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ECOLOGIE EVOLUTIVE DE LA TRANSMISSION MATERNELLE D'ANTICORPS

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Résumé

Chez les vertébrés, la réponse immunitaire acquise représente un mécanisme sophistiqué de réponse face aux parasites dont l'une des particularités est la possibilité qu'il offre aux mères de transférer certains de ses effecteurs à leurs nouveau-nés. Pourtant, malgré un intérêt croissant pour les effets maternels, les déterminants écologiques et évolutifs du transfert d'anticorps maternels n'ont pas encore été beaucoup étudiés. L'analyse d'un cadre théorique spécialement développé pour inclure le transfert transgénérationnel d'immunité montre que l'évolution de la capacité à transférer une immunité temporaire aux jeunes dépend des caractéristiques de l'hôte et du parasite. En particulier, l'augmentation de l'espérance de vie de l'hôte favorise l'évolution de réponses immunitaires acquises, et la protection conférée par ces réponses est aussi supposée durer plus longtemps chez les hôtes longévifs. En accord avec cette prédiction, une étude de vaccination transgénérationnelle chez une espèce d'oiseau de mer longévive a permis de mettre en évidence une demi-vie des anticorps maternels particulièrement longue. Les conditions sociales sont aussi un élément clé, et chez une espèce de mammifère, j'ai pu montrer qu'elles permettent un élargissement du répertoire d'anticorps maternels. Le transfert d'anticorps maternels est aussi à même de modifier les dynamiques épidémiologiques et pourrait présenter un atout non négligeable si la vaccination était utilisée en conservation. Enfin, ce mécanisme pourrait être mis à profit pour estimer l'exposition des mères, et ainsi inférer la dispersion entre différentes zones d'habitat.

Mots clés : Effets maternels ; Epidémiologie évolutive ; Immuno-écologie ; *Interactions hôte-parasite ; Transfert d'anticorps maternels ;*

Title: ***Evolutionary ecology of the maternal transmission of antibodies***

Summary

In vertebrate species, acquired immune response represents a sophisticated protection mechanism against parasites that has the particularity of enabling mothers to transmit part of its effectors to their newborns. Yet, despite an increasing interest in maternal effects, ecological and evolutionary determinants of the transfer of maternal antibodies remain poorly studied. The analysis of a theoretical framework specially developed to include a transgenerational transfer of immunity show that the evolution of an ability to temporarily protect offspring depends on the characteristics of both the host and the parasite. In particular, increasing the life span of the host favors the evolution of acquired immune responses and increases the duration of the protection offered by these mechanisms. Accordingly, a transgenerational vaccination study in a long-lived seabird revealed a particularly long half-life of maternal antibodies. Social conditions also proved important in a mammal species as they can allow for the broadening of the repertoire covered by maternal antibodies. The transfer of maternal antibodies could also modify epidemiological dynamics and could bbe an interesting asset if vaccination was used as a conservation tool. Finally, this mechanism could be used to estimate the exposure of mother and thus infer the dispersal rate between different habitat patches.

Keywords: Evolutionary epidemiology; Host-parasite interactions; Immuno-ecology; Maternal effects; Transfer of maternal antibodies

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Une thèse quand ça se termine, en fait, c'est un peu triste. Mais c'est juste le début : vivement la suite !!

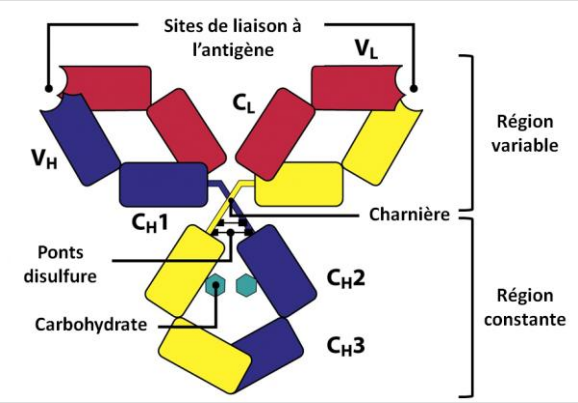
Sommaire

INTRODUCTION

Un effet maternel se définit comme l'influence causale que le génotype et/ou le phénotype de la mère peut avoir sur le phénotype de son jeune (Wolf & Wade 2009). La première mention du terme « effets maternels » est relativement ancienne (Dobzhansky 1935), mais c'est le développement de questionnements théoriques (Kirkpatrick & Lande 1989) et empiriques (Riska *et al.* 1985; Roach & Wulff 1987) sur les effets maternels qui a conduit à une augmentation de l'intérêt pour les implications écologiques et évolutives de ce phénomène au cours des vingt dernières années(e.g. Bernardo 1996; Mousseau & Fox 1998; Kruuk *et al.* 2000; Qvarnström & Price 2001; Räsänen & Kruuk 2007; Maestripieri & Mateo 2009). En réalité, l'un des premiers mécanismes décrit impliquant une influence du phénotype de la mère sur celui de son jeune est celui du transfert de molécules impliquées dans la protection du jeune contre les maladies, le transfert d'anticorps maternels (Ehrlich 1892). Les mécanismes impliqués dans ce transfert ainsi que la nature des molécules immunitaires transférées sont décrits depuis longtemps (e.g. Brambell & Halliday 1955; Halliday 1955; Brambell 1970). En revanche, l'étude du transfert d'anticorps maternels a peu bénéficié de l'essor des travaux sur les effets maternels et l'écologie et l'évolution de ce mécanisme restent peu étudiées (Gasparini *et al.* 2001; Grindstaff *et al.* 2003).

Pourtant, les conditions rencontrées par un jeune dans les premiers moments de sa vie influencent fortement sa valeur sélective (Lindström 1999). Les parasites, en particulier, font partie des pressions majeures influençant la valeur sélective individuelle dans les populations naturelles (Grenfell & Dobson 1995; Tompkins *et al.* 2002), et leur effet est potentiellement encore renforcé chez les jeunes vertébrés dont la réponse immunitaire n'est pas totalement fonctionnelle à la naissance (Frank 2002). Si la défense face aux parasites combine d'une manière générale de nombreux mécanismes (voir Schmid-Hempel 2011pour une revue), chez les vertébrés, elle repose en grande partie sur l'induction d'une réponse immunitaire (Frost 1999) combinant des mécanismes innés rapides généralement non spécifique et des mécanismes acquis d'expression plus tardive mais aussi plus spécifiques du parasite (Wakelin 1996). Une partie de cette réponse immunitaire acquise se traduit par la production de composés immunoactifs, les anticorps ou immunoglobulines (Ig, voir encadré 1), qui vont pouvoir être en partie transférés à la génération suivante et ainsi fournir une protection temporaire aux jeunes durant la phase critique de maturation de leur propre système immunitaire (Wallach *et al.* 1992; Smith *et al.* 1994; Carlier & Truyens 1995; Al-Natour *et al.*

Encadré 1 : Les immunoglobulines, effecteurs de l'immunité humorale



Représentation schématique d'une immunoglobuline de mammifère (adapté de Murphy et al. 2008)

Les immunoglobulines (Ig) font partie des protéines les plus importantes impliquées dans la réponse immunitaire et sont produites par des lymphocytes B après rencontre d'un antigène. Classiquement, leur structure de base comprend une région constante constituée de deux chaînes lourdes (en bleu et jaune dans la figure) et une région variable constituée de deux chaînes légères (en rouge dans la figure). La région variable interagit avec l'antigène, alors que la région constante est impliquée dans les relations avec les effecteurs cellulaires.

