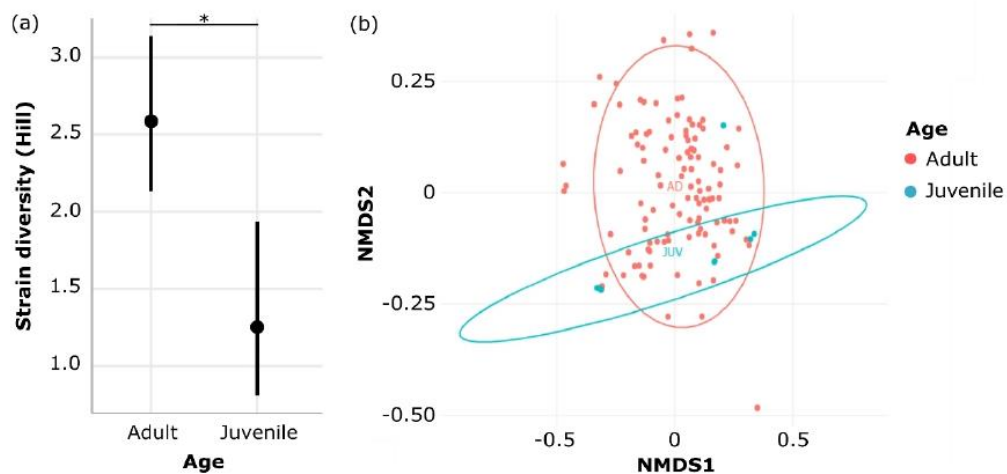
222 Beta-HSV was the most abundant (58.93% of total reads, Fig. S3) and diversified (12
 223 strains) subfamily in *M. francoismoutoui*. Our beta-HSV sequences grouped with viruses
 224 previously found in several molossid bat species, including *Tadarida brasiliensis*, *T. teniotis* and
 225 *Molossus temminckii*. Gamma-HSV (31.63% of total reads, Fig.S3) formed one monophyletic
 226 group, closely related to HSV from the mollosoid bat species *T. brasiliensis*. In contrast, alpha-
 227 HSV was less abundant (9.44% of total reads, Fig. S3) and grouped in a single cluster, not
 228 related to a particular species. The close genetic relationships with HSV from others members
 229 of the Mollosidae family illustrates the evolution of host specificity in bat HSV (Azab et al.,
 230 2018; Escalera-Zamudio et al., 2016). Our result also support a difference of diversity among
 231 subfamilies, with much lower diversity reported for alpha-HSV (Inagaki et al., 2020;
 232 Razafindratsimandresy et al., 2009; Sasaki et al., 2014), than for beta- and gamma-HSV
 233 (Griffiths et al., 2020; Harima et al., 2023; James et al., 2020; Moreira Marrero et al., 2021).
 234 This result could be explained by the distinct speed in lytic cycles and mechanisms of
 235 reactivation among subfamilies which may reduce the probability of detecting alpha-HSV in
 236 saliva (Weidner-Glunde et al., 2020). This is coherent with a modelling study suggesting that
 237 active phases are much longer than latency phases for beta-HSV dynamics (Griffiths et al.,
 238 2023), that could explain the higher abundance and diversity of beta-HSV observed in *M.*
 239 *francoismoutoui*.

240

241 3.2. Host-associated determinants of HSV strain and subfamily diversity

242 Strain diversity was strongly dependent of age, when both measuring the number of strains
 243 (M1: $\chi_1 = 15.25$, $p < 0.001$, Fig. S4a) and Hill numbers (M2: $\chi_1 = 6.48$, $p < 0.05$, Fig. 2a). The
 244 PERMANOVA test also detected a significant difference in strain composition between adults
 245 and juveniles ($F = 0.03$, $p < 0.01$) with reduced diversity observed in juveniles (Fig. 2b). Indeed,
 246 the majority of juveniles (8 over 9) excreted only one HSV subfamily (beta-HSV or gamma-HSV,
 247 Fig. S4b) and a reduced mean number of strains (mean = 2.56). Only one juvenile was infected
 248 with the three subfamilies (Fig. S4b). In comparison, almost half of adults were co-infected
 249 with the three subfamilies (52 over 112) and a higher number of strains (mean = 6.50).
 250 Interestingly, Griffiths et al. (2022) did not find an effect of bat's age on the number of HSV
 251 strains in *Desmodus rotundus*. This contrasting result could be due to species-specific

252 difference, or because our juvenile class was restricted to young up to 4 months-old, while
 253 Griffiths et al. (2022) also included subadults. These subadult bats had probably been exposed
 254 to a higher HSV diversity, supported by our previous study showing that juveniles get infected
 255 rapidly (Aguillon et al., *in prep.*). Thus, in addition to reduced HSV prevalence previously
 256 reported in juvenile bats (*e.g.* Aguillon et al., *in prep.*; Dietrich et al. 2018; Griffiths et al., 2020),
 257 we therefore demonstrate a lower genetic diversity of HSV in (young) juveniles compared to
 258 adult bats, which supports the protection of juveniles by maternal antibodies followed by an
 259 accumulation of multiple HSV latent-infections over time.

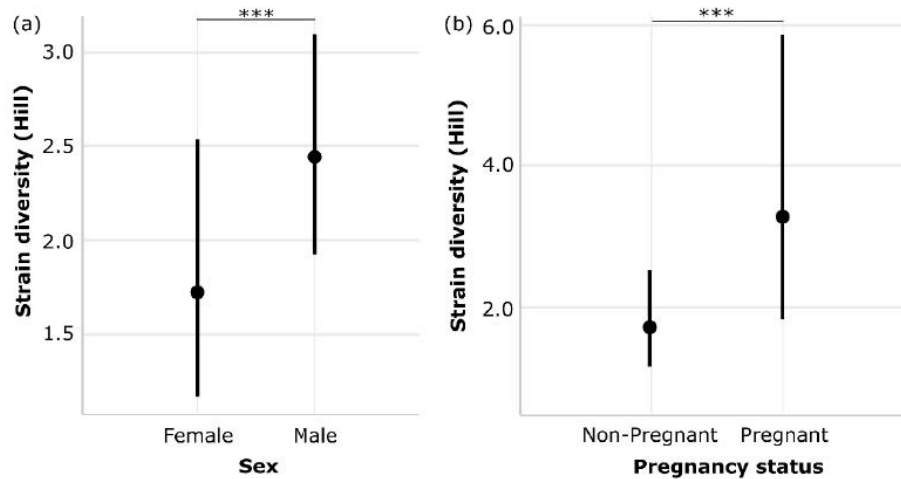


260 **Figure 2. Reduced herpesvirus diversity in juveniles of Reunion free-tailed bats.** (a) Hill
 261 diversity ($q = 1$) predicted by model M2, * $p < 0.05$. (b) NMDS plot.

262

263 We found an absence of geographical structure (among roosts) in HSV diversity
 264 supporting a widespread viral transmission in this island endemic bat metapopulation
 265 (Aguillon et al., 2023b). In adult bats, sex and reproductive status were major determinants of
 266 HSV diversity. Indeed, males harboured a higher strain diversity than females (M3: $\chi^2 = 24.99$,
 267 $p < 0.001$; Fig. 3a). This is coherent with a previous study showing male-biased prevalence in
 268 *M. francoismoutoui* (Aguillon et al., *in prep.*), and thus a probable higher transmission and/or
 269 susceptibility in males favouring HSV diversity. In contrast, the number of HSV strains was not
 270 affected by sex in a recent study on *Desmodus rotundus* (Griffiths et al. 2022), supporting that
 271 sex effect may also differ among bat species as shown by Sjodin et al., (2020). Host sex
 272 interacted with reproductive status as we detected a positive effect of reproduction only in

273 females, with pregnant adult females excreting a significantly higher HSV diversity compared
 274 to the non-pregnant ones (M3: $\chi_2 = 24.08$, $p < 0.001$; Fig. 3b). A similar pattern has been found
 275 in Puerto Rican bats (Sjodin et al., 2020), which suggests that reactivation of herpesvirus
 276 excretion during pregnancy leads to a diversification of the within-host viral community.



277 **Figure 3. Effect of sex and reproductive status on herpesvirus diversity in adults of Reunion**
 278 **free-tailed bats.** Hill diversity ($q = 1$) predicted by model M3, (a) by sex, and (b) by pregnancy
 279 status. *** $p < 0.001$.

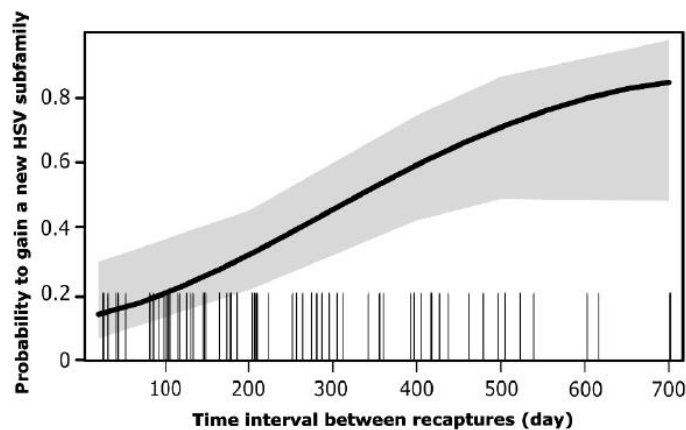
280

281 Patterns of co-infection between beta-HSV and the two others subfamilies were random,
 282 supporting low levels of cross-protective immunity for some pairwise HSV combinations
 283 (Griffiths et al., 2022). However, adult bats excreting alpha-HSV had less probability to excrete
 284 gamma-HSV at the same time (M4: $\chi_1 = 4.33$, $p < 0.05$, Fig. S5a), with the opposite correlation
 285 also being significant (M6: $\chi_1 = 4.95$, $p < 0.05$, Fig. S5b). Such negative interaction has already
 286 been reported in bats, but only for HSV strains within the beta-subfamily (Anthony et al.,
 287 2013). Further investigations on the infection mechanisms of HSV in bats are needed to
 288 explore factors leading to these subfamily-specific interaction patterns.

289

290 3.3. Within-host dynamics of HSV

291 Among 11 adult males recaptured after 25 to 702 days (Fig. S6), only two were initially HSV-
 292 negative (including one being juvenile at the first capture) and then became (and remained)
 293 infected. In majority of individuals (52.86%), bats excreted the same HSV subfamily over
 294 recapture events, although the gain of one or two subfamilies was also largely observed
 295 (38.57%). Therefore, we reported few loss or gain/loss events (8.57%). The changes in HSV
 296 excretion status had a different probability to occur over time ($\chi^2_3 = 16.06, P < 0.001$). Indeed,
 297 the probability that bats gain a subfamily increased with time (Fig. 4), while the probability to
 298 loss or gain/loss was low and stable over time (Fig. S7). These results support that HSV
 299 establish latent infection in bats, as suggested by previous results on prevalence data with
 300 bats remaining positive through time (Aguillon et al., *in prep.*; Griffiths et al., 2023).



301 **Figure 4. The probability of acquiring a new HSV subfamily increases over time in Reunion**
 302 **free-tailed bats.** Dashes across the x-axis indicate recapture of bats and the shading area
 303 represent the 95% CI on the mean probability (solid black line).

304

305 Investigation of within-host evolution of genetic diversity within each HSV subfamily
 306 revealed highly dynamic strain acquisition/release processes (Fig S8.). Indeed, 78.57% of bats
 307 both gained and loose strains between captures, and 14.29% of bats only gained strains over
 308 time. These frequent shifts is consistent with long-term HSV persistence and may be the result
 309 of latency phases and lytic reactivation (Griffiths et al. 2022), but do not exclude the
 310 occurrence of clearance processes as well.

311

312 **4. Conclusion**

313 Our work illustrates how herpesviruses provide an excellent study system to analyze within-
314 host viral community dynamics in bats. Widespread triple infections of HSV (alpha, beta and
315 gamma) in *M. francoismoutoui* shed light on individual factors (age, sex, reproduction)
316 influencing within-host viral diversity, and interactions between viruses. While the exact
317 mechanisms of persistence and reactivation are complex and not fully understood, HSV
318 infection can potentially drive other infections in bats (Aguillon et al., *in prep.*). Further studies
319 are needed to evaluate the role of HSVs in shedding patterns of pathogens of zoonotic
320 importance.

321

322 **Ethics**

323 Bat capture and manipulation techniques were evaluated by the ethic committee of Reunion
324 Island, approved by the Ministère de l'Enseignement Supérieur, de la Recherche et de
325 l'Innovation (APAFIS#10140-2017030119531267), and conducted under a permit
326 (DEAL/SEB/UBIO/2018-09) delivered by the Direction de l'Environnement, de l'Aménagement
327 et du Logement (DEAL) of Reunion Island.

328

329 **Authors' contributions**

330 **Samantha Aguillon**: Conceptualization, Formal analysis, Investigation, Visualization, Writing -
331 original draft. **Magali Turpin**: Formal analysis, Methodology, Writing - review & editing. **Gildas**
332 **Le Minter**: Investigation, Writing - review & editing. **Camille Lebarbenchon**: Investigation,
333 Writing - review & editing. **Axel Hoarau**: Investigation, Writing - review & editing. **Patrick**
334 **Mavingui**: Supervision, Writing - review & editing. **Muriel Dietrich**: Conceptualization, Formal
335 analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, Writing -
336 original draft.

337

338

339 **Conflict of interest declaration**

340 We declare we have no competing interests.

341

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353

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Discussion générale et perspectives

A l'heure où la pression anthropique s'intensifie sur les écosystèmes, la compréhension des processus écologiques qui influencent la circulation des agents infectieux chez les chauves-souris est primordiale si l'on veut, par la suite, mieux caractériser les processus en cascade menant à l'émergence de zoonoses (Jones *et al.*, 2013). L'objectif principale de mes travaux de recherche était d'étudier comment l'écologie des chauves-souris et la dynamique de leurs populations influencent la transmission de leurs agents infectieux, en utilisant comme modèle le Petit Molosse de la Réunion et trois de ses agents infectieux (paramyxovirus, bactéries *Leptospira* et herpesvirus).

I. Importance d'étudier l'écologie des chauves-souris

Nous nous sommes tout d'abord intéressés à l'écologie du Petit Molosse, qui malgré son abondance sur l'île de la Réunion, restait peu étudiée. Nos travaux de recherche ont permis de confirmer le caractère très hétérogène et dynamique des populations de chauves-souris d'un point de vue spatial et temporel. Par exemple, nous avons pu montrer que le Petit Molosse possède une importante diversité de gîtes (habitat, taille, sex-ratio, temporalité), bien que les paramètres structuraux (hauteur, volume) et environnementaux (température, hygrométrie) qui interviennent dans la sélection de ces gîtes n'aient pas été mesurés dans le cadre de cette thèse. Nos résultats suggèrent tout de même que les gîtes connus comme des maternités se trouvent dans des habitats accumulant la chaleur, qu'ils soient naturels (grotte) ou artificiels (toiture sous tôle, transformateur électrique), comme démontré chez d'autres espèces de chauves-souris (López-Baucells *et al.*, 2017 ; Lourenço & Palmeirim, 2004 ; Sedgely, 2001). Ainsi, évaluer les préférences de micro-habitats en fonction des besoins des différents types d'individus (reproduction, gîte hivernal), permettrait de mieux comprendre la distribution spatio-temporelle de cette espèce et ce qu'elle affectionne en milieu urbain en particulier, afin de pouvoir, par la suite, proposer des mesures de conservation adaptées à son écologie (*e.g.* installation de gîtes artificiels) (Hoeh *et al.*, 2018 ; Tillman *et al.*, 2021).

Ces travaux de recherche illustre également l'importance des suivis longitudinaux pour mieux décrypter le cycle biologique des espèces de la faune sauvage. En effet, la mise en place de suivi relativement fréquent dans le temps (environ toutes les 6 semaines), nous a permis de mettre en évidence des comportements dynamiques des différents groupes d'individus. Par exemple, nous avons pu montrer que les femelles gestantes sont observées de manière

synchrone dans les gîtes étudiés, mais qu'à la toute fin de gestation, elles rejoignent certains gîtes spécifiques pour mettre-bas. De plus, après la mise-bas, les femelles allaitantes occupent en journée des gîtes différents de ceux où sont leurs petits. Ce comportement pourrait résulter de stratégies permettant de limiter le parasitisme important observé dans les gîtes de mise-bas, probablement favorisé par la densité d'individus, la température et la susceptibilité des nouveau-nés (Bartonička & Gaisler, 2007 ; Lourenço & Palmeirim, 2007 ; Lucan, 2006 ; Reckardt & Kerth, 2009). Le taux de parasitisme n'a pas été mesuré au cours de cette thèse bien que des observations réalisées sur le terrain montre un parasitisme important des nouveau-nés (parfois complètement recouverts de minuscules acariens), notamment dans le plus grand gîte de mise-bas de l'île qui hébergent plusieurs dizaines de milliers d'individus. Les femelles allaitantes pourraient également préférer des gîtes plus frais en journée leur permettant de rentrer en torpeur journalière afin d'économiser de l'énergie, comme décrit chez la Petite chauve-souris brune (*Myotis lucifugus*) en milieu tempéré (Besler & Broders, 2019).

Avec l'observation et la capture des premiers juvéniles volants, nous avons pu estimer que le sevrage des juvéniles était rapide (après environ 6 semaines) chez le Petit Molosse, et qu'il coïncide avec une dispersion des juvéniles sur toute l'île. Ceci est cohérent avec la biologie d'autres espèces de Molossidés, comme chez le Molosse du Brésil (*Tadarida brasiliensis*) en Argentine (Boero *et al.*, 2020). De manière surprenante, la période de dispersion des juvéniles (février-avril) correspond également à la période d'accouplement supposée, pendant laquelle nous avons observé pour la première fois chez cette espèce, des mâles actifs pour la reproduction dans la quasi-totalité des gîtes étudiés, soit 7 mois avant le début de la gestation. Ces résultats suggèrent ainsi une interruption du cycle de reproduction, qui est un processus principalement décrit chez les chauves-souris de milieux tempérés (Pfeiffer & Mayer, 2013), mais par exemple aussi observé chez une autre espèce de Molossidés, vivant dans le sud de l'Australie (*Ozimops planiceps*) (Racey & Entwistle, 2000). De manière générale, plusieurs processus peuvent entraîner l'interruption du cycle de reproduction chez les chauves-souris : un délai dans le stockage du sperme, dans l'implantation de l'embryon ou même dans le développement du fœtus (Crichton, 2000 ; Pfeiffer & Mayer, 2013). Ces stratégies ont été associées à l'utilisation de torpeur journalière chez certaines espèces tropicales, comme chez *Afronycteris nanus* (Racey & Entwistle, 2000). Enfin, nos données montrent une baisse importante des effectifs en hiver dans la quasi-totalité des gîtes suivis (surtout des femelles

adultes), c'est pourquoi il a été difficile de suivre le comportement des individus à cette période. On ignore encore si le Petit Molosse passe l'hiver en petits groupes (Augros *et al.*, 2015) ou bien s'il s'agrège dans des gîtes hivernaux encore inconnus, se trouvant dans des endroits isolés (fissures et grottes en altitude) où on les remarque moins.

L'étude génétique avait pour objectif d'apporter des éléments nouveaux sur les mouvements intra et inter-saisons. Les résultats nous ont permis de mettre en évidence un important brassage des populations de Petits Molosses, qui semblent fonctionner à l'heure actuelle, comme une grande métapopulation. En effet, l'analyse des marqueurs microsatellites ne montre aucune structure génétique spatiale (entre gîtes), tandis que le marqueur mitochondrial suggère l'existence de plusieurs lignées, probablement anciennes, coexistant aujourd'hui en sympatrie dans les gîtes. Nous n'avons pas mis en évidence de comportements de dispersion différents entre sexes, même s'il pourrait exister un signal faible de philopatrie chez les femelles (Φ_{st} plus élevés en été lorsque les femelles gestantes se regroupent dans les gîtes). Les résultats discordants obtenus entre les marqueurs microsatellites et mitochondrial, laissent à penser qu'il pourrait exister des sites d'accouplements bien spécifiques sur l'île (correspondant aux groupes observés avec les microsatellites). En effet, les sites d'accouplement du Petit Molosse ne sont pas connus à ce jour, et on ignore de même s'ils sont différents des gîtes suivis dans ce projet. Ces groupes pourraient également résulter de l'évolution d'adaptations, potentiellement acoustiques et/ou morphologiques, comme cela a été décrit chez d'autres espèces de chauves-souris en Asie (Sun *et al.*, 2016). L'analyse d'autres marqueurs, comme par exemple les SNPs (*Single Nucleotide Polymorphism*), permettrait d'apporter des informations complémentaires et potentiellement plus précises (Norman & Spong, 2015). Par exemple, Richardson et collaborateurs (2021) ont utilisés des SNP chez des souris et chauves-souris pour analyser comment les capacités de dispersion des deux espèces impactent la connectivité génétique des individus dans un environnement urbain. Cependant, ce type d'outils nécessite en amont l'obtention d'un génome de référence de qualité pour le Petit Molosse, même si des méthodes alternatives ont aussi été proposées (Lopez-Maestre *et al.*, 2016).

Ainsi, du fait de l'absence de structure spatiale, les analyses génétiques n'ont pas pu fournir d'informations précises sur les déplacements des chauves-souris entre les gîtes. Des analyses de télémétrie pourraient ainsi être utilisées en complément de l'étude génétique

pour mieux comprendre et identifier les réseaux de gîtes et comment ils sont utilisés par les différents types d'individus (Rhodes *et al.*, 2006 ; Silvis *et al.*, 2014), mais aussi de résoudre le mystère des migrations hivernales. La télémétrie consiste à placer un émetteur sur le dos de la chauve-souris, et ensuite de trianguler sa position à l'aide de plusieurs antennes, et donc ses déplacements au cours de la nuit. En juin et juillet 2022, j'ai participé au premier essai de télémétrie chez le Petit Molosse, avec 12 individus équipés (Figure 14). Cette étude préliminaire a permis de valider cette méthode sur une espèce de petite taille et de montrer que sur plusieurs jours, le Petit Molosse utilise un réseau de gîtes, et qu'il peut même se déplacer entre ces différents gîtes au sein d'une même nuit (Ballet, 2022). En complément de cette méthode de suivi, le transpondage avec la pose de puces sous-cutanées (*pit-tag*) et l'utilisation de lecteurs automatiques à l'entrée des gîtes, pourrait permettre de suivre, à plus grande échelle et à plus long terme, les déplacements des chauves-souris et par exemple d'estimer des paramètres comme la fidélité des individus à un réseau de gîtes (Schorr & Siemers, 2021 ; Waag *et al.*, 2022). Cette technique est d'ailleurs utilisée en France hexagonale sur les Grands Rhinolophes (*Rhinolophus ferrumequinum*) pour estimer des taux de survie et étudier les déplacements entre gîtes afin de mieux protéger l'espèce (Vienne nature, 2023).

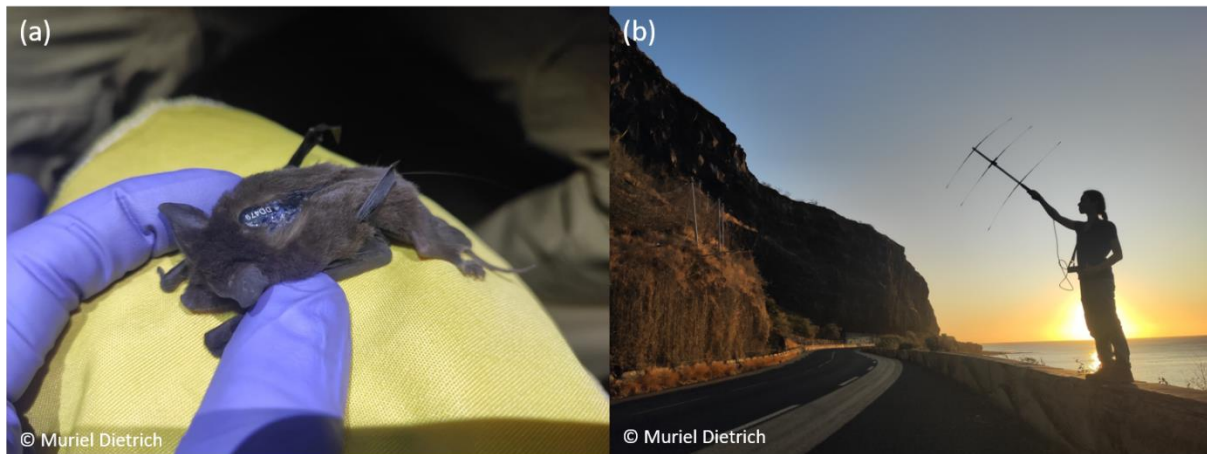


Figure 14. (a) Petit Molosse équipé d'un émetteur et (b) radiopistage à la tombée de la nuit des individus équipés à l'aide d'antennes réceptrices.

II. Déterminants écologiques des dynamiques d'excrétion

Les flux de gène et mouvements des individus peuvent avoir des conséquences sur la dispersion de l'infection (Archie *et al.*, 2009 ; Biek & Real, 2010). Par exemple, l'étude de Wilder et collaborateurs (2015) allie une approche de génétique des populations de l'hôte avec la construction d'un modèle statistique, afin de mieux prédire la propagation du champignon *Pseudogymnoascus destructans* (responsable du syndrome du museau blanc) dans les gîtes de la Petite chauve-souris brune (*M. lucifugus*). De même, Colombi et collaborateurs (2019) ont pu déterminer que la migration et les interactions entre différentes espèces de chauves-souris européennes contribuent de manière importante à la persistance du virus EBLV-1 chez le Minioptère de Schreibers (*Myotis schreibersii*). Dans certains cas, l'étude de la diversité des parasites peut également apporter de précieuses informations sur les déplacements de l'hôte (Nieberding & Olivieri, 2007). Dans cette thèse, l'analyse des séquences d'herpesvirus n'a révélé aucune structure génétique liée à la géographie et corrobore ainsi l'hypothèse de nombreux mouvements des individus qui favoriseraient la transmission des différents variants d'herpesvirus à l'échelle de l'île (Mazé-Guilmo *et al.*, 2016). De plus, des résultats préliminaires de séquençage des paramyxovirus (non détaillés dans cette thèse) montre l'existence de plusieurs groupes génétiques en sympatrie dans les gîtes. La comparaison des phylogénies de l'hôte (différentes lignées mitochondriales identifiées dans cette thèse) et des paramyxovirus ne révèle pas de concordance (Aguillon, 2020 ; Duchet, 2019), ce qui suggère également une importante transmission spatiale des paramyxovirus. Dans notre étude, nous n'avons pas exploré la diversité génétique des bactéries leptospires car une étude précédente, basée sur l'analyse de 5 gènes, n'avait détecté qu'un seul génotype, proche génétiquement de *Leptospira borgpetersenii* (Dietrich *et al.*, 2018). Une approche génomique pourrait par exemple être entreprise pour déceler des différences génétiques au sein du territoire.

L'étude de trois agents infectieux en parallèle nous a permis de mettre en évidence des processus écologiques communs, mais aussi agent-spécifique, qui influencent les dynamiques d'excrétion. Par exemple, les juvéniles étaient systématiquement moins infectés au début de leur vie que les adultes, suggérant la transmission d'anticorps maternels. Ce mécanisme est bien connu chez les mammifères et a été démontré chez les chauves-souris (Amman *et al.*, 2012 ; Baker *et al.*, 2014 ; Breed *et al.*, 2011 ; Hayman *et al.*, 2018 ; Peel *et al.*, 2012 ; Plowright *et al.*, 2008). Cependant le patron d'infection progressive des juvéniles était plus rapide et plus

important pour les herpesvirus, suggérant une quantité d'anticorps maternels initialement transmis moindre et une perte plus rapide, comparé aux paramyxovirus et bactéries leptospires. L'acquisition de données sérologiques permettrait de conforter cette hypothèse bien que dans le cas du Petit Molosse, des prélèvements sanguins n'ont pas été réalisés au cours du projet, mais seront collectés prochainement. Ces éléments sont importants car nous avons vu que les juvéniles dispersent de manière importante au sein de l'île. Les juvéniles pourraient donc jouer un rôle crucial dans la dispersion de l'infection, qui interviendrait de manière plus précoce pour les herpesvirus.

L'analyse des patrons d'excrétion à l'échelle des populations, mais aussi individuelle, suggèrent que les trois agents infectieux étudiés peuvent persister dans l'organisme du Petit Molosse. En effet, nous avons mis en évidence des prévalences d'excrétion importantes toute l'année (37-87%), ainsi qu'une excrétion récurrente chez des individus recapturés. De plus, nos résultats génétiques sur les herpesvirus sont cohérents avec un mécanisme de latence, puisque la probabilité d'excréter une nouvelle sous-famille (alpha-, beta-, gamma-HSV) est positivement corrélée au temps, tandis que très peu de pertes sont observées, suggérant ainsi que l'acquisition d'une nouvelle sous-famille persiste dans le temps. Les herpesvirus sont connus pour être latents dans l'organisme (Cohen, 2020) et ce mécanisme a été récemment suggéré pour les chauves-souris également (Gerow *et al.*, 2018 ; Griffiths *et al.*, 2023). En ce qui concerne les bactéries leptospires, les chauves-souris sont des hôtes reconnus de ces bactéries (Dietrich *et al.*, 2015a) et l'infection est souvent considérée comme chronique chez d'autres hôtes réservoirs comme les rongeurs (Cordonin *et al.*, 2020). Au contraire, la possibilité d'une persistance des paramyxovirus chez les chauves-souris est encore débattue (Glennon *et al.*, 2019 ; Plowright *et al.*, 2016), et nos données n'excluent d'ailleurs pas que les Petits Molosses puissent éliminer l'infection et se réinfecter par la suite, notamment pour les paramyxovirus et bactéries leptospires. L'utilisation de modèles épidémiologiques devraient permettre de tester ces différentes hypothèses comme cela a été réalisé chez la Roussette paillée africaine (*Eidolon helvum*) pour l'infection aux *Henipavirus* (Peel *et al.*, 2018). Des infections expérimentales utilisant des lignées cellulaires pourraient représenter une approche complémentaire permettant de mesurer la persistance de l'infection, comme cela a été réalisé sur des cellules de Sérotine brune (*Eptesicus fuscus*) qui ont été infectées au MERS-CoV (Banerjee *et al.*, 2020). La culture cellulaire est aussi un bon moyen d'identifier le potentiel

zoonotique des virus comme cela a été fait pour un gammaherpesvirus de chauves-souris (BGHV8) dont l'infection a montré qu'il serait capable de se répliquer dans des cellules humaines (Shabman *et al.*, 2016).

Nos données suggèrent que la reproduction (gestation et accouplement) jouerait un rôle dans la réactivation des infections persistantes chez les chauves-souris, notamment pour les paramyxovirus et bactéries leptospires. De tels résultats ont déjà été décrit chez les chauves-souris, bien que les études se soient essentiellement intéressées à la gestation des femelles, et qu'elles se basent surtout sur des données de séroprévalence (Plowright *et al.*, 2008 ; Pourrut *et al.*, 2009 ; Rahman *et al.*, 2013). En revanche, la reproduction ne semble pas influencer l'excrétion des herpesvirus, pourtant largement suspectés d'être latent chez les chauves-souris (Gerow *et al.*, 2018 ; Griffiths *et al.*, 2023) et donc susceptibles d'être réactivés lors de stress comme la reproduction. Ceci pourrait s'expliquer par la prévalence d'herpesvirus très élevée dans la population (87%), qui ne permettrait pas de mesurer précisément l'effet de la reproduction. En effet, ces travaux de thèse se sont basés sur des données de prévalence grâce à la détection par PCR des agents infectieux se trouvant dans les fluides (urine, salive) des chauves-souris. Cependant, nos données ne nous renseignent pas sur l'intensité d'excrétion, qui peut être évaluée à partir de la charge virale/bactérienne (détectable en PCR quantitative, qPCR), et qui est souvent corrélée de manière positive au développement de symptômes et à l'infectiosité (Lau *et al.*, 2010 ; Vega *et al.*, 2015). Par exemple, des études suggèrent que plus la charge virale au SARS-CoV-2 est importante, plus le virus est infectieux et va se propager rapidement chez l'homme (Marc *et al.*, 2021 ; Riediker *et al.*, 2022). En utilisant des données préliminaires de charge bactérienne obtenues à partir des valeurs de Ct (*cycle threshold*) de la qPCR leptospires, j'ai pu mesurer que les juvéniles avaient une charge bactérienne moins importante par rapport aux adultes ($t = 3.62$, $p < 0.001$, résultats non publiés) démontrant un rôle important des adultes dans la transmission de l'infection. De plus, lorsque les chauves-souris excrétaient un seul virus (paramyxovirus ou herpesvirus), la charge bactérienne en leptospires était plus importante (PMV : $t = -1.75$, $p = 0.08$; HSV : $t = -3.85$, $p < 0.001$, résultats non publiés), ce qui est cohérent avec les données de prévalence qui suggèrent des interactions positives lorsque que deux agents infectieux sont présents. Une analyse complémentaire incluant la quantification des charges virales des paramyxovirus et herpesvirus devraient apporter des éléments supplémentaires, mais n'a pas pu être réalisée

dans le cadre de cette thèse, car cela requiert la mise au point de qPCR spécifiques à ces deux virus, qui sont en cours de développement. Ce type d'information sur la charge virale et bactérienne est cruciale, comme souligné récemment par l'étude de Lunn et collaborateurs (2023) qui suggère qu'une charge virale plus importante au virus Hendra chez les chauves-souris frugivores augmenterait le risque de transmission à d'autres espèces (chevaux, hommes), remettant en question l'étude de la prévalence seule.

Enfin, cette thèse souligne l'importance d'étudier les interactions entre agents infectieux pour comprendre les dynamiques d'infection. En effet, Telfer et collaborateurs (2010) avait déjà montré, il y a plusieurs années, que de multiples interactions existaient au sein d'une communauté de quatre micro-parasites et que ces interactions pouvaient moduler la susceptibilité de l'hôte de manière bénéfique ou non. De manière intéressante, notre étude suggère des interactions différentes selon le nombre et l'agent infectieux considéré. En effet, dans le cas de deux agents infectieux, l'excrétion du premier pourrait faciliter l'excrétion du second. En revanche, lorsqu'un petit Molosse est déjà doublement infecté, il aurait moins de chance d'excréter un troisième agent infectieux. La prise en compte de telles interactions permettrait par exemple, d'affiner les modèles épidémiologiques. Cette approche multi-agents infectieux pourrait également être élargie avec l'analyse du virome (ensemble des virus) et bactériome (ensemble des bactéries) des chauves-souris, et permettraient d'étudier les interactions au sein d'une communauté virale ou bactérienne dans sa globalité (Wang *et al.*, 2023). Peu d'études ont exploré ce type d'interactions chez les chauves-souris (Dietrich *et al.*, 2018), mais des résultats récents suggèrent que la composition du microbiome bactérien influencerait, par exemple, la présence du parasite *Plasmodium* spp. chez des chauves-souris africaines (Lutz *et al.*, 2022).