Chez les mammifères, la classification des Igs en fonction de leur structure et de leur fonction permet de distinguer cinq classes : IgM, IgG, IgA, IgE et IgD. Seule l'IgG, effecteur principal de la réponse immunitaire acquise, peut être transférée via le placenta (Mix *et al.* 2006) mais d'autres classes, notamment les IgA et E, sont rencontrées dans le colostrum et le lait. Chez les oiseaux (Davison *et al.* 2008), l'effecteur principal de la réponse est l'IgY (qui représente un équivalent fonctionnel de l'IgG mammalienne) et les IgM et IgA sont retrouvées avec des structures et fonctions identiques. En revanche, les IgE et IgD sont absentes, leurs rôles étant assurés respectivement par des IgY et des IgM.

Il faut enfin signaler que les immunoglobulines sont les protéines sériques présentant la plus longue demi-vie plasmatique. Elles sont en effet protégées des mécanismes de catabolisme habituels par des récepteurs (Brambell *et al.* 1964). Ces récepteurs, le FcRN chez les mammifères (Ghetie & Ward 2000) et le FcRY chez les oiseaux (West *et al.* 2004), se lient à la région constante des Igs et empêchent leur dégradation (Roopenian & Akilesh 2007).

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2004; Kariyawasam *et al.* 2004; Pravieux *et al.* 2007) avant d'être éliminé du système circulatoire du juvénile par catabolisme.

Ces anticorps peuvent atteindre le système circulatoire du jeune avant ou après la naissance. Chez les espèces ovipares et en particulier chez les oiseaux où le transfert d'anticorps a été bien étudié, les anticorps sont accumulés dans le vitellus au cours de l'oviposition et l'absorption par le jeune débute peu de temps avant l'éclosion (Kowalczyk *et al.* 1985). Des anticorps maternels sont aussi présents dans l'albumen (Rose *et al.* 1974; Hamal *et al.* 2006) mais ils ne sont que pas ou peu absorbés par le jeune. Chez les mammifères, les anticorps peuvent provenir d'un passage à travers le placenta au cours de la gestation ou d'un passage à travers la barrière intestinale au cours de l'allaitement (Baintner 2007). Au cours de la lactation, deux phases successives doivent être distinguées. Tôt après la naissance, les Igs représentent les protéines principales de la sécrétion que l'on appelle alors colostrum. Il se produit ensuite une transition vers le lait qui contient toujours des Igs mais en proportion

moindre. (Wheeler *et al.* 2007). L'importance relative du transfert pré- et post-natal est très variable chez les mammifères, notamment du fait d'une variabilité dans la structure anatomique du placenta (Chucri *et al.* 2010).

Chez les mammifères comme chez les oiseaux, le transfert d'anticorps maternels implique un récepteur qui se lie spécifiquement aux Igs. Chez les mammifères, le FcRn (« Neonatal Fc receptor ») permet spécifiquement le passage des immunoglobulines G (IgG) à travers le placenta et à travers la barrière intestinale (Ghetie & Ward 2000), à l'exception des ongulés chez lesquels le colostrum est absorbé très rapidement par des mécanismes d'endocytose massive (Baintner 2007). Chez les oiseaux, les travaux menés chez le poulet ont montré que c'est l'absorption spécifique des IgY (l'équivalent aviaire des IgG des mammifères) à partir du vitellus par le jeune qui est dépendante d'un récepteur d'identification plus récente, nommé par analogie FcRY (West *et al.* 2004). L'accumulation des anticorps dans ce vitellus est en revanche soit passive soit dépendante d'un autre récepteur, moins sélectif, comme en témoigne la possibilité de transfert d'Ig de mammifères injectés à des oiseaux (Morrison *et al.* 2002). Quoi qu'il en soit, les récepteurs FcRn et FcRY limitent les sous-types d'anticorps qui peuvent atteindre la circulation du jeune : seules certaines IgG (chez les mammifères) et IgY (chez les oiseaux) ont cette possibilité. Il est aussi intéressant de noter que chez les oiseaux et chez les mammifères, les récepteurs FcRn et FcRY ne jouent pas un rôle seulement dans le transfert d'anticorps maternels mais sont aussi impliqués dans la régulation du catabolisme des Igs (Roopenian & Akilesh 2007; Tesar *et al.* 2008). Bien que remplissant des fonctions extrêmement similaires, ces deux récepteurs appartiennent à des familles de molécules très différentes, ce qui laisse penser à la possibilité d'une convergence évolutive impliquant les mécanismes de transfert d'anticorps maternels.

Les anticorps transférés présentent aussi des effets similaires chez les jeunes. A court terme, les anticorps maternels ont la capacité d'empêcher l'activation du système immunitaire du jeune (Glezen 2003; Staszewski *et al.* 2007a). Cette réponse permet ainsi au jeune d'éviter de détourner une partie de ses ressources pour produire une réponse immunitaire, ce qui peut expliquer l'amélioration de la croissance des jeunes soumis à une pression parasitaire et ayant reçu des anticorps maternels (Heeb *et al.* 1998; Buechler *et al.* 2002; Uller *et al.* 2006; Grindstaff 2008). En revanche, l'effet bloquant des anticorps maternels empêche le développement de la réponse mémoire du jeune, ce qui doit notamment être pris en compte pour établir des plans de vaccination contre des pathogènes d'intérêt vétérinaire (e.g. Naqi *et al.* 1983; Griot *et al.* 2004) ou humain (e.g. Mooi & de Greef 2007; Metcalf *et al.* 2011).

Quand la décroissance des anticorps maternels est en cours, un niveau intermédiaire de protection par les anticorps maternels pourrait fournir un environnement optimal pour la mise en place d'une réponse mémoire à moindres coûts pour le jeune (Zinkernagel 2001, 2003; Navarini *et al.* 2010). Les anticorps maternels pourraient aussi avoir un effet éducationnel sur le système immunitaire après avoir disparu de la circulation du jeune : lorsque l'individu est exposé à un parasite contre lequel il a reçu des anticorps maternels, la réponse immunitaire serait alors plus forte (Lemke *et al.* 2003; Grindstaff *et al.* 2006; Reid *et al.* 2006; Fink *et al.* 2008). Les anticorps, présents dans le nouveau-né pendant la phase critique de maturation du système immunitaire, pourraient en effet avoir un rôle d'imprégnation immunologique sur les différents composants du système immunitaire (Lemke *et al.* 2004; Lemke *et al.* 2009). Le transfert d'anticorps maternels représente donc un mécanisme important dans les stratégies de défense face aux parasites, et à ce titre, ses déterminants écologiques et évolutifs, pour le moment peu étudiés (Grindstaff *et al.* 2003; Boulinier & Staszewski 2008), méritent une attention particulière.

D'un point de vue théorique, les parasites attirent d'une manière générale beaucoup d'attention en tant que force influençant la dynamique des populations d'hôtes (Anderson & May 1978; May & Anderson 1978) mais aussi comme pression de sélection importante influençant l'évolution des traits d'histoire de vie de leurs hôtes (e.g. Gandon *et al.* 2002; Altizer *et al.* 2003). La réponse immunitaire en particulier est considérée comme un facteur épidémiologique d'importance (Anderson & May 1991) et son évolution et sa coévolution avec d'autres mécanismes de résistance aux parasites ont été largement étudiées par des méthodes de modélisation allant de la génétique des populations (e.g. Harding *et al.* 2005) à la dynamique adaptative (van Baalen 1998; Boots & Bowers 1999, 2004; Miller *et al.* 2007; voir Boots *et al.* 2009 pour une revue). L'une des informations principales que l'on peut retirer des études de dynamique adaptative, c'est que la prise en compte des rétrocontrôles épidémiologiques est indispensable à la bonne description de l'évolution et de la coévolution des mécanismes de résistance aux parasites. Le transfert d'anticorps maternels est susceptible de modifier l'accessibilité des jeunes pour le parasite ainsi que la proportion d'individus sensibles dans la population. Pourtant, l'importance potentielle de ce mécanisme de protection a été jusqu'à présent ignorée dans les études théoriques. Mettre en place un cadre théorique autorisant la prise en compte d'un transfert transgénérationnel d'immunité et plusieurs autres mécanismes de résistance aux parasites a donc constitué le premier objectif de cette thèse. A partir de ce modèle théorique, à l'aide du « théorème de la génération suivante » ("Next

generation theorem", NGT; van den Driessche & Watmough 2002; Hurford *et al.* 2010), un critère d'invasion a permis de s'intéresser à l'évolution du transfert d'anticorps maternels et à sa coévolution avec d'autres mécanismes de défenses face aux parasites. Enfin, ce critère d'invasion a pu être utilisé pour obtenir des prédictions sur l'évolution d'autres traits directement liés à l'efficacité des réponses immunitaires (maternelles et acquises) comme la persistance de la protection conférée par les anticorps.

La stratégie de résistance aux parasites d'un individu peut ainsi être vue comme le résultat de processus d'optimisation résultant des bénéfices apportés par cette stratégie et des coûts imposés en termes écologiques ou de compromis évolutifs entre traits d'histoire de vie (Sheldon & Verhulst 1996). La mise au point de techniques immunologiques adaptées aux populations naturelles a permis le développement des approches d'immuno-écologie (Martin *et al.* 2011) visant à comprendre la variabilité des stratégies de défense face aux parasites ainsi que les conséquences en termes de valeur sélective de cette diversité (Graham *et al.* 2011). Le transfert d'anticorps maternels fait partie de ces stratégies, et en tant que tel, l'allocation d'anticorps maternels aux jeunes pourrait présenter des patrons de variabilité intéressants. Cependant, malgré l'intérêt croissant porté à cet effet maternel (voir les revues par Grindstaff *et al.* 2003; Boulinier & Staszewski 2008; Hasselquist & Nilsson 2009), la variabilité de ce trait entre différentes espèces n'a reçu que peu d'attention (Addison *et al.* 2009). Un second objectif de cette thèse a été d'aborder certains des aspects de cette variabilité, en relation avec les mécanismes mis en jeu. L'existence de compromis évolutifs impliquant le transfert d'anticorps maternels présuppose l'existence de coûts associés à ce mécanisme. En particulier, des coûts énergétiques ont été associés à la production d'une réponse immunitaire chez l'adulte (Lochmiller & Deerenberg 2000; Norris & Evans 2000) et, pour compenser le drain important exercé par le jeune, le transfert d'anticorps maternels implique la production *de novo* d'anticorps dirigés contre les parasites qui constituent l'histoire parasitaire de la mère. Par exemple, chez la poule, 100 à 200 mg d'Ig sont retrouvés dans le vitellus, ce qui correspond à 10 à 20% de la quantité d'anticorps circulants de la femelle (Kowalczyk *et al.* 1985). De plus, l'expression et le maintien des récepteurs nécessaires au transfert d'anticorps sont aussi probablement à l'origine de coûts. Le transfert d'anticorps maternels est donc probablement coûteux, et en tant que tel son évolution pourrait conduire à des stratégies d'allocations différentes entre espèces à traits d'histoire de vie contrastés (Ricklefs & Wikelski 2002). En particulier, les espèces longévives pourraient présenter une persistance des anticorps maternels prolongés, adaptée à la fois à la durée de

leur période d'élevage généralement plus longue et à la mise en place d'un système immunitaire acquis supposé plus fort du fait des bénéfices des réponses mémoires (Lee 2006). Une expérience utilisant la vaccination pour contrôler l'exposition des mères (Staszewski & Boulinier 2004) dans une espèces d'oiseau marin longévive m'a permis de m'intéresser à cette question dans le cadre de cette thèse.

Par ailleurs, les bénéfices liés au transfert d'anticorps maternels vont dépendre de l'environnement parasitaire que le jeune va rencontrer pendant les premiers moments de sa vie. Ainsi, la vie en groupe pourrait favoriser le transfert d'anticorps maternels. En effet, la socialité ou la colonialité augmentent la transmission des parasites en augmentant la densité locale des hôtes (voir encadré 2), et les individus d'un même groupe rencontrent des conditions parasitaires plus similaires (Delahay *et al.* 2000). Dans les espèces sociales ou coloniales, les anticorps transférés par les mères seraient donc susceptibles de fournir une protection potentiellement importante au jeune. De plus, chez les mammifères sociaux, les jeunes peuvent obtenir du lait de plusieurs mères du groupe social et ainsi à la fois renforcer quantitativement le répertoire d'anticorps obtenus de leur mère biologique mais aussi potentiellement élargir ce répertoire en obtenant des anticorps contre des pathogènes rares rencontrés seulement par certains membres du groupe social (Roulin & Heeb 1999). Cependant, ce transfert croisé d'anticorps entre jeunes et femelles non liés biologiquement n'a jamais été mis en évidence empiriquement. Une expérience en laboratoire chez une espèce de petit mammifère sociale m'a permis de considérer ce mécanisme.

L'importance du transfert d'anticorps maternels dans les dynamiques épidémiologiques a été mis en évidence dans des études théoriques (Fouchet *et al.* 2006; Fouchet *et al.* 2007; Fouchet *et al.*

Encadré 2 : Vie en groupe et parasitisme

Vivre en groupe est une stratégie relativement répandue dans le monde animal. Les associations peuvent être de longue durée chez les espèces sociales, ou limitées à une période donnée du cycle annuel chez des espèces qui se concentrent temporairement sur des colonies de reproduction ou des zones d'hivernage. Ces deux modes de vie ont toutefois en commun de favoriser les contacts entre individus et ainsi d'augmenter les possibilités de transmission des parasites (Loehle 1995; Altizer *et al.* 2003).