III. Changements globaux et zoonoses à l'ère de l'Anthropocène

Les changements globaux (activités anthropiques et changements climatiques) menacent l'équilibre des écosystèmes et peuvent altérer le comportement, la physiologie, la phénologie et la mortalité des espèces (Mawdsley *et al.*, 2009 ; Quratulann *et al.*, 2021). De plus, des études rapportent que les changements globaux entraînent, par des effets en cascade, l'augmentation des risques d'émergence de maladies zoonotiques (Bradley & Altizer, 2007 ; Jones *et al.*, 2013 ; Semenza *et al.*, 2022 ; Suk *et al.*, 2020). En utilisant des données spatiales

de haute résolution, Warmuth et collaborateurs (2023) ont récemment montré que la modification des habitats (essentiellement liée à l'urbanisation et l'agriculture) augmentait la prévalence en coronavirus chez les chauves-souris. De même, le risque de transmission du virus Hendra des chauves-souris *Pteropus* aux chevaux est plus important après des événements d'El Niño (Eby *et al.*, 2023), et serait lié à une modification du comportement de recherche de nourriture. La Réunion est une île qui subit de plus en plus de pressions anthropiques avec une urbanisation croissante en raison d'une continuelle augmentation de la population estimée à 30% d'ici 2030 (David *et al.*, 2011). Ainsi, des études visant à évaluer l'impact à long terme de l'urbanisation sur l'écologie du Petit Molosse de La Réunion, permettraient de mieux comprendre comment les populations de chauves-souris répondent aux changements anthropiques et les conséquences sur l'infection.

L'urbanisation et l'augmentation croissante de la population entraîne le rapprochement des villes sur les milieux naturels, et contraignent les espèces de la faune sauvage à répondre à ces changements. Certaines espèces sont particulièrement sensibles à ces modifications d'habitat et ne réussissent pas à s'y adapter (dites *urban avoiders*), d'autres espèces sont capables d'exploiter les ressources urbaines (*urban adapters*) tandis que certaines autres sont capables de s'adapter au milieu urbain en exploitant ces ressources et en y devenant dépendantes (*urban exploiters*) (Blair, 1996 ; Kark *et al.*, 2007). Dans le cas des Molossidés, l'étude de Jung & Kalko (2011) suggère que la plupart des espèces seraient des *urban exploiters*, ce qui semble être le cas pour le Petit Molosse dont de nombreux gîtes se trouvent en milieu urbain. La disponibilité de ces nouveaux gîtes urbains ne semble pourtant pas avoir favorisé la taille de sa population. Au contraire, notre étude génétique montre que, même si la population semble avoir connu une expansion depuis 55 000 ans, celle-ci stagne, voire se réduit depuis environ 500 ans, ce qui pourrait coïncider avec l'arrivée de l'homme sur l'île. En complément des résultats génétiques, un suivi acoustique sur plusieurs années permettrait, par exemple, d'évaluer avec plus de précision l'évolution actuelle de la taille de population de Petits Molosses (Marques *et al.*, 2013).

Au-delà de la taille de la population, l'urbanisation peut aussi influencer les comportements de dispersion, l'utilisation de l'habitat et la connectivité entre les gîtes (Russo & Ancillotto, 2015). Par exemple, la lumière artificielle des villes est suspectée d'entraîner une discontinuité au sein du territoire de chasse des animaux nocturnes qui l'évitent (Hale *et al.*,

2015 ; Marín-Gómez *et al.*, 2020). Ainsi, une étude réalisée en France montre que certaines espèces de chauves-souris sont attirées par les éclairages de nuit en ville, en raison d'une densité en insectes plus importante, tandis que d'autres vont plutôt les éviter probablement à cause d'un risque de prédation accrue ou parce que la lumière perturbe leur orientation (Lacoeuilhe *et al.*, 2014). De plus, une étude de modélisation suggère que la densité et la distance entre les gîtes des roussettes en Australie (*Pteropus* spp.) peut modifier la dynamique d'excrétion (Lunn *et al.*, 2021). En effet, une connectivité réduite entre gîtes engendrerait des épidémies du virus Hendra moins fréquentes chez les chauves-souris, mais de magnitude plus importante (Lunn *et al.*, 2021). Un suivi télémétrique et par transpondage, visant à quantifier les déplacements et la connectivité entre gîtes dans des milieux plus ou moins anthropisés, permettrait d'évaluer la plasticité comportementale du Petit Molosse face à l'urbanisation croissante de l'île de la Réunion. Ce type de données peut ensuite alimenter des modèles mathématiques pour tester les conséquences potentielles en termes de transmission.

Des études suggèrent que la vie urbaine peut également entraîner un stress physiologique chez certaines espèces et affecter négativement, par exemple, leur condition corporelle (Brunton *et al.*, 2020 ; Potratz *et al.*, 2019 ; Robertson *et al.*, 2023). Le stress peut également impacter fortement l'organisme, notamment dans la réponse à l'infection, puisqu'il peut entraîner une augmentation de la susceptibilité de l'hôte (Murray *et al.*, 2019) ou encore des changements de la composition du microbiome (Yan *et al.*, 2021). Une étude préliminaire réalisée pendant la période de gestation, et à laquelle j'ai participé, a mis en évidence des variations de la condition corporelle et du niveau de stress (cortisol urinaire) du Petit Molosse, selon le niveau d'urbanisation et d'agriculture autour des gîtes (Toshkova *et al.*, en préparation, Annexe 5). En effet, les chauves-souris en milieu agricole étaient en meilleure condition corporelle et moins stressées, et en milieu urbain, seuls les femelles répondaient de manière similaire. De plus, les résultats suggèrent que le stress chez les femelles entraîne une augmentation de la prévalence d'excrétion des paramyxovirus et de l'intensité d'excrétion des bactéries leptospires, et impacte négativement la diversité du microbiome urinaire. Le Petit Molosse de La Réunion semble ainsi bien tolérer la vie urbaine (meilleure condition corporelle, moins de stress, microbiome plus diversifié) avec des conséquences sur l'infection (réduction de la prévalence et de l'intensité d'excrétion). Cependant, cette étude reste limitée à la période de gestation, et ne concerne qu'un nombre limité d'échantillons. Les conséquences des

variations du stress selon l'habitat méritent d'être explorées plus largement au cours du cycle biologique, grâce notamment aux données d'infection produites dans cette thèse. De plus, il serait intéressant de mener une étude comparative avec son espèce sœur, le Petit Molosse de Maurice (*M. acetabulosus*) qui, étonnamment, n'est inféodée qu'aux grottes et n'ai quasiment jamais retrouvée dans des milieux urbains (Bergmans *et al.*, 2017), en dépit de la forte urbanisation du littoral mauricien. Ainsi, l'étude de modèles insulaires, proches phylogénétiquement, permet également d'avoir des répliques naturels pour étudier l'impact des changements globaux sur les populations de chauves-souris et sur la dynamique de leurs agents infectieux.

IV. L'approche *One Health* : une solution pour prévenir l'émergence de zoonoses ?

Le terme *One Health* (ou « une seule santé ») a commencé à être utilisé à la suite de l'émergence du SRAS en 2003 et implique que la santé humaine, des animaux et de l'environnement sont interconnectées (Mackenzie & Jeggo, 2019). Cette approche encourage les travaux interdisciplinaires afin de prévenir et lutter contre la transmission des zoonoses. En effet, il est important d'utiliser une approche à l'interface de la santé de l'environnement, des animaux et de l'homme, puisqu'il a été montré que la modification des habitats perturbe l'écologie des espèces et de leurs agents infectieux, ce qui augmente les risques de transmission des zoonoses chez l'homme (Bradley & Altizer, 2007 ; Jones *et al.*, 2013 ; Semenza *et al.*, 2022 ; Suk *et al.*, 2020). Par exemple, l'étude de Dini et collaborateurs (2023) sur le parasite *Toxoplasma gondii* (provoquant la toxoplasmose), transmis à l'homme essentiellement par de la nourriture contaminée, visait à comparer la séroprévalence chez l'homme avec celles d'espèces d'élevage, de la faune sauvage et domestique. Cette étude a permis d'identifier les sources potentielles de contamination et de mieux comprendre les dynamiques d'infection du parasite, dans l'optique de mettre en place des mesures de contrôle et des campagnes de prévention adaptées. Chez les chauves-souris, grâce à une collaboration entre vétérinaires, anthropologues et épidémiologistes, des solutions simples, comme l'utilisation de protections en bambou autour des troncs d'arbres sont aujourd'hui proposées pour réduire le risque de transmission du virus Nipah entre les chauves-souris et les populations humaines (Figure 15) (Nahar *et al.*, 2013).



Figure 15. Protection en bambou des seaux qui récoltent la sève des dattiers, pour éviter leur contamination avec le virus Nipah, présent dans l'urine des chauves-souris qui viennent manger les dattes (Nahar *et al.*, 2017).

Dans ce contexte, la protection de l'habitat naturel de la faune sauvage et la réduction des contacts avec l'homme apparaissent comme des enjeux forts dans la lutte contre les zoonoses. Cela peut se traduire, par exemple, par la préservation des lieux importants pour le cycle biologique des espèces, comme les lieux de reproduction ou d'élevage des jeunes. Cette problématique est d'autant plus importante que le Petit Molosse semble mettre bas dans des gîtes très localisés, dont le plus grand gîte connu de l'île accueille jusqu'à 100 000 individus chaque année. Ainsi, protéger des sites d'importance biologique pour les chauves-souris, en sensibilisant l'homme aux questions de dérangement et en limitant l'accès, permettraient de protéger ces espèces menacées et de réduire les risques d'émergence de maladies. De plus, les grottes pouvant parfois être des lieux de visites pour les touristes, le port d'équipement de protection est largement recommandé pour limiter la transmission potentielle d'agents infectieux, comme cela est fait dans les grottes et mines où gîtent les chauves-souris frugivores porteuses du virus Marburg (Dhama *et al.*, 2022).

Les constructions humaines constituent un habitat très important pour le Petit Molosse, d'où la nécessité, de prendre en compte cette espèce en amont dans l'aménagement du territoire urbain. Ainsi, la création d'un guide à La Réunion a été mis en place afin de fournir des recommandations pour les aménageurs, visant à limiter en amont l'installation des

chauves-souris, et donc les risques de dérangement ou de mortalité, tout en limitant la gêne engendrée pour les habitants des bâtiments (Suarez & Amirault, 2021). Cependant, lorsqu'aucune solution d'évitement n'est trouvée, la délocalisation d'une colonie peut être entreprise et encadrée réglementairement (arrêté préfectoral), avec des mesures compensatoires qui consistent à proposer des gîtes artificiels après fermeture de la colonie (Figure 16). Cependant, il est important que ces gîtes répondent à toutes les préférences de micro-habitats des chauves-souris afin de ne pas impacter leur comportement et survie, et d'augmenter ainsi le succès des délocalisations (Crawford & O'Keefe, 2023). Aujourd'hui, on n'ignore si ces interventions humaines affecte l'écologie du Petit Molosse et les conséquences épidémiologiques que cela peut entraîner. Un suivi du comportement (télémétrie ; *e.g.* Stone *et al.*, 2015), de l'état physiologique (cortisol, microbiome) et de l'infection (prévalence et intensité d'excrétion), avant, pendant et après la délocalisation permettra sans doute de répondre à cette question.



Figure 16. Exemple de gîtes artificiels de différentes tailles mis en place sur le toit de l'aéroport à La Réunion. Ces gîtes sont occupés comme on peut le voir avec le guano en-dessous (flèche rouge).

Augmenter la sensibilisation et l'engagement de la société représente un atout certain dans la lutte contre les zoonoses. Dans le cas des chauves-souris, ce sont des animaux qui ont depuis longtemps mauvaise réputation, certainement en raison de leur caractère nocturne qui les rend mystérieuses, des légendes qui les associent aux vampires, ou encore des idées reçues

comme celle qu'elles se prennent dans les cheveux (Sieradzki *et al.*, 2022). Ainsi, afin d'assurer une meilleure cohabitation entre l'homme et les chauves-souris, il est important d'étudier comment les hommes perçoivent ces animaux. Par exemple, une étude réalisée dans des Parcs Nationaux aux Etats-Unis a montré que les visiteurs qui reçoivent un message de sensibilisation incluant les bénéfices apportés par les chauves-souris, sont plus enclin à adopter les comportements recommandés en matière de prévention de l'exposition à la rage sans nécessairement blâmer les chauves-souris (Lu *et al.*, 2016). De telles approches sociologiques sont en cours sur le Petit Molosse (thèse de Rachel Leong, UMR PIMIT) et permettront d'étudier les connaissances du grand public et leur perception des chauves-souris de l'île, afin d'apporter des informations nouvelles sur les éléments de sensibilisation à utiliser à La Réunion. La vulgarisation des résultats de la recherche auprès du grand public et en particulier des jeunes, est un bon moyen d'encourager l'engagement de la société, par exemple à travers des événements nationaux (*e.g.* Déclics, Expérimentarium, Fête de la Science, MT180, Nuits des chercheurs) auxquels j'ai pu participer pendant ma thèse. Cela passe également par la diffusion des résultats auprès d'acteurs plus spécifiques comme les autorités sanitaires et de l'environnement (Agence Régionale de la Santé ; Direction Régionale de l'Environnement, de l'Aménagement et du Logement), les structures de protection de l'environnement (associations naturalistes, Parc National de la Réunion, Office Français pour la Biodiversité, Muséum d'Histoire Naturelle), et des entreprises privées (bureaux d'études). Lors de ma thèse, j'ai ainsi pu participer à de telles réunions afin de discuter de l'articulation des enjeux de santé publique et de conservation, avec l'objectif d'améliorer les stratégies de gestion du Petit Molosse et de surveillance épidémiologique notamment.

En effet, aujourd'hui, le lien entre l'épidémiologie des agents infectieux hébergés par le Petit Molosse et le développement de maladies chez l'homme à La Réunion n'a été que peu exploré. Une seule étude portant sur la leptospirose a montré que la souche hébergée par le Petit Molosse était génétiquement distincte de celle retrouvée dans les cas humains graves (hospitalisés) à La Réunion (Guernier *et al.*, 2016). Néanmoins, des événements de transmission pourraient avoir lieu entre les chauves-souris et l'homme, sans mener pour autant à des symptômes graves ou des épidémies, expliquant qu'ils ne soient pas détectés par les services de surveillance habituels. Dans le cas des *Henipavirus* que l'on pensait cantonnées à l'Asie et l'Australie, des anticorps ont été retrouvés chez les chauves-souris et dans les

populations humaines africaines, sans que cela soit associé au développement de maladies grave chez l'homme (Pernet *et al.*, 2014). De la même manière, une étude sérologique récente suggère que les populations humaines vivant à proximité de grottes au Vietnam sont exposées à des virus proches du virus Marburg, initialement décrit en Afrique (Latinne *et al.*, 2023). Dans notre cas, il serait indispensable de développer des nouveaux outils de diagnostic, adaptés à la spécificité des virus de Petit Molosse (Dietrich *et al.*, 2015b ; Joffrin *et al.*, 2020). La mise en place de tests sérologiques permettrait de mesurer l'exposition aux virus de petits Molosses dans la population générale mais également parmi les personnes les plus exposées, tels que les chiroptérologues ou les personnels d'entretiens des ponts et bâtiments par exemple. A plus long terme, ces informations permettraient d'informer les autorités sanitaires sur les potentiels risques de transmission des zoonoses et d'adapter les stratégies de prévention et de diagnostic.

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Annexes

Annexe 1

Matériels supplémentaires pour l'article 1 :

Aguillon S., Le Minter G., Lebarbenchon C., Hoarau A.O.G., Toty C., Joffrin L., Ramanantsalama R.V., Augros S., Tortosa P., Mavingui P. & Dietrich M. 2023. A population in perpetual motion: Highly dynamic roosting behaviour of a tropical island endemic bat. *Ecology and Evolution*, 13(2), e9814. <https://doi.org/10.1002/ece3.9814>

Table S1. Sampling periods characteristics.

Year	Sampling period	Capture dates (MM/DD) (number of captured individuals)
2018	November - December	From 10/26 to 12/07 (n = 601)
	December	From 12/10 to 12/17 (n = 164)
2019	January - February	From 01/24 to 02/27 (n = 555)
	March - April	From 03/07 to 04/09 (n = 534)
	April - May	From 04/23 to 05/13 (n = 478)
	June	From 06/04 to 06/19 (n = 317)
	July	From 07/16 to 07/30 (n = 229)
	September	From 09/03 to 09/17 (n = 325)
	October - November	From 10/21 to 11/07 (n = 510)
	November - December	From 11/25 to 12/10 (n = 379)
2020	January - February	From 01/15 to 02/05 (n = 525)
	March	From 03/02 to 03/20 (n = 413)
	May - June	From 05/19 to 06/04 (n = 331)
	July	From 07/06 to 07/21 (n = 190)
	September	From 09/07 to 09/24 (n = 310)
	October	From 10/14 to 10/29 (n = 453)
	November - December	From 11/23 to 12/09 (n = 407)

Table S2. Summary of the statistical models used to analyse the Reunion free-tailed bat data set, including the analysis of 19 roosts.

Complete models include all tested variables while selected models include only significant variables. N is the number of observations (captured bats) included in the model according to the different roosts and types of individuals analysed. Variables in brackets represent random effects and nested effects are represented with a slash. The interaction between two variables is represented with a double point. For the GAMM, the "period" variable was coded as numeric.