Réduire les conséquences du parasitisme est donc critique dans ces modes de vie. La migration est ainsi souvent évoquée comme un moyen d'éviter une partie du risque parasitaire rencontré sur les zones à fortes densités (Altizer *et al.* 2011), que ce soient des colonies de reproduction ou des zones d'hivernage. De la même façon, les espèces sociales ont développé des mécanismes comportementaux sophistiqués d'évitement des parasites (Hart 1990). En revanche, les conséquences évolutives de ces modes de vie sur le système immunitaires ont été très peu étudiées (Schmid-Hempel 2011).

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2008) mais aussi évoqué dans les populations naturelles (Kallio *et al.* 2006; Kallio *et al.* 2010). Dans de nombreuses populations animales, la reproduction intervient de façon synchrone, en particulier dans les espèces à reproduction coloniale. D'un point de vue épidémiologique, cela se traduit par un pic de productions de juvéniles généralement considérés comme sensibles et dont l'importance dans les dynamiques épidémiologiques est largement évoquée (Roberts & Kao 1998; Keeling & Rohani 2008). La prise en compte du transfert d'anticorps maternels pourrait ainsi profondément modifier le niveau d'immunité de groupe (Anderson & May 1985) à une période cruciale après la naissance des jeunes, lorsque les conditions écologiques seraient les plus favorables à la circulation des parasites. Un troisième objectif de cette thèse a été d'aborder ces aspects. Par des approches théoriques, je me suis ainsi intéressé aux conséquences que le transfert d'anticorps maternels pouvait avoir sur la récurrence d'épidémies, en association avec la synchronie de la période de reproduction. Lorsqu'un pathogène menace la survie d'une population (Daszak *et al.* 2000), la manipulation des niveaux de protection des mères par une stratégie de vaccination pourrait permettre de protéger les juvéniles et ainsi maintenir une taille de population viable. Une approche théorique a permis une exploration de cette question dans un contexte de biologie de la conservation. Enfin, la relative facilité d'accès aux anticorps maternels pourrait permettre d'obtenir des informations sur l'historique d'exposition aux parasites des mères. Un cadre théorique a été développé et pourrait permettre, à terme, d'utiliser ces données immunologiques pour obtenir des informations sur la dispersion entre différentes populations ou entre différents groupes à plus fine échelle.

Ce document représente donc une synthèse des travaux que j'ai effectués sur différents aspects de l'évolution, l'écologie et l'éco-épidémiologie de la transmission maternelle d'anticorps, à l'interface entre des approches théoriques et empiriques. Les résultats sont discutés lors de leur présentation, et des perspectives sont évoquées dans une dernière partie de ce document. Les 6 manuscrits présentés en annexe et auxquels il est fait référence dans cette synthèse présentent les résultats obtenus plus en détail.

MODELE EPIDEMIOLOGIQUE

Dans la première partie de cette thèse, un modèle épidémiologique a été utilisé. Ce modèle théorique considère quatre catégories d'individus qui se reproduisent de manière asexuée et qui sont exposés à plusieurs souches de parasite, chacune caractérisée par sa force d'infection. Les hôtes peuvent être sensibles (S), infectés par soit le parasite 1 (I_1) soit le parasite 2 (I_2), résistants et ayant développé leur immunité (respectivement R_1 ou R_2), et enfin protégés par des anticorps maternels (respectivement M_1 ou M_2). Ces individus M perdent leur protection à un taux δ_M et deviennent alors sensibles. Ils peuvent aussi s'infecter avec la souche de parasite contre laquelle leur mère n'a pas fourni d'anticorps, en fonction des forces d'infection h_1 et h_2 et d'un paramètre χ qui mesure l'immunité croisée conférée par les anticorps maternels. Les individus sensibles peuvent eux s'infecter à un taux qui dépend à la fois de la force de l'infection du parasite (h_1 ou h_2) et de la capacité de l'hôte à réduire la probabilité d'infection en évitant d'entrer en contact avec le parasite, π. Les individus infectés peuvent guérir à un taux γ décrivant la réponse immunitaire acquise mais subissent un excédent de mortalité liée à la virulence du parasite α. Enfin les individus guéris (R) conservent cette protection pour une durée déterminée par le taux de perte d'immunité, δ_R. Tous les individus, peu importe leur statut épidémiologique, subissent une mortalité naturelle μ. Pour simplifier, le parasite ne se transmet que de manière horizontale et la coinfection des individus R est impossible. Ces transitions (à l'exception de la mortalité naturelle) sont schématisées dans la figure 1.