Scale of analysis	Category of individuals included	N	Model number	Complete model	Selected model
<u>1) Roost size</u>					
Log (Roost Size)	All individuals	6721	GAMM ₁	Period + (Roost) + (EstimSizeMethod)	Period + (Roost)
<u>2) Sex-ratio</u>					
Sex-ratio	All individuals	6721	LMM ₂	Period + (Roost)	Period + (Roost)
<u>3) Sexual dimorphism</u>					
Forearm length	Adults (excluding data from recaptures)	5241	LMM ₃	Sex + (Roost)	Sex + (Roost)
Forearm length	Juveniles (excluding data from recaptures)	953	LMM ₄	Sex + Date + Sex : Date + (Roost) + (Year)	Sex + Date + (Roost) + (Year)
<u>4) Body condition</u>					
BCI	All individuals	6721	LMM ₅	Sex + Age + Sex : Age + (Period) + (Roost)	Sex + Age + Sex : Age + (Period) + (Roost)
BCI	Adult females	2471	LMM ₆	Repro + (Period) + (Roost)	Repro + (Period) + (Roost)
BCI	Adult males	3292	LMM ₇	Repro + (Period) + (Roost)	Repro + (Period) + (Roost)
BCI	Adult females (Gestation period)	584	LMM ₈	logSize + Repro + logSize : Repro + (Year/Date) + (Roost)	Repro + logSize : Repro + (Year/Date)
BCI	Adult females (Gestation period) without TBA	498	LMM ₉	logSize + Repro + logSize : Repro + (Year/Date) + (Roost)	Repro + (Year/Date)
BCI	Adult males (Mating period)	294	LMM ₁₀	logSize + Repro + logSize : Repro + (Year/Date) + (Roost)	Repro + (Year/Date)

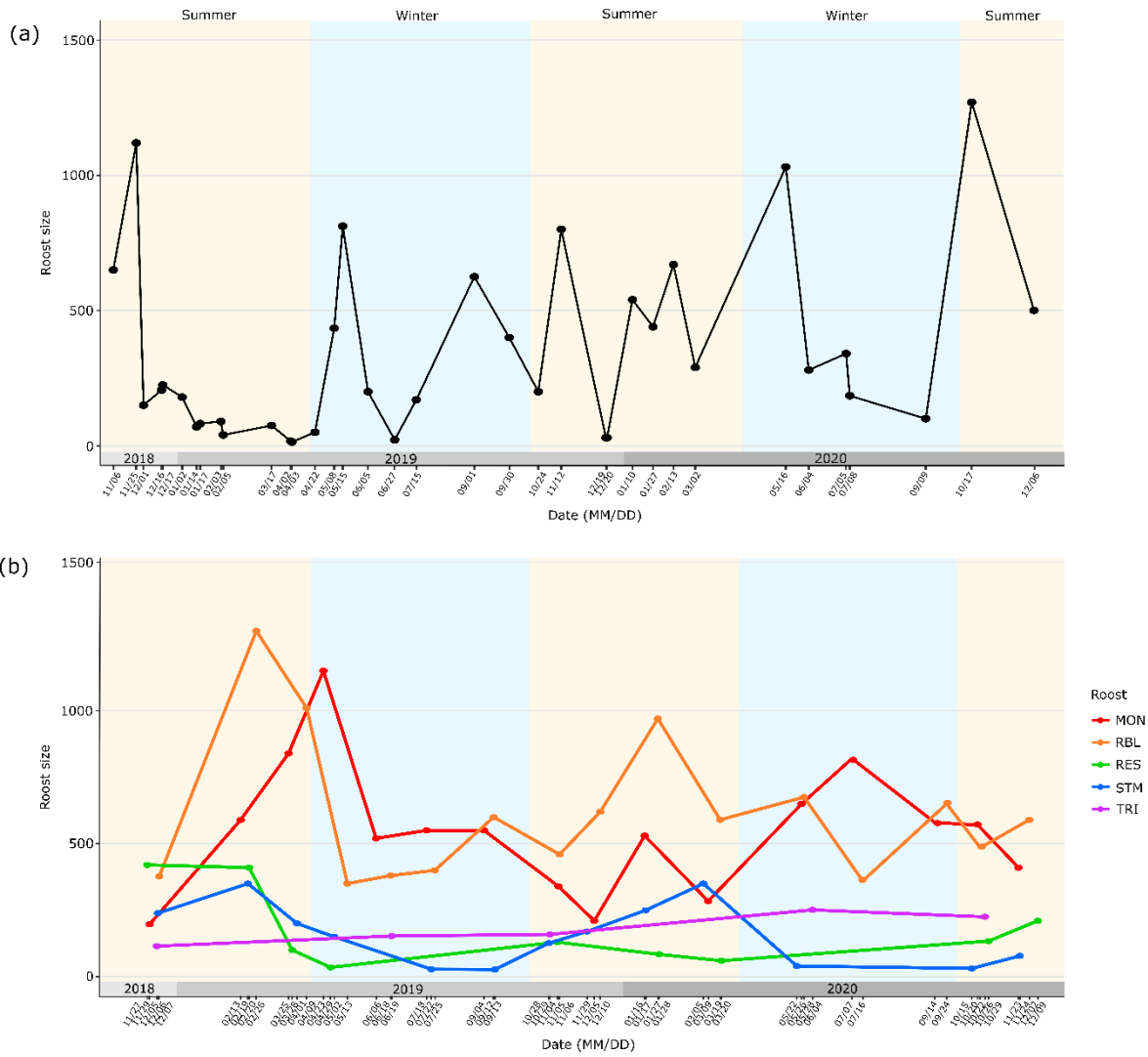


Figure S1. Temporal variation of roost size.

a) Data for the TGI roost were plotted separately as the roost size estimation was performed more frequently. b) Roost size is presented for five colonies, data for others roosts can be found in Figure 3a. Summer and winter periods correspond to rainy and dry seasons and are indicated by yellow and blue backgrounds.

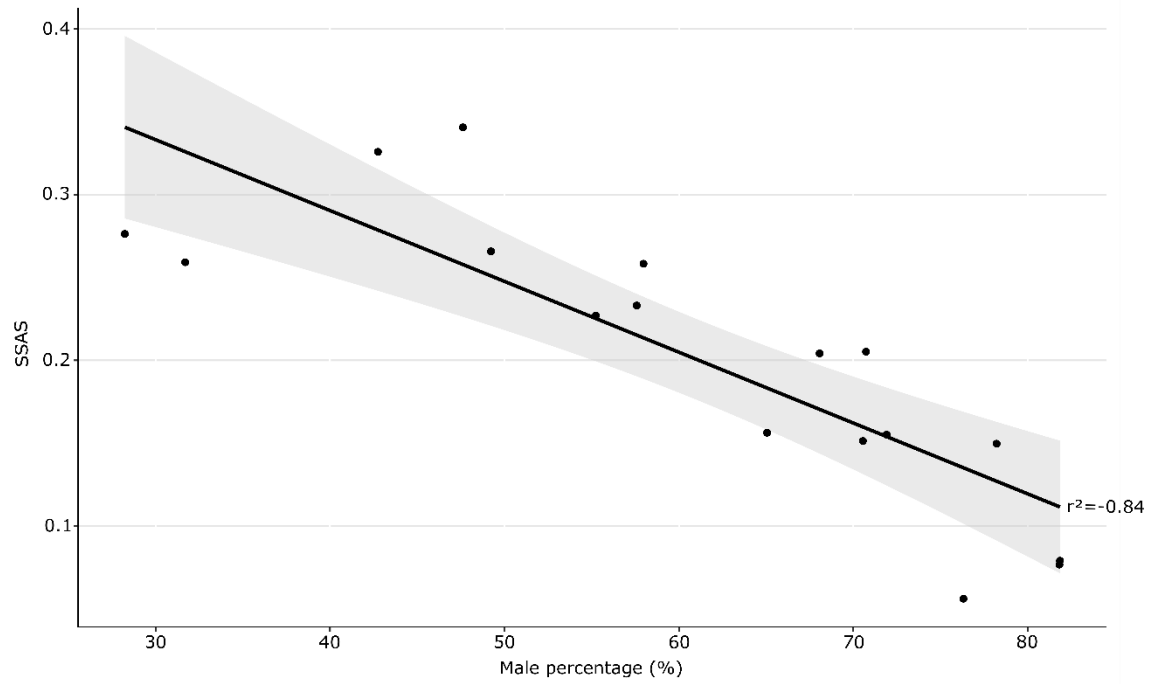


Figure S2. Correlation between the Sexual Segregation and Aggregation Statistic (SSAS) between sexes in adults and the percentage of adult males.

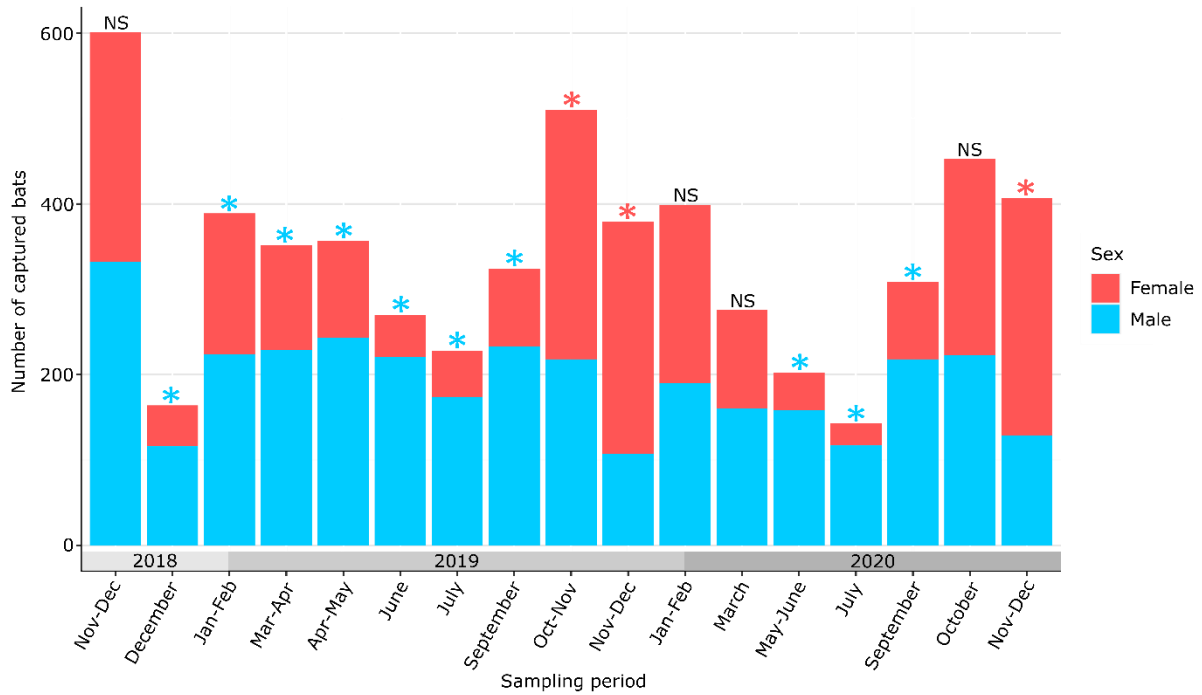


Figure S3. Temporal variation of the sex-ratio in adults.

Bars indicate the total number of captured bats per sampling periods. Asterisks indicate significant deviations from a ratio 1:1 ($P < 0.05$) and are coloured according to the majority sex. NS = not significant.

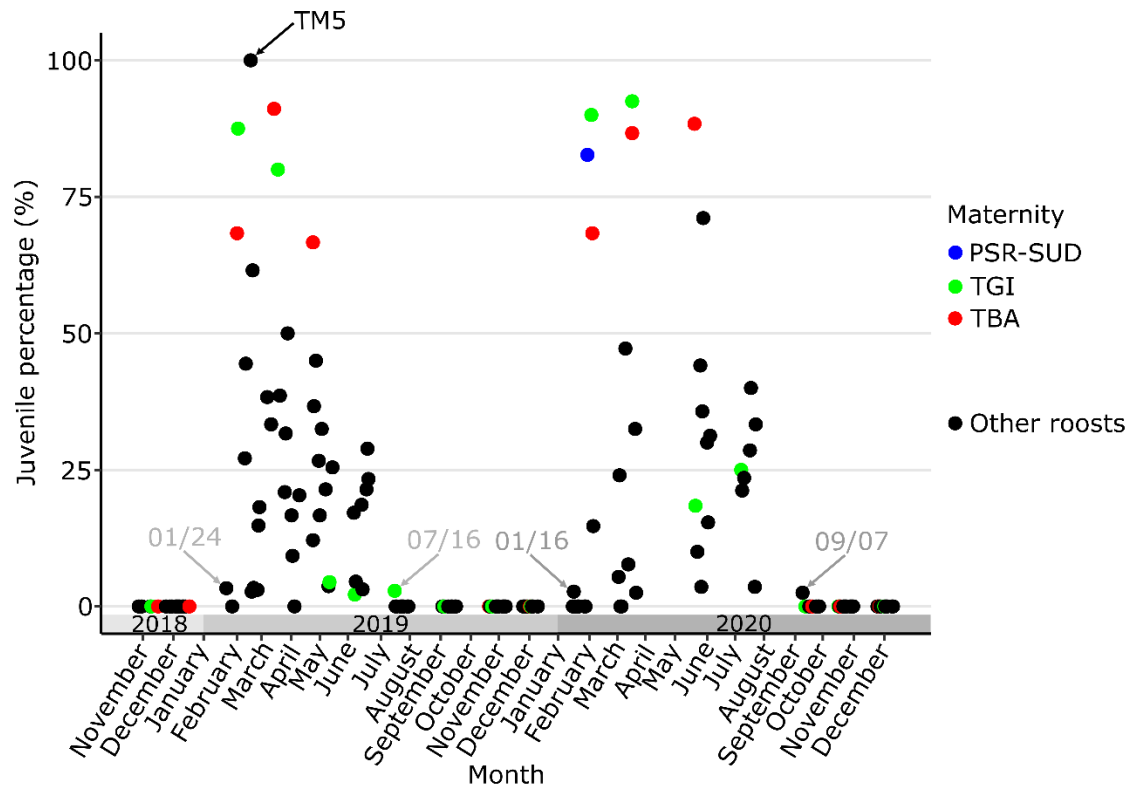


Figure S4. Temporal changes in the proportion of flying juveniles. Each black dot corresponds to the sampling of a roost at one date.

Coloured dots correspond to the three maternity roosts: PSR-SUD, TGI, and TBA. TM5 roost (exclusively composed of juveniles) is denoted with a black arrow. The first and the last date (MM/DD) of juvenile's observation are based on the capture of flying juveniles and are indicated with grey arrows for each year.

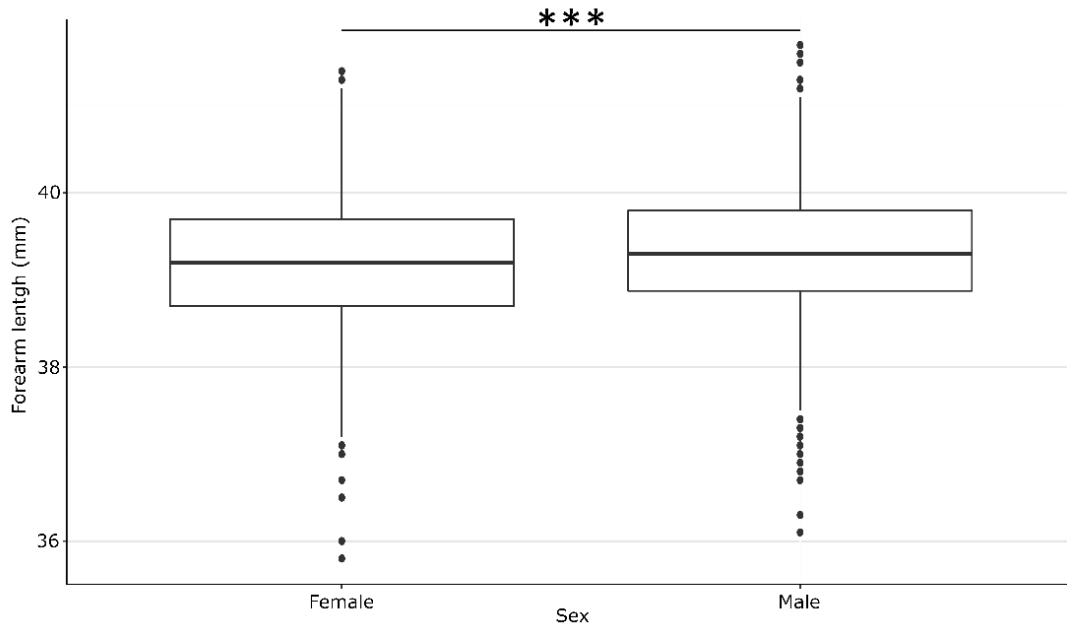


Figure S5. Differences in forearm length between sexes in adults.

*** $P < 0.001$ significance of the LMM test.

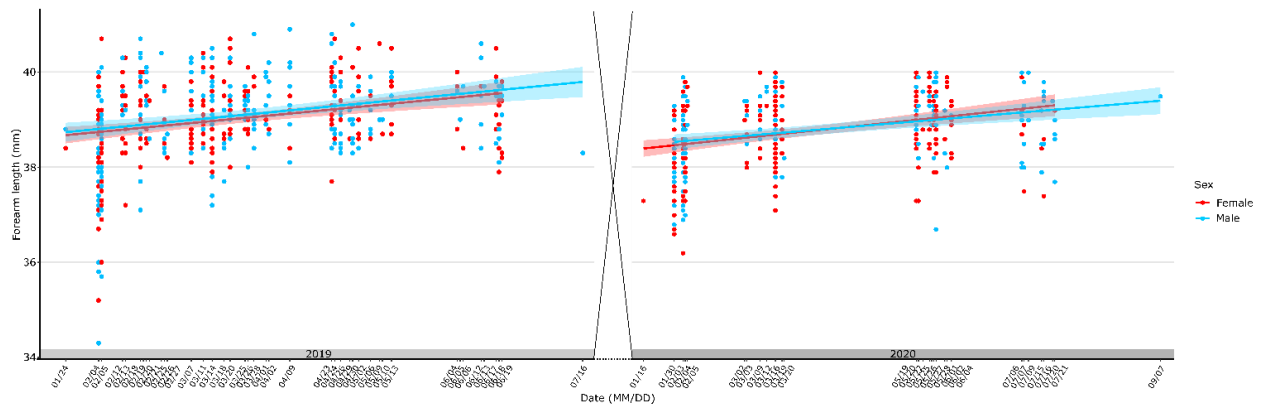


Figure S6. Differences in forearm length between sexes in juveniles.

The cross line represents the month gap (July to January) when juveniles can't be distinguishing from adults because of the epiphysis fusion in finger articulations, and thus no data for juveniles were collected.

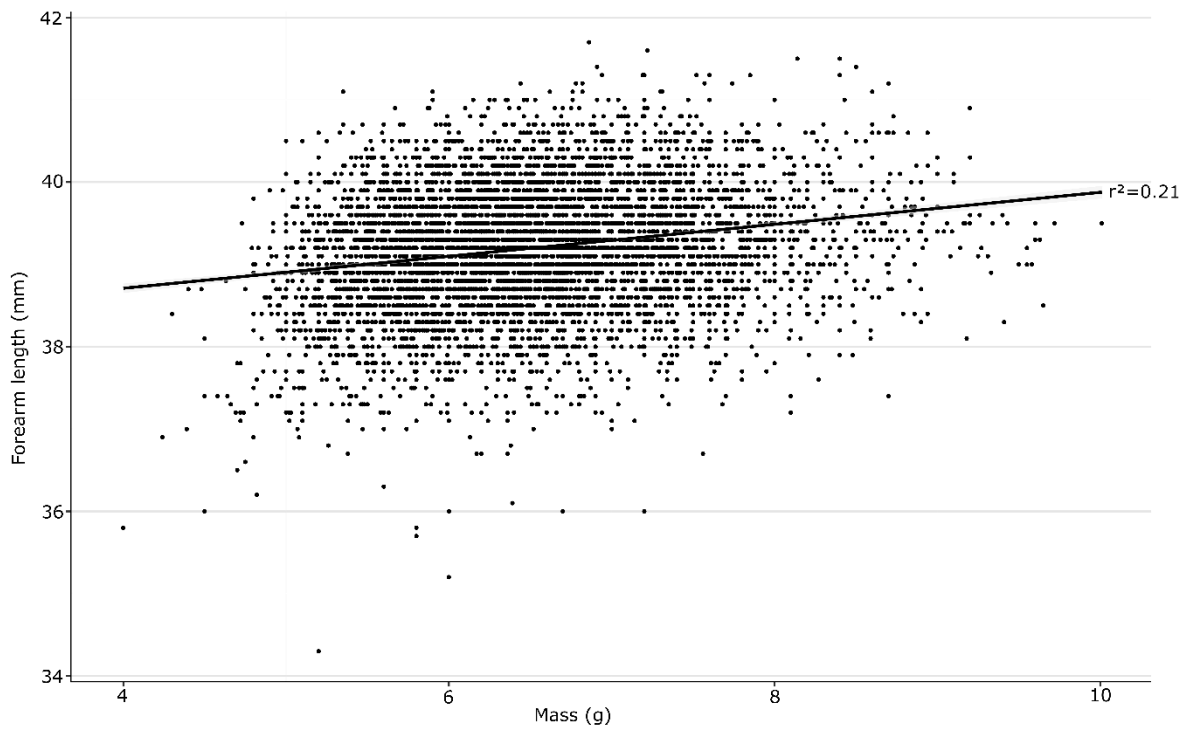


Figure S7. Correlation between the forearm length and the mass (including both adult and juvenile bats).

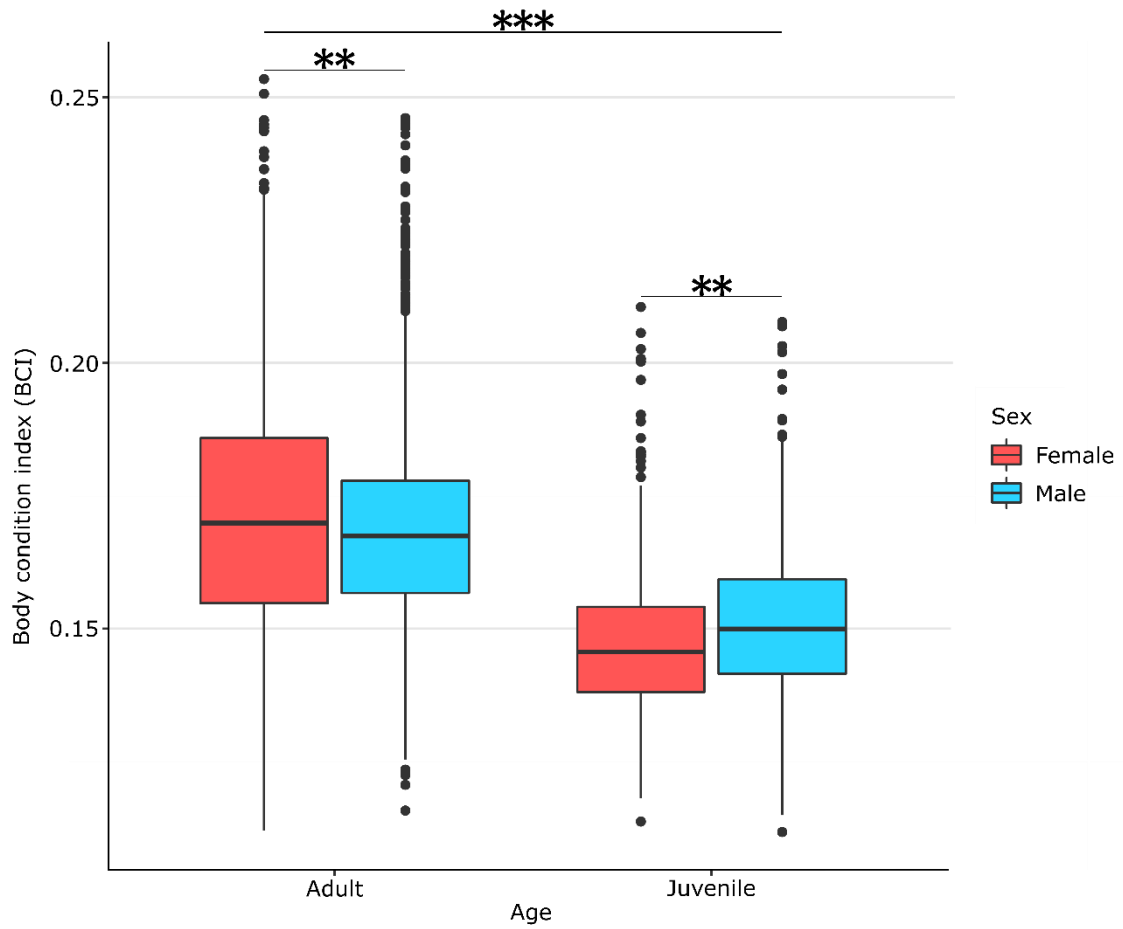


Figure S8. Differences in body condition between ages and sexes.

** $P < 0.01$ and *** $P < 0.001$ significance of the LMM and Tukey's post-hoc tests.

Annexe 2

Matériels supplémentaires pour l'article 2 :

Aguillon S., Castex C., Duchet A., Turpin, M., Le Minter G., Lebarbenchon C., Hoarau A.O.G.,
Toty C., Joffrin L., Tortosa P., Mavingui P., Goodman S.M. & Dietrich M. Stuck on a small
tropical island: wide *in-situ* diversification of an urban-dwelling bat.

Table S1. Characteristics of the 12 microsatellite loci developed for *Mormopterus francoismoutoui*.

Na is the number of alleles per locus, Ho is the observed heterozygosity, He is the expected heterozygosity, and Fis is the fixation index.

Locus	Na	Ho	He	Fis
MF_loc03	17	0.820	0.826	0.008
MF_loc04	20	0.743	0.881	0.157
MF_loc05	16	0.804	0.824	0.025
MF_loc06	13	0.818	0.848	0.036
MF_loc07	16	0.830	0.840	0.013
MF_loc13	15	0.874	0.852	-0.025
MF_loc14	14	0.831	0.814	-0.020
MF_loc15	17	0.786	0.846	0.071
MF_loc18	13	0.785	0.759	-0.035
MF_loc28	7	0.479	0.481	0.006
MF_loc36	15	0.789	0.794	0.006

Table S2. Genetic diversity indices calculated with mitochondrial DNA (D-loop) of *Mormopterus francoismoutoui* separately for females and males.

N is the number of sequences used for calculation, Hd is the haplotype diversity, and π is the nucleotide diversity. Roost sites are defined in Table 1.

Roost	Females			Males		
	N	Hd	π	N	Hd	π
AOM	8	0.964	0.0256	6	1.000	0.0288
CIT	/	/	/	15	1.000	0.0309
EGI	16	1.000	0.0310	25	0.997	0.0274
ESA	26	1.000	0.0263	20	0.995	0.0284
MON	18	1.000	0.0304	30	1.000	0.0278
PBV	4	1.000	0.0251	7	1.000	0.0336
PSR	15	1.000	0.0293	33	0.998	0.0307
RAC	4	1.000	0.0249	12	1.000	0.0298
RBL	22	1.000	0.0292	24	1.000	0.0280
RES	14	1.000	0.0290	16	1.000	0.0292
RPQ	22	1.000	0.0297	23	0.992	0.0298
STJ	8	1.000	0.0258	7	1.000	0.0298
STM	13	1.000	0.0295	30	1.000	0.0284
TBA	36	0.998	0.0292	10	1.000	0.0303
TGI	26	0.997	0.0313	23	0.996	0.0292
TM5	7	1.000	0.0307	8	1.000	0.0305
TRI	9	1.000	0.0296	21	1.000	0.0284
VSP	21	1.000	0.0286	25	0.997	0.0299
TOTAL	269	0.998	0.0290	334	0.998	0.0282

Table S3. Genetic diversity indices calculated with mitochondrial DNA (D-loop) of *Mormopterus francoismoutoui* captured during three different periods.

N is the number of sequences used for calculation, Hd is the haplotype diversity, and π is the nucleotide diversity. Roost sites are defined in Table 1.

Roost	Pregnancy 2018 (summer)			Non-reproductive 2019 (winter)			Mating 2019 – 2020 (March)		
	N	Hd	π	N	Hd	π	N	Hd	π
AOM	/	/	/	/	/	/	14	0.989	0.0264
CIT	15	1.000	0.0296	/	/	/	/	/	/
EGI	26	1.000	0.0288	/	/	/	15	0.990	0.0289
ESA	15	0.990	0.0264	16	1.000	0.0277	15	0.990	0.0279
MON	18	1.000	0.0291	15	1.000	0.0296	15	1.000	0.0293
PBV	/	/	/	/	/	/	/	/	/
PSR	17	1.000	0.0304	15	0.990	0.0306	15	1.000	0.0306
RAC	/	/	/	/	/	/	/	/	/
RBL	16	0.992	0.0305	15	1.000	0.0293	15	1.000	0.0279
RES	15	1.000	0.0270	/	/	/	15	1.000	0.0306
RPQ	15	0.990	0.0295	15	1.000	0.0298	15	1.000	0.0313
STJ	/	/	/	15	1.000	0.0278	/	/	/
STM	15	1.000	0.0317	13	1.000	0.0287	15	1.000	0.0280
TBA	31	0.998	0.0299	/	/	/	15	1.000	0.0272
TGI	19	1.000	0.0329	15	1.000	0.0294	15	1.000	0.0292
TM5	/	/	/	/	/	/	/	/	/
TRI	15	1.000	0.0279	15	1.000	0.0310	/	/	/
VSP	16	1.000	0.0286	15	1.000	0.0268	15	1.000	0.0304
TOTAL	233	0.998	0.0287	149	0.998	0.0287	179	0.998	0.0285

Table S4. Genetic diversity indices calculated with 11 microsatellite markers of *Mormopterus francoismoutoui* separately for females and males.

N is the number of genotypes used, Ho is the observed heterozygosity, and He is the expected heterozygosity. Standard error is given following \pm . The roost sites are defined in Table 1.

Roost	Females			Males		
	N	Ho	He	N	Ho	He
AOM	15	0.781 \pm 0.039	0.776 \pm 0.033	15	0.743 \pm 0.038	0.776 \pm 0.038
CIT	/	/	/	29	0.787 \pm 0.035	0.791 \pm 0.029
EGI	26	0.750 \pm 0.047	0.793 \pm 0.033	38	0.777 \pm 0.039	0.807 \pm 0.027
ESA	57	0.767 \pm 0.033	0.802 \pm 0.033	39	0.788 \pm 0.038	0.803 \pm 0.033
MON	34	0.795 \pm 0.040	0.785 \pm 0.033	54	0.797 \pm 0.030	0.801 \pm 0.036
PBV	4	0.803 \pm 0.062	0.721 \pm 0.036	7	0.732 \pm 0.045	0.738 \pm 0.025
PSR	21	0.799 \pm 0.034	0.785 \pm 0.032	75	0.784 \pm 0.039	0.795 \pm 0.037
RAC	4	0.894 \pm 0.064	0.688 \pm 0.043	10	0.814 \pm 0.077	0.773 \pm 0.033
RBL	44	0.768 \pm 0.026	0.793 \pm 0.038	49	0.758 \pm 0.032	0.802 \pm 0.032
RES	22	0.743 \pm 0.052	0.801 \pm 0.030	40	0.744 \pm 0.036	0.793 \pm 0.035
RPQ	45	0.795 \pm 0.039	0.799 \pm 0.034	55	0.782 \pm 0.030	0.803 \pm 0.033
STJ	13	0.800 \pm 0.027	0.766 \pm 0.034	17	0.769 \pm 0.037	0.793 \pm 0.036
STM	23	0.829 \pm 0.042	0.792 \pm 0.036	47	0.782 \pm 0.042	0.808 \pm 0.031
TBA	53	0.788 \pm 0.038	0.799 \pm 0.031	10	0.756 \pm 0.062	0.746 \pm 0.057
TGI	48	0.765 \pm 0.049	0.804 \pm 0.034	55	0.769 \pm 0.045	0.802 \pm 0.035
TM5	12	0.727 \pm 0.051	0.769 \pm 0.033	16	0.772 \pm 0.048	0.777 \pm 0.034
TRI	10	0.742 \pm 0.054	0.757 \pm 0.029	48	0.790 \pm 0.037	0.803 \pm 0.031
VSP	40	0.789 \pm 0.036	0.800 \pm 0.036	61	0.773 \pm 0.030	0.800 \pm 0.033
TOTAL	471	0.784 \pm 0.011	0.778 \pm 0.008	665	0.773 \pm 0.010	0.789 \pm 0.008

Table S5. Genetic diversity indices calculated with 11 microsatellite markers of *Mormopterus francoismoutoui* captured during three different periods.

N is the number of genotypes used, Ho is the observed heterozygosity, and He is the expected heterozygosity. Standard error is given following \pm . Roost sites are defined in Table 1.

Roost	Pregnancy 2018 (summer)			Non-reproductive 2019 (winter)			Mating 2019 – 2020 (March)		
	N	Ho	He	N	Ho	He	N	Ho	He
AOM	/	/	/	/	/	/	30	0.762 \pm 0.035	0.793 \pm 0.037
CIT	29	0.787 \pm 0.035	0.791 \pm 0.029	/	/	/	/	/	/
EGI	34	0.783 \pm 0.029	0.808 \pm 0.025	/	/	/	30	0.748 \pm 0.063	0.794 \pm 0.036
ESA	36	0.798 \pm 0.025	0.809 \pm 0.034	34	0.757 \pm 0.048	0.788 \pm 0.032	26	0.768 \pm 0.038	0.796 \pm 0.032
MON	33	0.796 \pm 0.033	0.800 \pm 0.033	30	0.804 \pm 0.031	0.791 \pm 0.034	25	0.784 \pm 0.049	0.777 \pm 0.039
PBV	/	/	/	/	/	/	/	/	/
PSR	34	0.796 \pm 0.033	0.797 \pm 0.031	34	0.781 \pm 0.040	0.775 \pm 0.041	28	0.784 \pm 0.046	0.792 \pm 0.036
RAC	/	/	/	/	/	/	/	/	/
RBL	31	0.764 \pm 0.038	0.783 \pm 0.037	34	0.760 \pm 0.033	0.797 \pm 0.034	28	0.773 \pm 0.027	0.800 \pm 0.031
RES	32	0.745 \pm 0.035	0.791 \pm 0.035	/	/	/	30	0.744 \pm 0.048	0.795 \pm 0.032
RPQ	37	0.783 \pm 0.039	0.795 \pm 0.038	33	0.780 \pm 0.036	0.788 \pm 0.034	30	0.802 \pm 0.038	0.804 \pm 0.030
STJ	/	/	/	30	0.782 \pm 0.029	0.796 \pm 0.034	/	/	/
STM	34	0.794 \pm 0.039	0.803 \pm 0.032	13	0.846 \pm 0.051	0.794 \pm 0.033	23	0.774 \pm 0.050	0.794 \pm 0.031
TBA	34	0.799 \pm 0.039	0.791 \pm 0.033	/	/	/	29	0.764 \pm 0.048	0.797 \pm 0.035
TGI	38	0.783 \pm 0.040	0.792 \pm 0.037	36	0.762 \pm 0.057	0.798 \pm 0.030	29	0.748 \pm 0.052	0.801 \pm 0.040
TMS	/	/	/	/	/	/	/	/	/
TRI	29	0.772 \pm 0.035	0.803 \pm 0.030	29	0.789 \pm 0.048	0.784 \pm 0.035	/	/	/
VSP	38	0.798 \pm 0.035	0.790 \pm 0.035	35	0.770 \pm 0.036	0.799 \pm 0.035	28	0.766 \pm 0.046	0.797 \pm 0.031
TOTAL	439	0.784 \pm 0.009	0.796 \pm 0.009	308	0.783 \pm 0.013	0.791 \pm 0.010	336	0.768 \pm 0.013	0.795 \pm 0.009

Table S6. Pairwise differentiation between roost sites among global population of *Mormopterus francoismoutoui* with mitochondrial DNA values (Φ_{st}) below the diagonal and microsatellite markers (F_{st}) above. No significant p-values after Holm corrections. Roost sites are defined in Table 1.