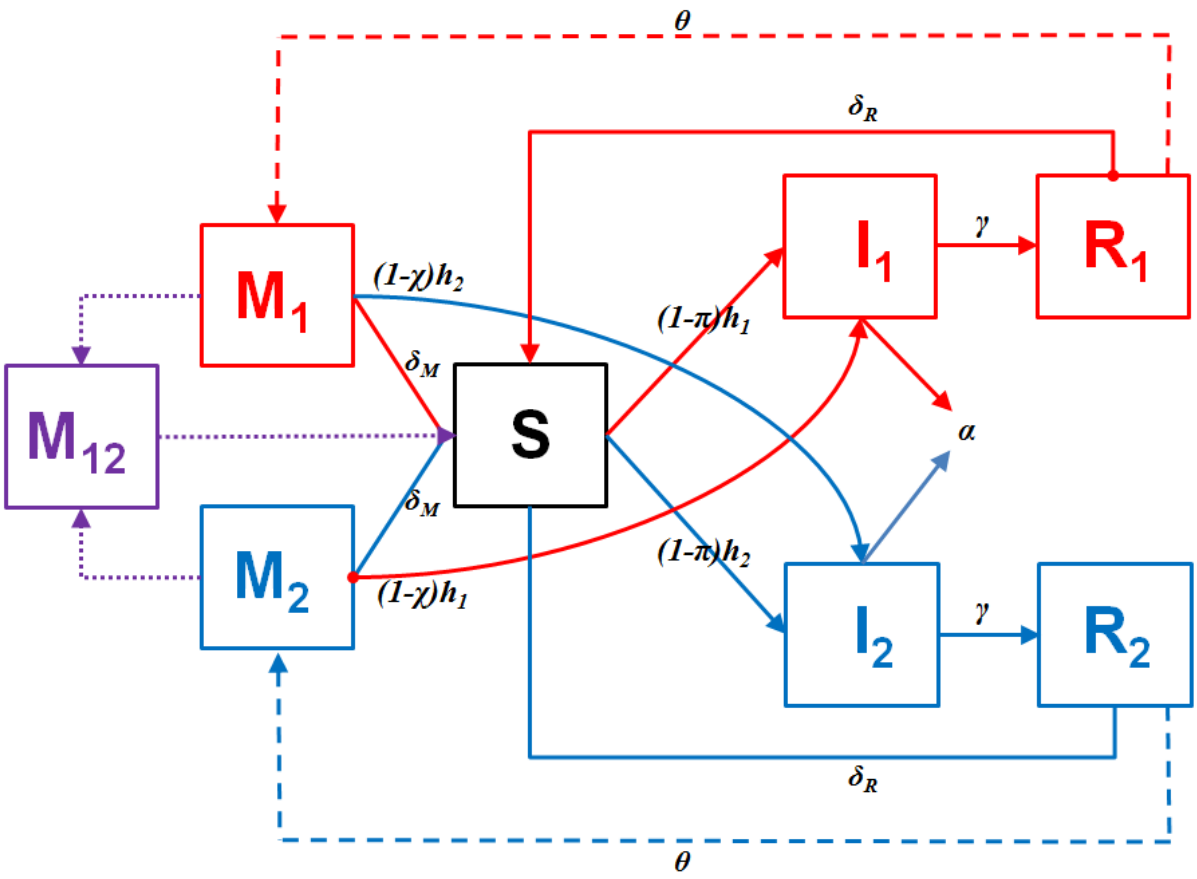


Figure 1 : Diagramme des transitions du modèle MSIRS à 2 souches. Les transitions correspondant à la mortalité naturelle et à la reproduction ne sont pas représentées, à l'exception du transfert d'anticorps maternels (*ϑ*) qui traduit la capacité des individus résistants à transférer cette résistance à la génération suivante (voir la figure 1 de l'Annexe 1 pour un diagramme incluant toute la mortalité et la reproduction). Le modèle distingue quatre catégories d'individus : *M*, protégés par les anticorps Maternels ; *S*, Sensibles ; *I*, Infectés ; *R*, Résistants. Pour les statuts infectés et résistants, les individus sont distingués en fonction de la souche de parasite qu'ils ont rencontrée (1 ou 2). La transition des individus M_1 et M_2 vers les individus M_{12} décrit les conséquences épidémiologiques d'un transfert d'anticorps par une mère non biologique dans le cadre par exemple de l'allosuckling chez les mammifères sociaux.

La reproduction des individus est gouvernée par un taux de reproduction $\lambda = r - \kappa N$, avec r représentant la fécondité de l'hôte, $N = M + S + I + R$ la population d'hôtes totale et κ un paramètre d'occupation du milieu (qui peut être directement relié à la capacité de charge). Toutes les catégories d'hôtes sont capables de se reproduire de manière identique, et l'infection par le parasite n'a aucun effet sur cette fécondité. De plus par souci de simplification, aucune transmission verticale du parasite n'est considérée. Les nouveau-nés produits sont sensibles à leur naissance, sauf une fraction θ des jeunes produits par les individus ayant développé leur immunité qui reçoit une protection par des anticorps maternels et qui sont ainsi temporairement protégés. Dans le cas du modèle à une souche de parasite, la

reproduction et les transitions entre les différents états peuvent être exprimés par le système d'équations différentielles suivant (voir aussi l'annexe 1, figure 1) :

$$\frac{dM}{dt} = \lambda\theta R - (\delta_M + \mu)M$$

$$\frac{dS}{dt} = \lambda(M + S + I + (1-\theta)R) + \delta_M M + \delta_R R - (\mu + (1-\pi)h)S \quad (1)$$

$$\frac{dI}{dt} = (1-\pi)hS - (\mu + \alpha + \gamma)I$$

$$\frac{dR}{dt} = \gamma I - (\mu + \delta_R)R$$

Dans un tel système, un parasite est capable d'envahir une population d'hôte à sa densité d'équilibre $\hat{S}$ entièrement sensible si son nombre reproducteur de base est supérieur à 1 : $R_0 = \frac{(1-\pi)h}{\mu+\alpha+\gamma}\hat{S} > 1$. Lorsque cette invasion est possible, le système atteint un nouvel équilibre qui se caractérise par une coexistence des différentes catégories d'hôtes à des densités correspondant à leur équilibre endémique (respectivement $\bar{M}, \bar{S}, \bar{I}, \bar{R}$). Seule l'expression à l'équilibre endémique de $\bar{S}$ peut être déterminée simplement en remarquant que l'équilibre endémique correspond à une situation où $R_0 = 1$, ce qui donne $\bar{S} = \frac{\mu+\alpha+\gamma}{(1-\pi)h}$. Les densités à l'équilibre endémique des autres catégories d'hôtes ne sont en revanche pas simples à exprimer analytiquement, même si leur détermination numérique ne pose pas de problème pour les calculs.

I. EVOLUTION DU TRANSFERT D'ANTICORPS MATERNELS

Dans le but d'étudier l'évolution du transfert d'anticorps maternel, un critère d'invasion dépendant des différents mécanismes de résistance considérés a été dérivé, quand une seule souche ou deux souches de parasites sont considérées (voir l'Annexe 1, et particulièrement l'appendice 1 pour les détails du calcul de critère d'invasion). Bien que des coûts puissent être associés à la survie et à la fécondité, seule une fonction de coût impliquant la fécondité sera étudiée ici. Un compromis légèrement accélérant est utilisé, car cette forme de compromis empêche l'apparition de branchement évolutif (Boots & Haraguchi 1999; Hoyle *et al.* 2008) et simplifie ainsi l'étude de l'optimisation de l'investissement dans les différents mécanismes de résistance.

Le critère d'invasion R_m représente le nombre moyen de jeunes produits à la génération suivante par un individu mutant (dénoté par l'indice m) dans une population résidente. Son expression est relativement compréhensible quand une seule souche de parasite est considérée :

$$R_m = \left(\tau^m_{S\to S} + \sqrt{4\tau^m_{M\to S}\tau^m_{S\to M} + {\tau^m_{S\to S}}^2} \right)/2$$

avec $\tau^m_{S\to S}$, $\tau^m_{M\to S}$ et $\tau^m_{S\to M}$ correspondant aux transitions résultant dans la production de nouveau-nés dans les catégories sensibles (S) et protégés par les anticorps maternels (M). Quand deux souches de parasites circulent dans la population d'hôtes, ce critère d'invasions devient plus compliqué et seule une analyse numérique sera réalisée dans ce cas. Dans tous les cas, en se plaçant dans les conditions de la dynamique adaptative, ce critère d'invasion peut être utilisé pour déterminer l'investissement optimal dans le transfert d'anticorps maternels et dans les autres mécanismes de résistance aux parasites. Tous les équilibres rapportés ici sont à la fois stable évolutivement et par convergence, et correspondent donc à des stratégies continuellement stables (sensu Geritz *et al.* 1998).