Roost	AOM	CIT	EGI	ESA	MON	PBV	PSR	RAC	RBL	RES	RPQ	STM	STJ	TBA	TGI	TMS	TRI	VSP
AOM	-	0.018	0.011	-0.017	0.017	0.006	0.026	0.022	-0.0005	0.015	0.015	0.003	0.043	0.033	0.006	-0.018	0.0008	0.011
CIT	-0.002	-	-0.001	0.009	-0.006	-0.027	0.015	-0.020	-0.002	0.002	0.001	-0.002	0.006	0.0007	-0.016	-0.013	-0.003	-0.003
EGI	-0.0008	-0.003	-	-0.0006	-0.010	-0.031	0.0016	-0.025	-0.003	-0.0005	-0.013	-0.015	0.010	-0.0002	-0.004	-0.020	-0.009	-0.004
ESA	0.002	0.004	-0.0009	-	0.004	0.0008	0.023	-0.004	-0.0007	-0.003	0.007	-0.004	0.018	0.011	0.010	-0.025	-0.0007	-0.0007
MON	0.0006	0.001	-0.00005	0.002	-	-0.030	0.010	-0.029	-0.008	0.001	-0.008	-0.008	0.011	-0.001	-0.005	-0.014	-0.008	-0.010
PBV	0.003	-0.007	0.0007	0.006	0.004	-	-0.026	-0.040	-0.020	-0.007	-0.035	-0.025	-0.012	-0.022	-0.028	-0.033	-0.032	-0.023
PSR	-0.00002	-0.0006	-0.001	0.003	0.0002	0.003	-	-0.013	0.0129	0.004	-0.005	0.003	-0.004	-0.0001	0.004	0.005	0.010	0.005
RAC	0.008	-0.007	0.0009	0.005	0.005	-0.003	0.003	-	-0.018	-0.016	-0.022	-0.021	-0.008	-0.019	-0.018	-0.023	-0.021	-0.022
RBL	-0.0008	-0.0006	-0.0009	0.002	0.0001	0.004	0.002	0.002	-	-0.007	-0.003	-0.006	0.006	-0.001	-0.009	-0.016	-0.008	-0.013
RES	-0.0008	-0.0009	-0.001	-0.0007	0.0009	0.003	-0.0002	-0.0003	-0.0003	-	-0.008	-0.003	-0.017	-0.016	-0.003	-0.012	0.005	-0.011
RPQ	-0.002	-0.002	-0.0009	0.0006	0.001	0.003	0.0008	0.004	0.00003	-0.0002	-	-0.012	-0.003	-0.010	-0.005	-0.015	-0.007	-0.009
STM	-0.001	-0.004	-0.001	0.002	-0.00003	0.003	0.001	-0.0006	-0.001	-0.0009	-0.001	-	0.005	-0.001	-0.006	-0.022	-0.011	-0.008
STJ	0.002	-0.005	-0.0006	0.005	0.004	-0.001	0.0007	0.0006	0.002	-0.002	0	-0.002	-	-0.018	-0.0004	-0.006	0.008	-0.006
TBA	0.0005	-0.002	-0.003	-0.001	-0.002	0.005	-0.002	-0.002	-0.001	-0.004	-0.001	-0.0008	0.0004	-	0.0002	-0.010	0.0004	-0.011
TGI	0.002	-0.003	-0.0006	0.001	0.0001	0.0002	0.0004	0.003	0.001	0.0002	-0.0007	-0.001	0.001	-0.002	-	-0.015	-0.0007	-0.006
TMS	0.004	0.005	0.003	0.005	0.003	0.017	0.003	0.010	0.003	0.003	0.003	0.005	0.008	0.006	0.004	-	-0.025	-0.015
TRI	-0.001	-0.001	-0.002	0.001	-0.002	0.005	-0.001	0.004	-0.001	-0.002	-0.0003	-0.0002	0.001	-0.003	-0.001	0.002	-	-0.007
VSP	0.0007	-0.0002	-0.001	0.0008	-0.0005	0.008	-0.001	0.003	-0.0003	-0.002	-0.0004	-0.0004	0.0002	-0.002	-0.0003	0.003	-0.001	-

Table S7. Summary of isolation by distance tests for each sex and season for both mtDNA and microsatellite markers in *Mormopterus francoismoutoui*.

r is the regression coefficient and p is the significance of the test.

	Mitochondrial data		Microsatellites data	
	r	p	r	p
Females	0.06	0.27	-0.03	0.63
Males	-0.12	0.88	0.009	0.44
Pregnancy 2018 (summer)	-0.03	0.536	0.03	0.42
Non-reproductive 2019 (winter)	0.21	0.12	0.06	0.39
Mating 2019 – 2020 (March)	-0.26	0.97	-0.15	0.87

Table S8. Summary of effective sample size (ESS) for each dimension group parameters of *Mormopterus francoismoutoui* implemented in the Coalescent Bayesian Skyline model from mitochondrial data (D-loop).

The model with five groups (in red) have the best likelihood ESS followed by six and ten group models.

Dimension group	Posterior ESS	Likelihood ESS	Prior ESS	Bayesian Skyline ESS
3	2290	1160	3497	3499
4	2501	816	3726	3576
5	3073	1634	3354	3200
6	2480	1618	3069	2929
7	3030	1010	3623	3400
8	2382	1130	3336	2789
9	2543	1168	4035	3717
10	2745	1617	3253	3042

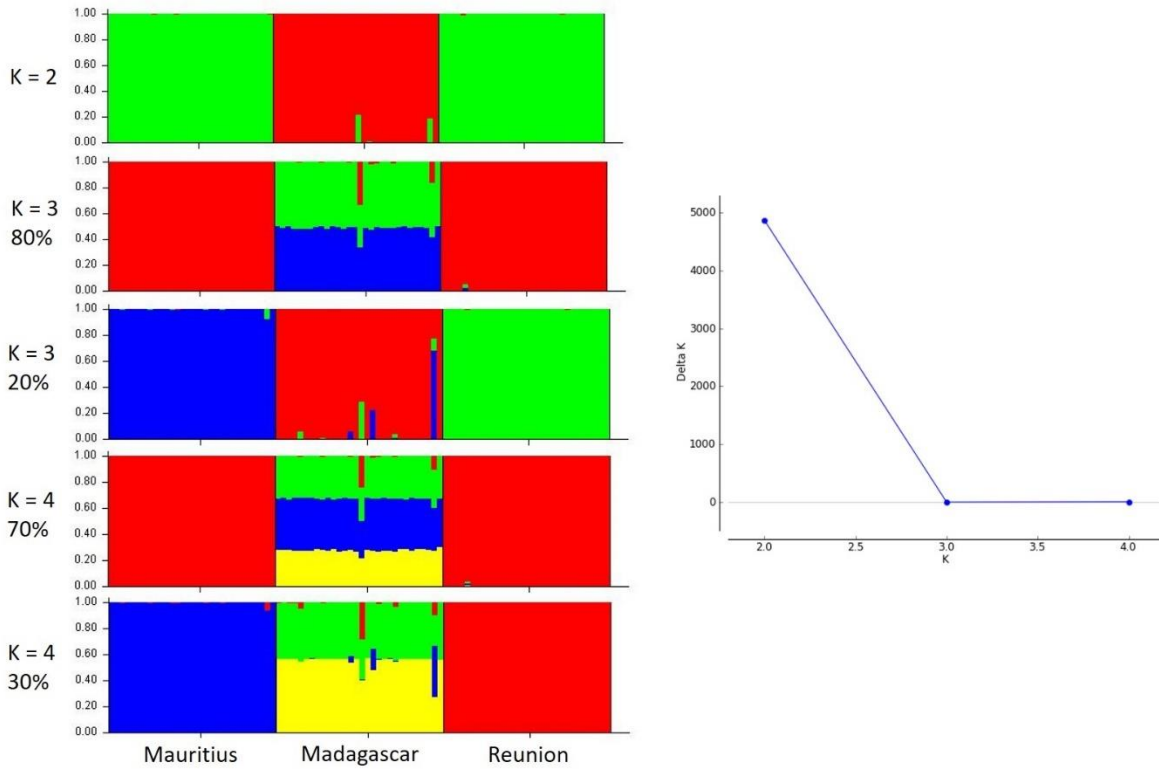


Fig. S1. Assignment plots among regional *Mormopterus* performed in STRUCTURE for K from 2 to 4.

The best K (K = 2) was determined following the Evanno method (Delta K). Each vertical bar represents one individual and colours indicates genetic clusters. Islands are indicated below the plot and percentages runs are indicated below the K.

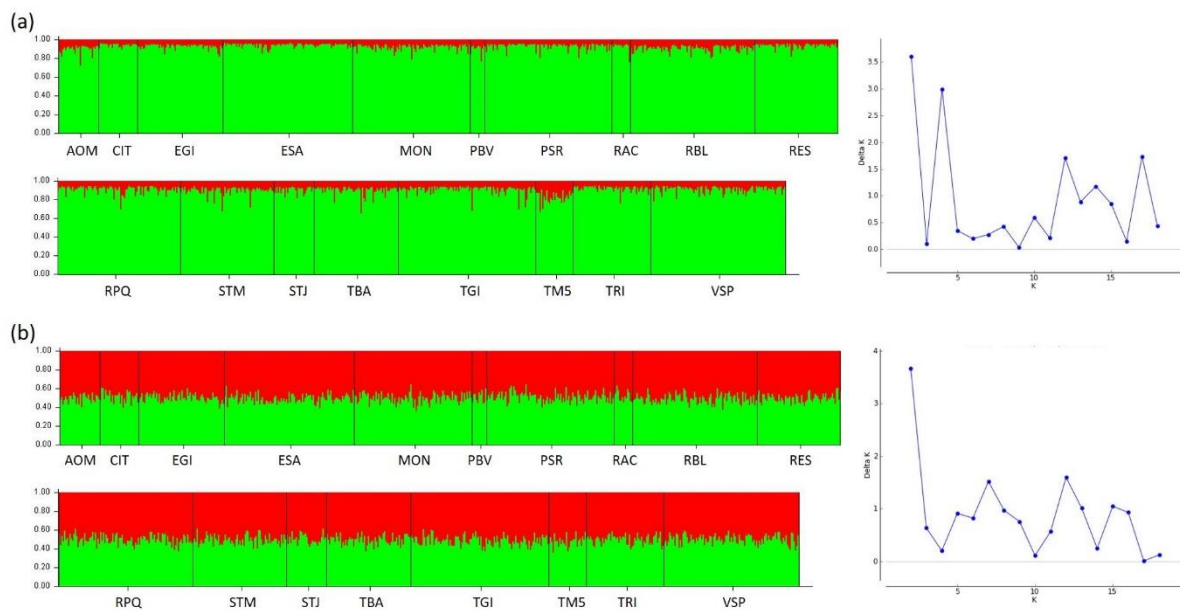


Fig. S2. Assignment plot among Reunion population of *Mormopterus francoismoutoui* in STRUCTURE for the best $K = 2$ following the Evanno Method (Delta K).

Results are shown (a) with LocPrior and (b) without LocPrior model.

Each vertical bar represents one individual and colours indicate genetic clusters. Roost sites are indicated below the plot and these are defined in Table 1.

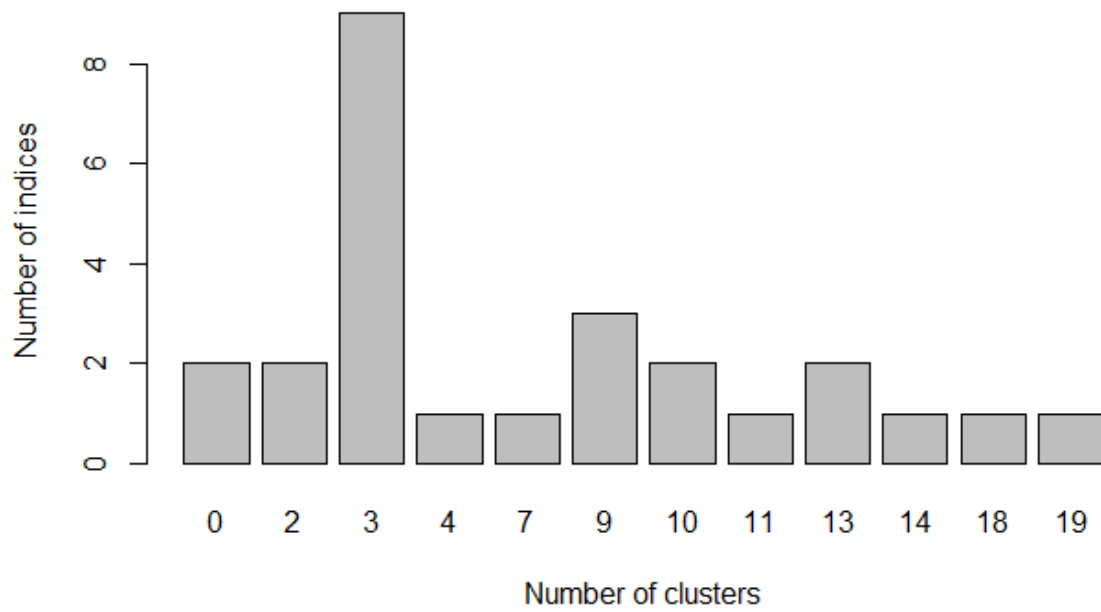


Fig. S3. Bar plot showing the best number of genetic clusters of *Mormopterus francoismoutoui* according to 26 indices evaluated with the k-means clustering.

The greatest frequency of indices found the best number of clusters being $K = 3$.

Annexe 3

Matériels supplémentaires pour l'article 3 :

Aguillon S., Turpin M., Le Minter G., Lebarbenchon C., Hoarau A.O.G., Toty C., Duchet A., Joffrin L., Ramanantsalama R.V., Tortosa P., Mavingui P. & Dietrich M. Fine-scale spatio-temporal monitoring of an island endemic bat reveals how individual and population heterogeneity shapes triple co-excretion dynamics.

Table S1. Summary of the statistical models used to analyse the mono-excretion dynamics in *M. francoismoutoui*.

Significant variables are in bold and the double point represent the interaction between two variables. PMV: paramyxovirus, LEPTO: *Leptospira* bacteria, HSV: herpesvirus.

Category of individuals (number)	Infectious agent	Model type and number	Full model	% explained deviance
All individuals (N = 5,949)	PMV	GAMM M1	Smooth: Sampling Period Linear: logSize + Sex-ratio + Age + Sex + Age : Sex + HSV + LEPTO + HSV-LEPTO Random : Site + JulDate + Year	14.3
	LEPTO	GAMM M2	Smooth: Sampling Period Linear: logSize + Sex-ratio + Age + Sex + Age : Sex + PMV + HSV + PMV-HSV Random : Site + JulDate + Year	12.9
	HSV	GAMM M3	Smooth: Sampling Period Linear: logSize + Sex-ratio + Age + Sex + Age : Sex + PMV + LEPTO + PMV-LEPTO Random : Site + JulDate + Year	45.4
Pregnancy period - Adult females (N= 548)	PMV	GLMM M4	Linear: Pregnancy Random : Site + JulDate + Year	/
	LEPTO	GLMM M5	Linear: Pregnancy Random : Site + JulDate + Year	/
	HSV	GLMM M6	Linear: Pregnancy Random : Site + JulDate + Year	/
Mating period - Adult males (N=388)	PMV	GLMM M7	Linear: Repto Random : Site + JulDate + Year	/
	LEPTO	GLMM M8	Linear: Repto Random : Site + JulDate + Year	/
	HSV	GLMM M9	Linear: Repto Random : Site + JulDate + Year	/

Table S2. Summary of the statistical models used to analyse the bi- and tri-excretion dynamics in *M. francoismoutoui*.

Significant variables are in bold and the double point represent the interaction between two variables. PMV: paramyxovirus, LEPTO: *Leptospira* bacteria, HSV: herpesvirus

Category of individuals (number)	Infectious agent	Model type and number	Full model	% explained deviance
All individuals tested for the three infectious agents (N = 3,784)	PMV-LEPTO	GAMM M10	Smooth: Sampling Period Linear: logSize + Sex-ratio + Age + Sex + Age : Sex Random : Site + JulDate + Year	10.1
	LEPTO-HSV	GAMM M11	Smooth: Sampling Period Linear: logSize + Sex-ratio + Age + Sex + Age : Sex Random : Site + JulDate + Year	13.1
	PMV-HSV	GAMM M12	Smooth: Sampling Period Linear: logSize + Sex-ratio + Age + Sex + Age : Sex Random : Site + JulDate + Year	11.1
	PMV-LEPTO-HSV	GAMM M13	Smooth: Sampling Period Linear: logSize + Sex-ratio + Age + Sex + Age : Sex Random : Site + JulDate + Year	10
Pregnancy period - Adult females tested for the three infectious agents (N = 370)	PMV-LEPTO	GLMM M14	Linear: Pregnancy Random : Site + JulDate + Year	/
	LEPTO-HSV	GLMM M15	Linear: Pregnancy Random : Site + JulDate + Year	/
	PMV-HSV	GLMM M16	Linear: Pregnancy Random : Site + JulDate + Year	/
	PMV-LEPTO-HSV	GLMM M17	Linear: Pregnancy Random : Site + JulDate + Year	/
Mating period - Adult males tested for the three infectious agents (N = 214)	PMV-LEPTO	GLMM M18	Linear: Repro Random : Site + JulDate + Year	/
	LEPTO-HSV	GLMM M19	Linear: Repro Random : Site + JulDate + Year	/
	PMV-HSV	GLMM M20	Linear: Repro Random : Site + JulDate + Year	/
	PMV-LEPTO-HSV	GLMM M21	Linear: Repro Random : Site + JulDate + Year	/

Table S3. Sampling period characteristics and coding for GAMM models.