1. Evolution avec 2 souches de parasite

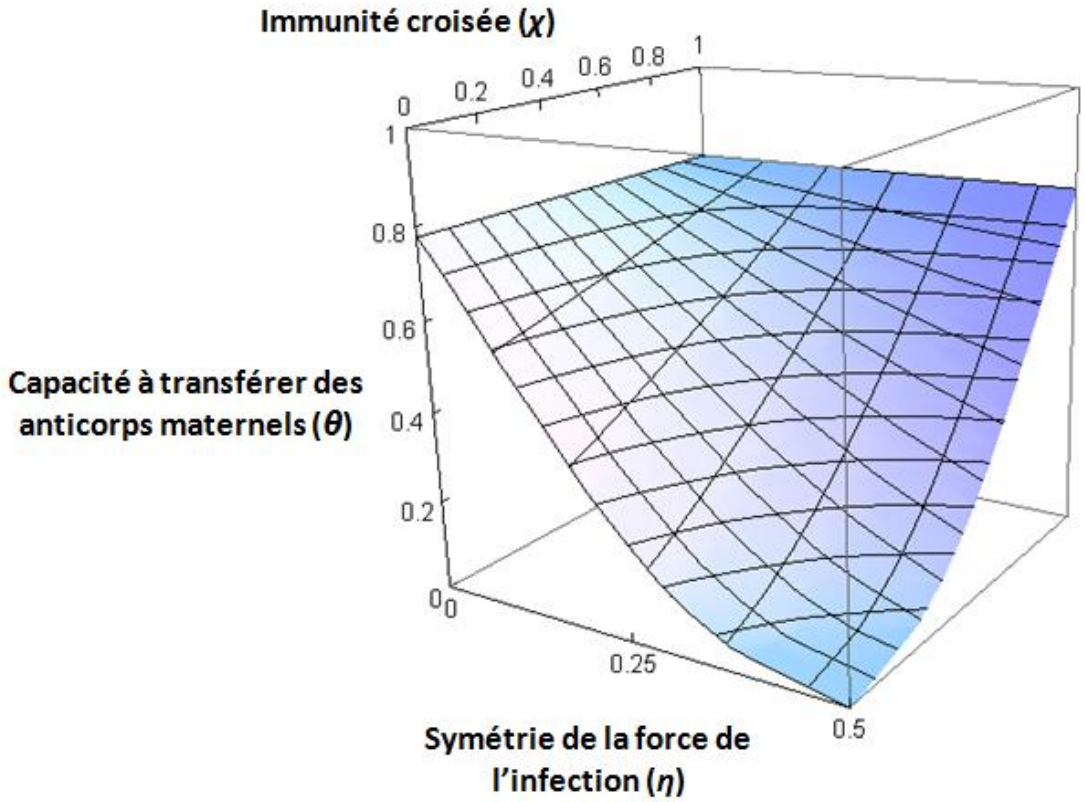


Figure 2 : Investissement évolutivement stable dans la capacité à transférer des anticorps maternels (ϑ) en fonction de l'immunité croisée des anticorps maternels (χ) et de la symétrie de la force de l'infection (η) dans le modèle avec 2 souches de parasites telles que $h_1 = \eta h$ et $h_2 = (1-\eta)h$.

Le modèle à deux souches permet d'illustrer l'importance de l'efficacité de la protection conférée par les anticorps maternels pour l'évolution de la capacité à transmettre des anticorps (figure 2). Ainsi, lorsque l'immunité croisée diminue, la valeur évolutivement stable de la capacité à transmettre des mères diminue aussi. Une immunité croisée forte revient à dire que la protection des mères prédit bien l'immunité que les anticorps maternels vont offrir au jeune, ce qui augment les bénéfices du transfert d'anticorps maternels. Dans ce modèle, la force de l'infection totale h peut être distribuée plus ou moins également en fonction d'un paramètre de symétrie η de telle sorte que $h_1 = \eta h$ et $h_2 = (1-\eta)h$. Ainsi, lorsque $\eta = 0.5$, les deux souches de parasite sont strictement identiques alors que lorsque ce paramètre s'éloigne de 0.5, la force de l'infection totale est biaisée en fonction de l'une ou l'autre souche. Augmenter ce biais revient donc à augmenter la prédictibilité de

l'environnement que le jeune va rencontrer et sélectionne pour des niveaux plus élevés de capacité à transférer des anticorps maternels (figure 2).

Ce résultat illustre bien l'importance pour l'évolution du transfert d'anticorps maternel de la corrélation entre l'environnement rencontré par la mère et celui rencontré par le jeune dans les premiers moments de sa vie. En effet, une autre manière d'appréhender ce résultat est de considérer les souches de parasite 1 et 2 non plus comme des souches mais comme des environnements parasitaires différents. Dans chaque environnement, la mère serait alors exposée à un certain nombre de parasites contre lesquels elle transmettrait son immunité acquise à la génération suivante. Dans cet ordre d'idée, le paramètre d'immunité croisée représenterait alors une mesure de recouvrement entre l'environnement parasitaire du jeune et celui de sa mère. Ainsi, lorsque l'immunité croisée est nulle, les parasites auxquels la mère a été exposée ne seraient pas ceux auxquels le jeune est exposé pendant la durée de protection des anticorps maternels. Lorsque l'immunité croisée augmente, le nouveau-né et sa mère partagent un nombre croissant de parasites. Ce qui est donc important pour l'évolution du transfert d'anticorps maternels, c'est que les anticorps maternels transférés soient dirigés contre des parasites présents dans l'environnement du jeune. Ainsi, la prise en compte de la variabilité dans le temps et dans l'espace des parasites (Holt & Boulinier 2005) pourrait aussi modifier l'évolution du transfert d'anticorps maternels. A l'inverse, le transfert d'anticorps maternels pourrait aussi constituer un frein à l'évolution de mécanismes qui pourraient résulter en un décalage entre l'environnement rencontré par la mère et celui rencontré par le jeune. La dispersion des adultes (Greenwood & Harvey 1982) entre deux zones de reproduction, en particulier dans des systèmes coloniaux où le risque parasitaire est structuré dans l'espace (e.g. Gasparini *et al.* 2001), pourrait par exemple être soumise à un compromis évolutif avec le transfert d'anticorps maternels.

L'évolution d'autres stratégies de résistance aux parasites est aussi une source probable de modifications de l'évolution du transfert d'anticorps maternels. En particulier, le modèle développé ici permet de s'intéresser à la coévolution de l'aptitude à transférer avec l'aptitude à guérir, et de ces deux mécanismes de résistance acquise avec l'aptitude des individus à éviter l'infection par le parasite. De plus, comme cela a été souligné par Boots *et al.* (2009), la prise en compte de rétrocontrôles épidémiologiques est aussi une force agissant sur l'évolution de stratégies de défense face aux parasites. Cet effet est aussi ajouté dans la section suivante.

2. Coévolution du transfert d'anticorps maternels avec la réponse immunitaire et l'évitement

Dans cette section, la force de l'infection va maintenant dépendre directement du taux de transmission du parasite (β) et de la densité d'individus infectés dans la population (I) de telle sorte que $h = \beta I$. Pour rester simple, l'analyse sera restreinte au modèle à une souche. Le critère d'invasion défini plus haut permet dans ce cas de déterminer les stratégies co-évolutivement stables (« CoEvolutionary Stable Strategies », CoESS; van Baalen 1998).