Year	Sampling period	Sampling dates (MM/DD)	GAMM code
2019	January - February	From 01/24 to 02/27	1
	March - April	From 03/07 to 04/09	2
	April - May	From 04/23 to 05/13	3
	June	From 06/04 to 06/19	4
	July	From 07/16 to 07/30	5
	September	From 09/03 to 09/17	6
	October - November	From 10/21 to 11/07	7
	November - December	From 11/25 to 12/10	8
2020	January - February	From 01/15 to 02/05	9
	March	From 03/02 to 03/20	10
	May - June	From 05/19 to 06/04	11.5
	July	From 07/06 to 07/21	13
	September	From 09/07 to 09/24	14
	October	From 10/14 to 10/29	15
	November - December	From 11/23 to 12/09	16

Table S4. Summary of the statistical models used to analyse recaptured Reunion free-tailed bat data.

Significant variables are in bold.

Category of individuals	Infectious agent	Model Number	Final model
All individuals recaptured at least one time (N = 477)	PMV	M22	Time + Sex + Repro
	LEPTO	M23	Time + Sex + Repro

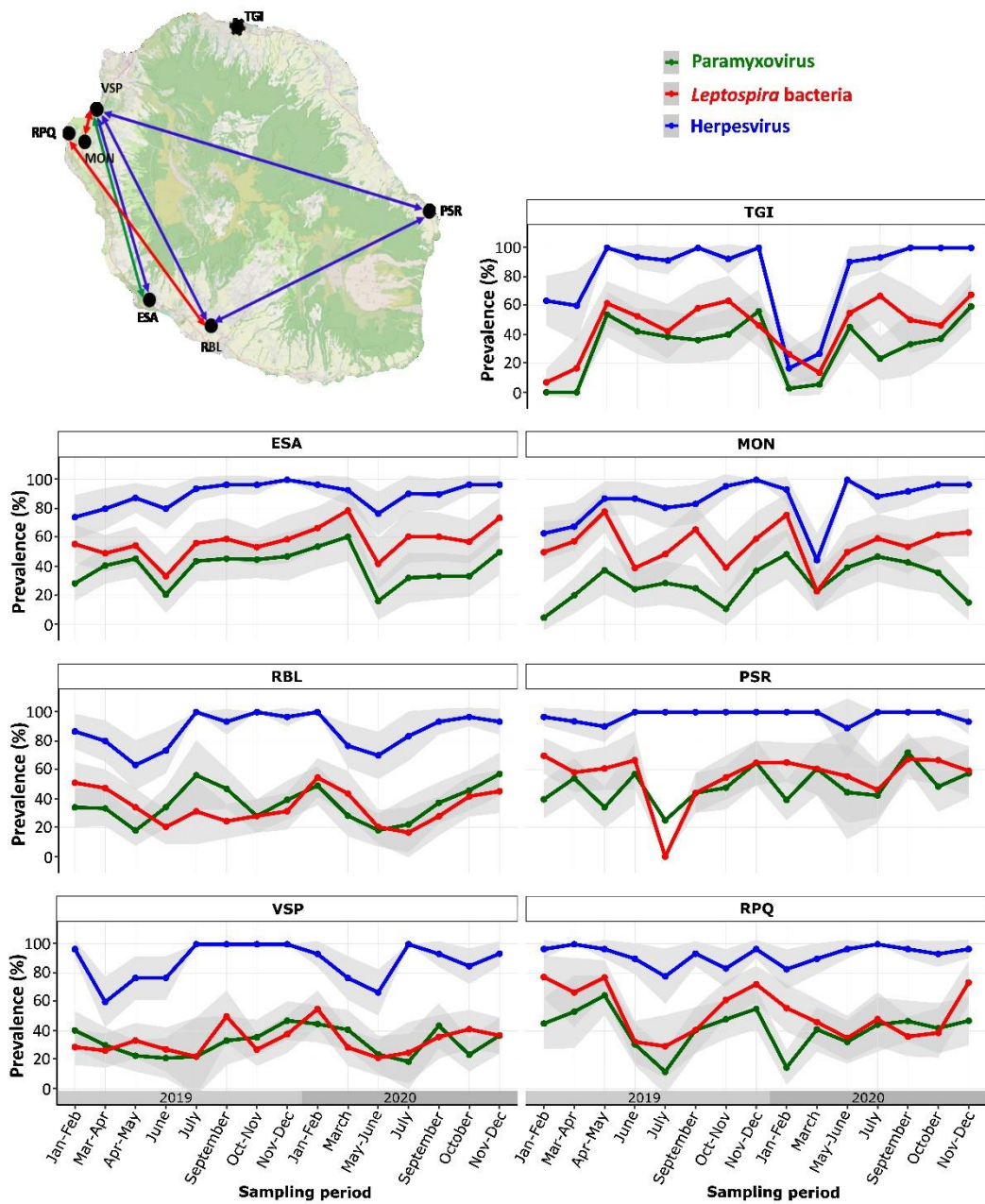


Figure S1. Observed prevalence of paramyxovirus, *Leptospira* bacteria and herpesvirus in the seven roosts analysed for temporal synchrony.

Shaded areas correspond to 95% confidence intervals. Significant pairwise temporal synchrony are indicated by coloured arrows on the map.

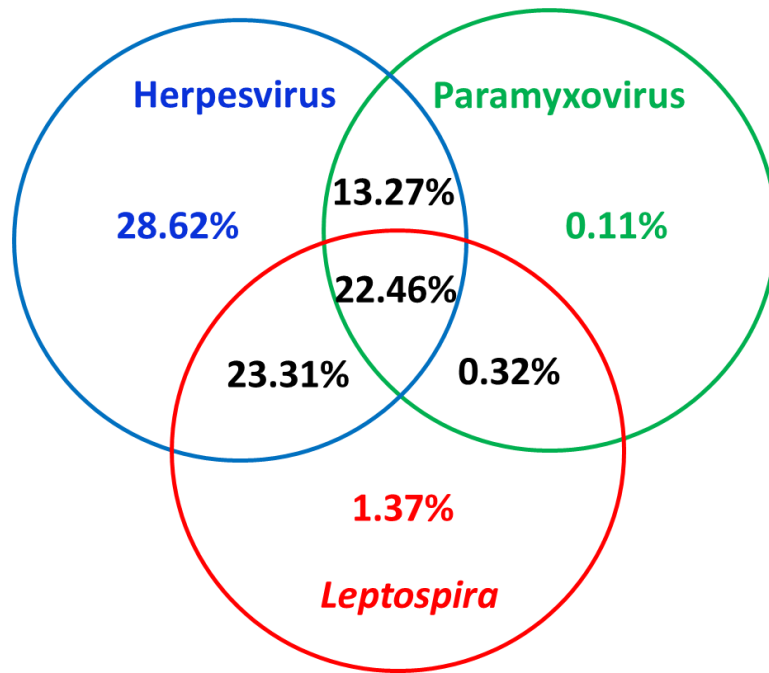


Figure S2. Venn diagram of strict mono-, bi- and tri-excretion in *M. francoismoutoui*

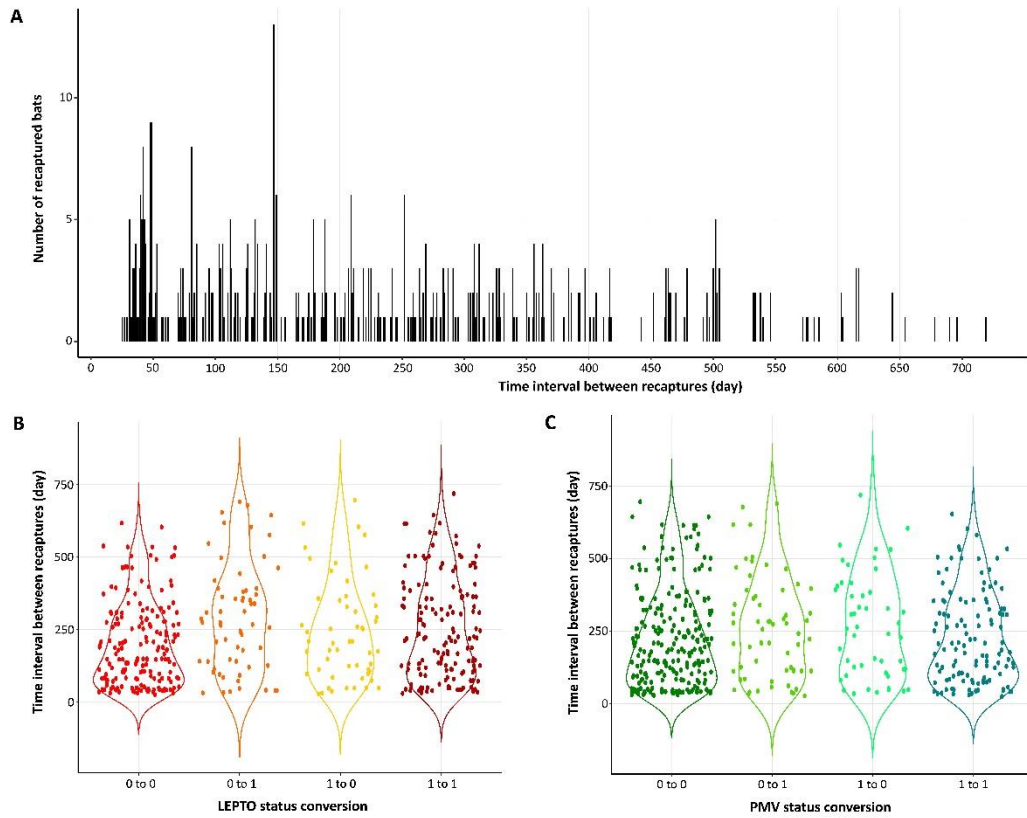


Figure S3. Details of recaptured bats.

(A) Distribution of time interval between recaptures. Probability of changing infectious agent excretion status through time for LEPTO (B) and (C) PMV. Excretion status is coded with 0 for non-excreting and 1 for excreting bats.

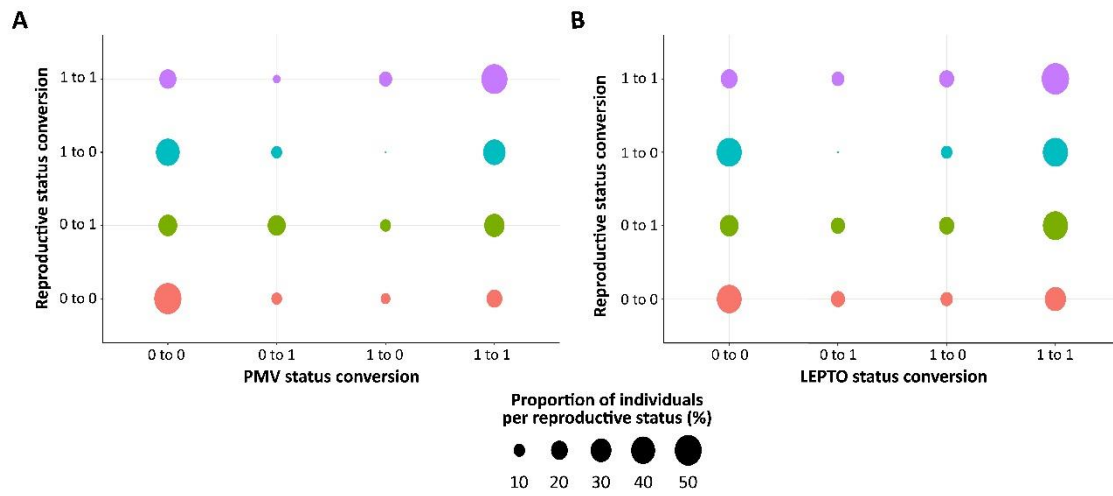


Figure S4. Probability of changing excretion status according to change in reproductive status for (A) PMV and (B) LEPTO. Excretion status is coded with 0 for non-excreting and 1 for excreting bats. Reproductive status is coded with 0 for non active and 1 for active bats.

Annexe 4

Matériels supplémentaires pour l'article 4 :

Aguillon S., Turpin M., Le Minter G., Lebarbenchon C., Hoarau A.O.G., Mavingui P. & Dietrich M. Bat things comes in threes: within-host dynamics of herpesvirus triple infection in bats.

Table S1. Summary of the statistical models used to analyse herpesvirus genetic diversity.

Complete model include all tested variables, with interaction indicated by the double point and significant effect being in bold.

Response variable	Scale of analysis	Distribution	N	Model Number	Complete model
Number of strain	All individuals	Poisson	121	M1	Fixed: Age + Sex + Age:Sex Random: Roost + Period + Jday + Bat's ID
Hill diversity (strain level)	All individuals	Gaussian (log)	121	M2	Fixed: Age + Sex + Age:Sex Random: Roost + Period + Jday+ Bat's ID
Hill diversity (strain level)	Adult bats Pregnancy and mating periods	Gaussian (log)	96	M3	Fixed: Repro + Sex + Repro:Sex Random: Roost + Period + Jday+ Bat's ID
Alpha prevalence	Adult bats Pregnancy and mating periods	Binomial	96	M4	Fixed: Repro + Sex + Repro:Sex + Beta + Gamma Random: Roost + Period + Jday + Bat's ID
Beta prevalence	Adult bats Pregnancy and mating periods	Binomial	96	M5	Fixed: Repro + Sex + Repro:Sex + Alpha + Gamma Random: Roost + Period + Jday+ Bat's ID
Gamma prevalence	Adult bats Pregnancy and mating periods	Binomial	96	M6	Fixed: Repro + Sex + Repro:Sex + Alpha + Beta Random: Roost + Period + Jday
Conversion of subfamily	Recaptured bats	Multinomial	70 (capture events)	M7	Time

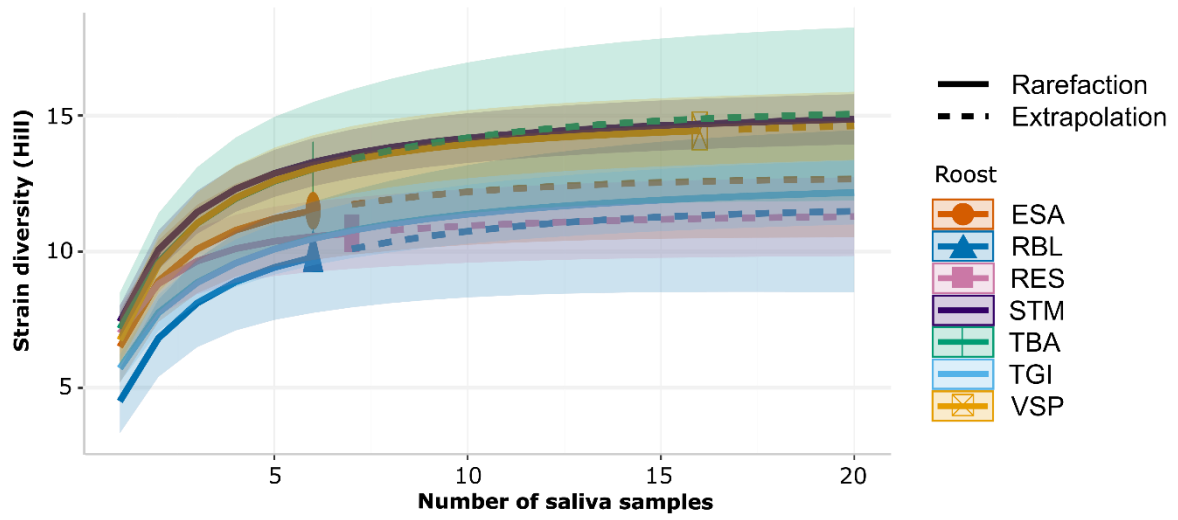


Figure S1. Accumulation curves of strain diversity (Hill, $q = 1$) according to the number of saliva sample collected per roost.