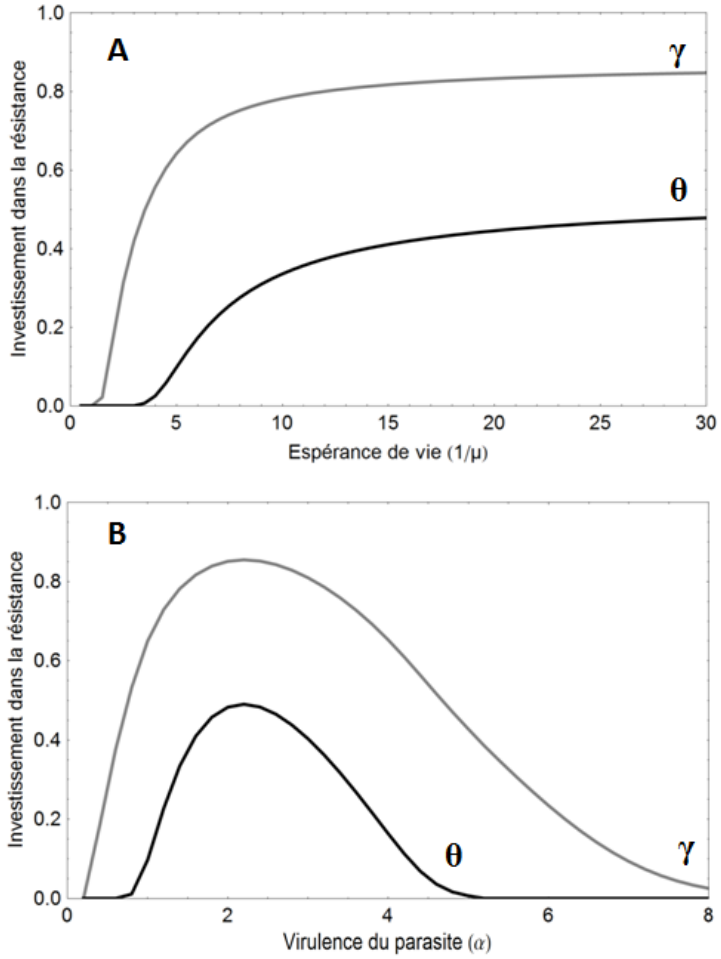


Figure 3 : Coévolution entre le transfert d'anticorps maternels (ϑ, courbe noire) et le taux de guérison (γ, courbe grise). (A) Effet de l'espérance de vie ($1/\mu$) sur le résultat de la coévolution entre le transfert d'anticorps maternels et le taux de guérison. (B) Effet de la virulence (α) sur le résultat de la coévolution entre le transfert d'anticorps maternels et le taux de guérison. Voir l'Annexe 1 pour les valeurs par défaut des paramètres.

La CoESS entre le transfert d'anticorps maternels et le taux de guérison est une fonction croissante de la durée de vie moyenne (figure 3A). Le transfert d'anticorps maternels

(courbe noire) commence à augmenter pour des durées de vie supérieures par rapport au taux de guérison. Cette saturation provient de l'effet de l'augmentation de la durée de vie sur la prévalence du parasite qui augmente la force de l'infection (Miller *et al.* 2007). Ainsi, la probabilité de rencontrer un même parasite augmente avec la durée de vie. C'est ce qui explique qu'il est rentable d'investir de plus en plus dans les réponses acquises avec l'augmentation de l'espérance de vie. En revanche, la virulence du parasite a un effet non monotone sur l'investissement dans les deux mécanismes de réponse acquise (figure 3B). Lorsque la virulence est faible, une augmentation sélectionne pour un investissement accru dans le transfert d'anticorps maternels et dans la guérison. Au-delà d'une valeur intermédiaire de virulence, l'investissement dans ces deux traits diminue avant d'atteindre zéro. Ce point au-delà duquel il n'y a plus d'investissement dans le trait est obtenu à des valeurs de virulence moindres pour le transfert d'anticorps maternels. Une augmentation importante de la virulence conduit en effet à une mortalité rapide des individus infectés, ce qui cause une chute rapide de la force de la probabilité d'infection et à une sélection maximale des mécanismes de réponse immunitaire acquise pour des virulences intermédiaires. De plus, lorsque la virulence augmente, la proportion d'individus résistants dans la population tend à diminuer (du fait d'une mortalité accrue) ce qui tend à faire diminuer de manière plus rapide le transfert d'anticorps maternels.

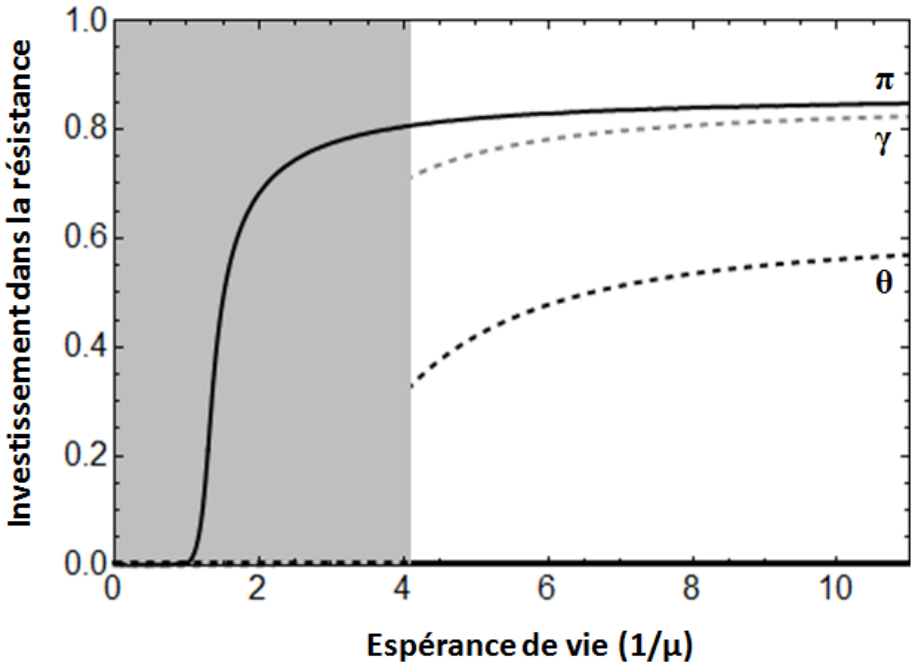


Figure 4 : Effet de l'espérance de vie l'hôte (1/μ) sur le résultat de la coévolution entre l'évitement du parasite (*π*, courbe noire), le taux de guérison (*γ*, courbe gris clair) et le transfert d'anticorps maternels (*ϑ*, courbe gris foncé) en fonction des conditions initiales (courbes pleines : $\pi_{début}$ = 0.7; $\gamma_{début}$ = 0.01; $\theta_{début}$ = 0.01; courbes pointillées: $\pi_{début}$ = 0.01; $\gamma_{début}$ = 0.7; $\theta_{début}$ = 0.7). Quand l'espérance de vie est courte, l'investissement se fait seulement dans l'évitement du parasite (zone à fond grisé). Quand l'espérance de vie augmente (zone à fond blanc), une bistabilité apparait : l'hôte investit soit dans l'évitement seul soit dans une capacité à guérir et à transférer l'immunité à la génération suivante, en fonction des conditions initiales.