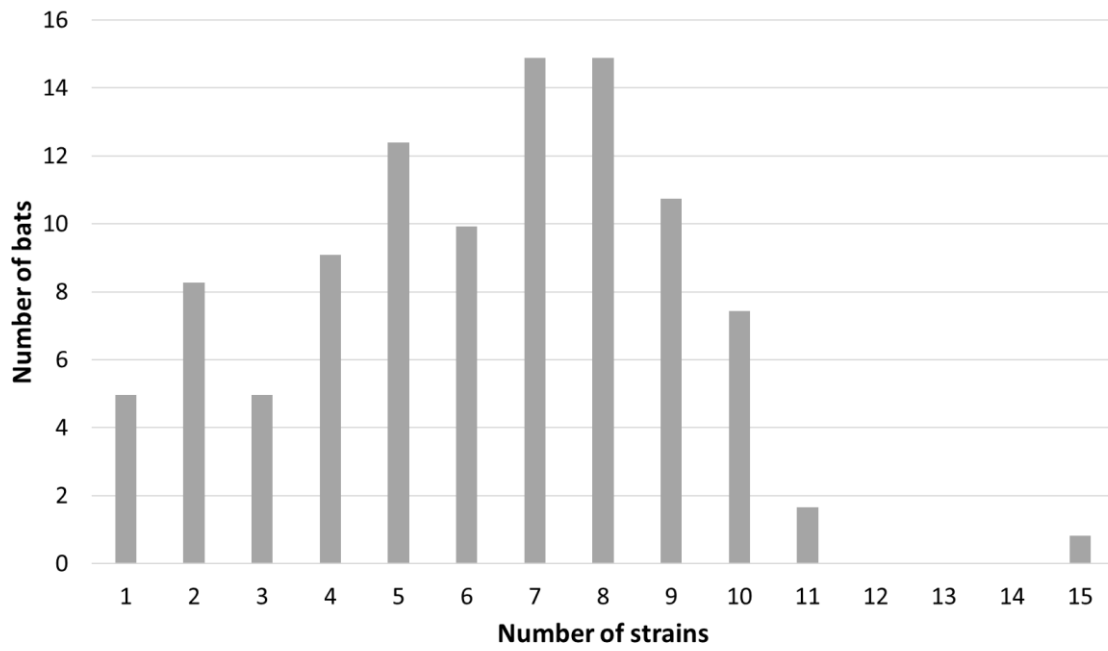


Figure S2. Number of strains detected in each Reunion free-tailed bats.

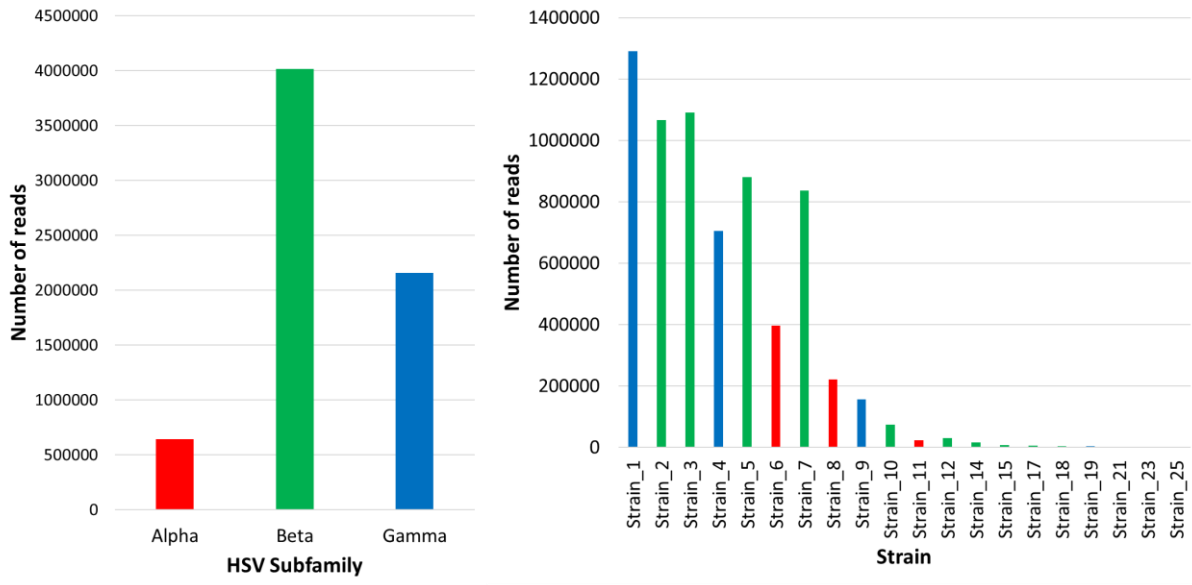


Figure S3. Abundance of the three herpesvirus (HSV) subfamilies and strain in Reunion free-tailed bats.

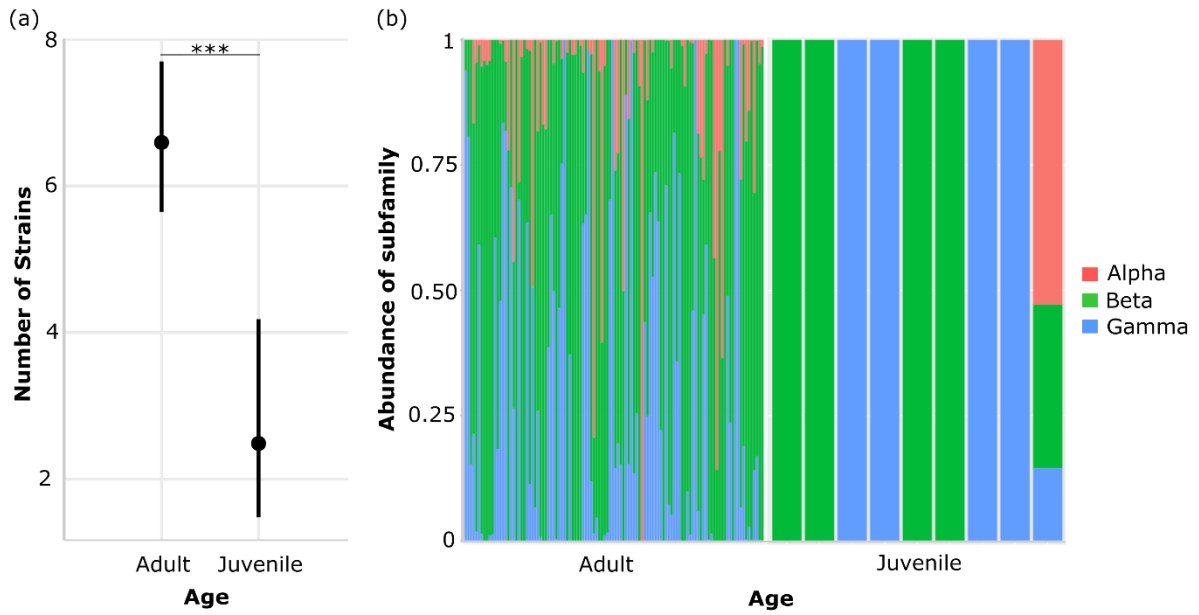


Figure S4. Diversity and abundance of herpesvirus strain in Reunion free-tailed bats according to bat's age.

(a) Strain number predicted by model M1. *** $p < 0.001$. (b) Composition of subfamilies in each bats, with one column corresponding to one bat.

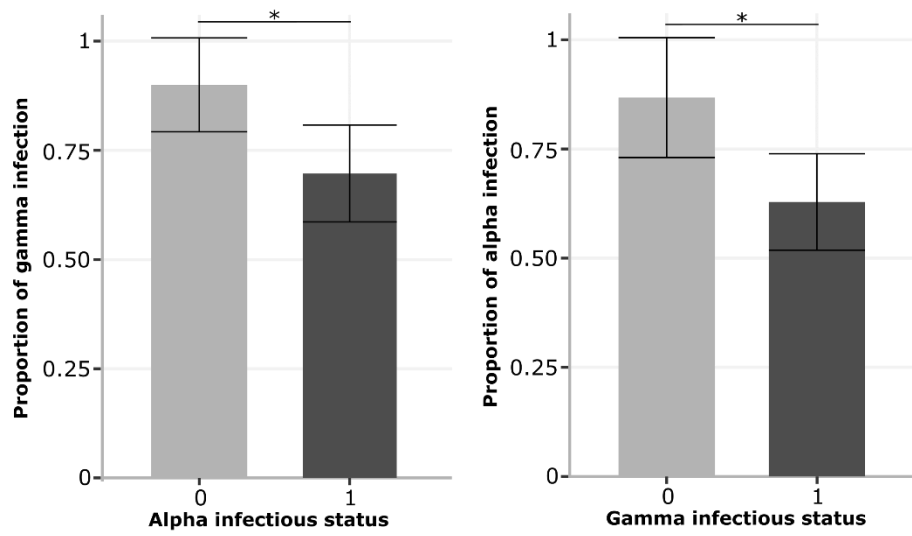


Figure S5. Negative interaction between alpha- and gamma-herpesvirus in Reunion free-tailed bats.

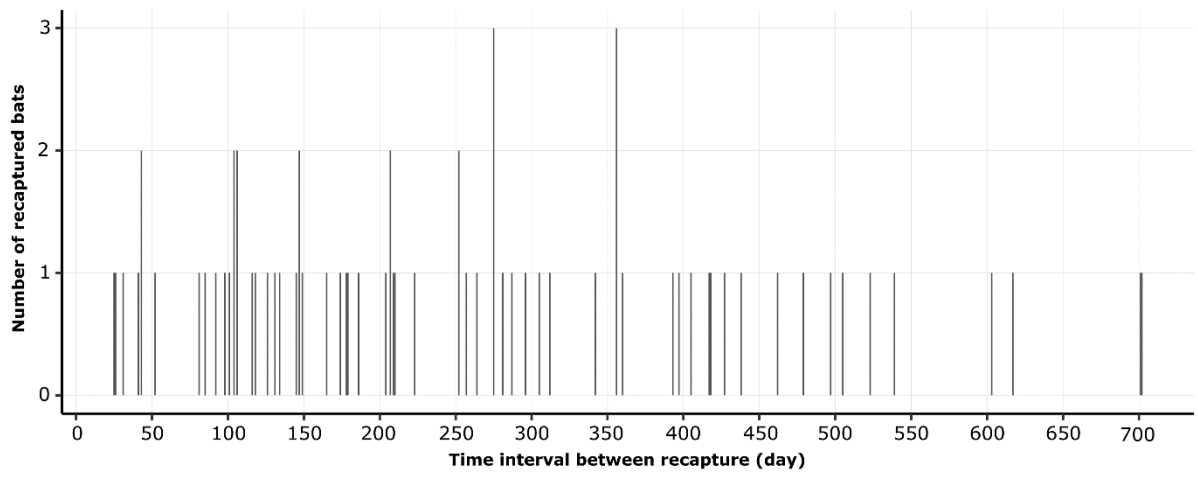


Figure S6. Distribution of time interval between recaptures.

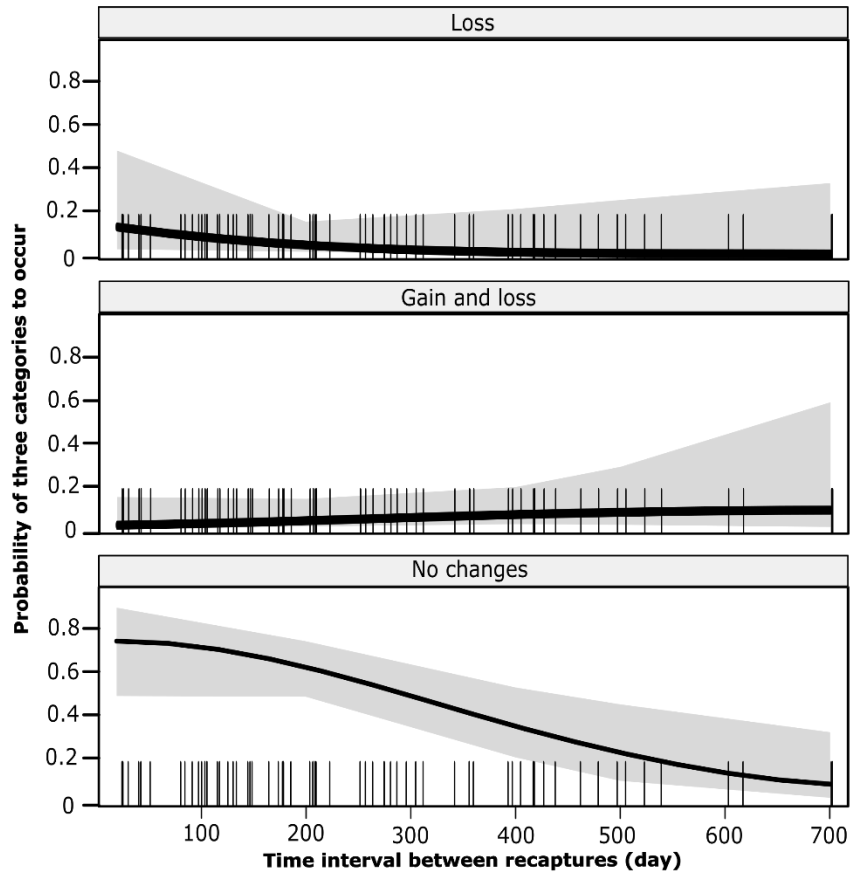


Figure S7. Probability of the three categories of excretion status change, with 95% confidence interval in grey. Probability of the fourth category (gain) is presented in Figure 3.

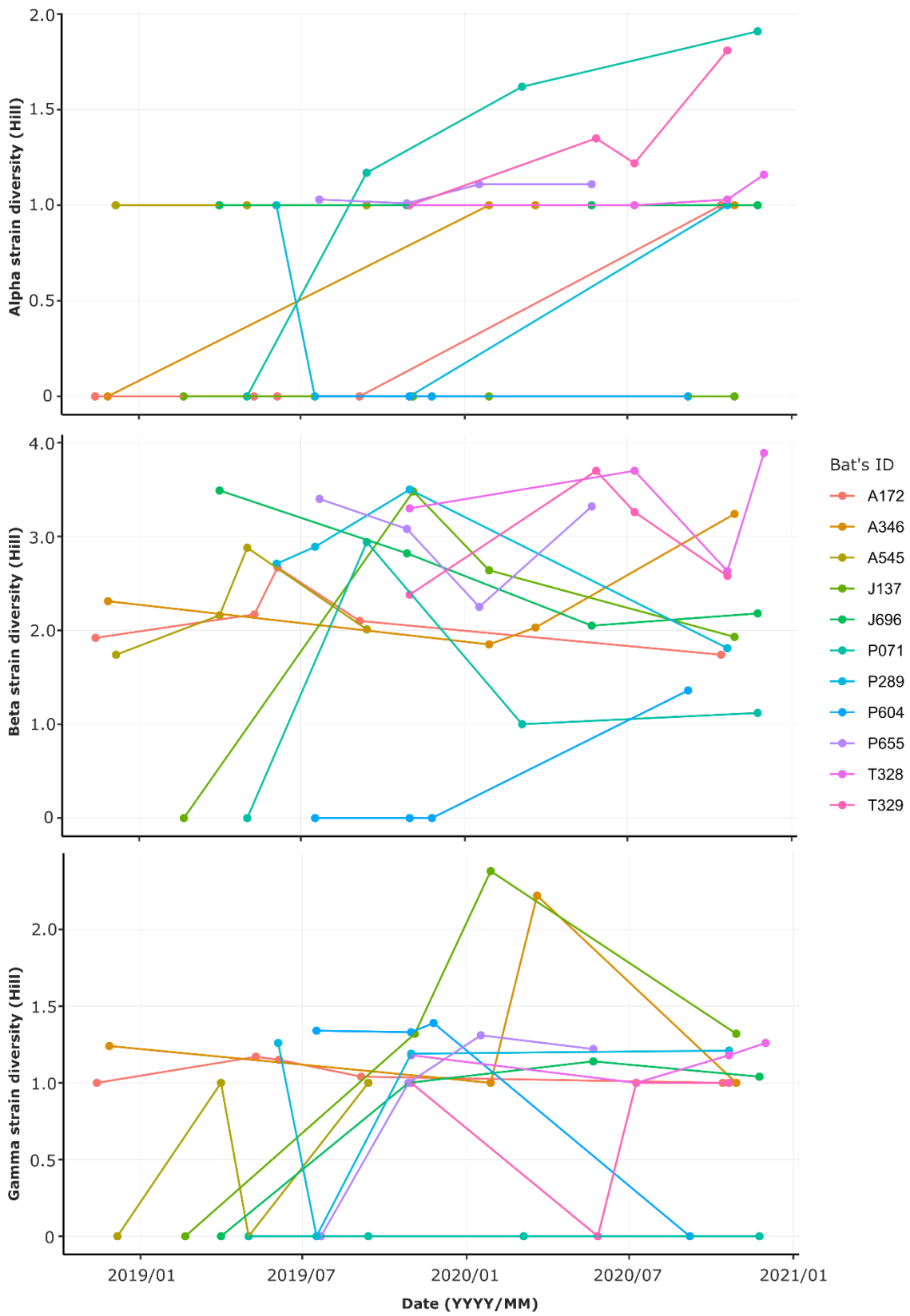


Figure S8. Intra-subfamily diversity (Hill, $q = 1$) of HSV strains over time.

Annexe 5

Résumé de l'article en préparation :

Toshkova N., Aguillon S., Turpin M., Lebarbenchon C. & Dietrich M. How urban-dwelling bats cope with urbanization? Sex-dependent plasticity of stress, microbiome and pathogen excretion.

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How urban-dwelling bats cope with urbanization?

Sex-dependent plasticity of stress, microbiome and pathogen excretion

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ABSTRACT

In our rapidly urbanizing world, stress and infection response patterns of wildlife remains poorly understood. Bats are known to be very sensitive to habitat destruction and land use change, yet some of them well thrive in human-modified landscapes. Here, we used the Reunion free-tailed bat, *Mormopterus francoismoutoui*, to analyze how urban-dwelling bats cope with urbanization. We assessed urinary cortisol levels, bacterial microbiome and the shedding of both *Leptospira* bacteria and paramyxovirus in 412 urine samples across 11 roosts throughout Reunion Island, encompassing a gradient of urbanization and agricultural levels. After controlling for natural variation of cortisol levels associated to circadian rhythm, age and capture-induced stress, we found evidence that bats roosting in agricultural areas were in better condition and had lower cortisol levels, while in urbanized areas, only females responded positively. We showed that reduced stress was globally associated with increased urinary microbiome diversity. Our results also revealed pathogen- and sex-specific association between stress and infection. Indeed, stressed-females excreted more frequently paramyxovirus and higher load of *Leptospira* bacteria in urine.

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