Lorsque l'on prend en compte dans le modèle précédent la possibilité d'un mécanisme d'évitement, le résultat de la coévolution entre les trois traits de résistance montre un patron fortement dépendant de l'espérance de vie de l'hôte (figure 4). Quand l'hôte a une durée de vie courte, l'investissement se fait tout le temps dans l'évitement. Quand la durée de vie augmente, l'investissement se fait en fonction des conditions initiales soit toujours dans l'évitement soit dans une association de la guérison avec le transfert d'anticorps maternels. L'espérance de vie a effectivement été suggérée comme un trait d'histoire de vie à même de modifier l'évolution de processus physiologiques coûteux comme la résistance aux parasites (Ricklefs & Wikelski 2002). L'augmentation de l'espérance de vie peut en effet se traduire par une probabilité accrue de rencontrer le même parasite plusieurs fois au cours de sa vie. De ce fait, les espèces longévives sont supposées investir plus dans les réponses acquises alors que les espèces à vie plus courte devraient investir dans des réponses innées (Lee 2006), une hypothèse soutenue par le résultat prédit par le modèle développé ici. Par ailleurs, cet

investissement dans des réponses acquises pourrait aussi s'accompagner d'un accroissement de la persistance conférée.

3. Evolution de la persistance des réponses acquises

Le critère d'invasion développé précédemment peut être utilisé pour s'intéresser à l'évolution de n'importe quel trait présent dans le modèle. Ainsi, il est par exemple possible de se concentrer sur l'évolution des traits décrivant la persistance de la protection immunitaire chez les individus résistants (R), δ_R, et chez les individus protégés par les anticorps maternels (M), δ_M.

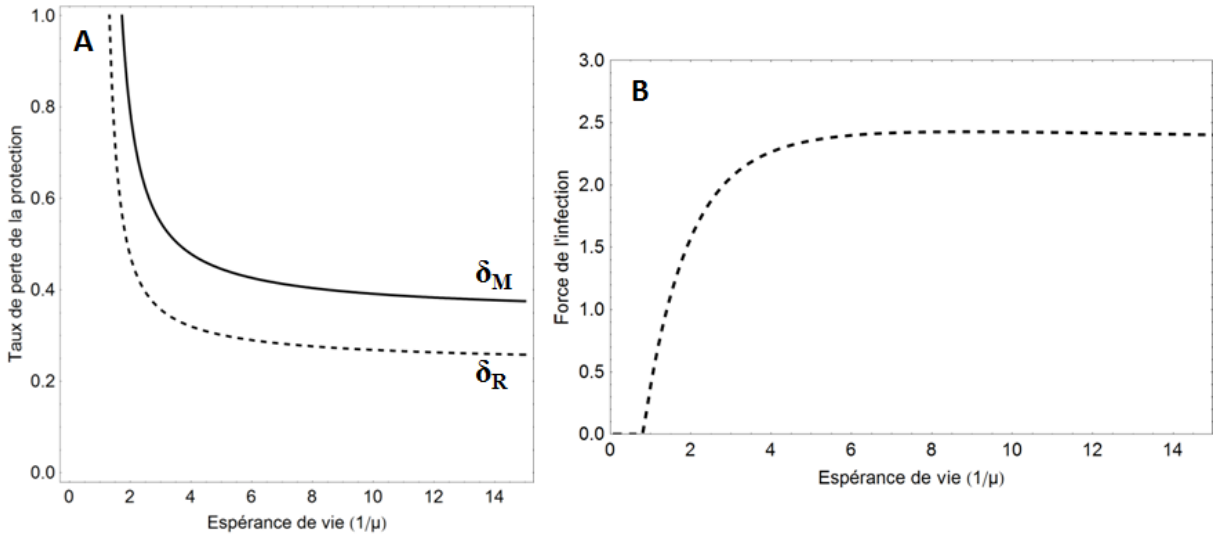


Figure 5 : (A) Investissement évolutivement stable dans la persistance de la protection acquise par transfert transgénérationnel (δ_M, courbe pleine) ou après guérison (δ_R, courbe pointillée) en fonction de l'espérance de vie (1/μ) de l'hôte. (B) Force de l'infection du parasite (βI) correspondant à la valeur évolutivement stable de perte de la protection après gurérison présentée dans la figure A en fonction de l'espérance de vie de l'hôte.

L'évolution du taux de perte de la protection après guérison (δ_R) ou après transfert maternel (δ_M) suit le même patron de décroissance avec l'espérance de vie l'hôte (figure 5A). En d'autre termes, les espèces plus longévives sont supposées investir dans une persistance prolongée des anticorps, maternels ou non. Toutefois, la protection des jeunes par les anticorps maternels (courbe pleine) est toujours perdue plus vite que la protection conférée par la guérison après une rencontre avec le parasite (courbe pointillée), même si cette différence est moindre pour des durées de vie courtes. L'augmentation de l'espérance de vie

se traduit en effet par une augmentation de la densité des individus infectés, qui augmente la force de l'infection du parasite (figure 5B). Cet effet augmente le risque pour les espèces longévives de rencontrer le parasite plusieurs fois au cours de leur vie, et sélectionne du même coup pour des taux de perte de protection plus faibles. En accord avec ce résultat, l'augmentation du taux de transmission du parasite (β) produit le même patron de sélection vers une persistance de la protection immunitaire plus longue, qu'elle soit acquise après guérison ou passivement par des anticorps maternels (voir l'Annexe 2).

Là encore, le résultat de modélisation va dans le sens des hypothèses d'évolution des mécanismes immunitaires évoquées dans le cadre de la théorie des traits d'histoire de vie (Lee 2006). Cependant, d'un point de vue empirique, les données concernant la persistance des réponses immunitaires en populations naturelles sont très peu disponibles et rendent difficiles le test empirique de ces hypothèses. L'une des raisons de la faible disponibilité est probablement la difficulté à estimer la décroissance des réponses immunitaires, notamment car cela nécessite d'échantillonner de manière répétée les mêmes individus, ce qui peut être compliqué sur le terrain. La mesure de la décroissance des anticorps maternels chez les jeunes, généralement plus accessibles pour des captures répétées, pourrait représenter un moyen d'obtenir ce genre de données (Davison *et al.* 2008). En effet, les anticorps maternels présentent l'intérêt de ne pas pouvoir être renouvelés par le jeune, à la différence des taux d'anticorps des adultes qui sont le résultat d'une balance entre le catabolisme et la production d'anticorps (Manz *et al.* 2005). Chez les oiseaux, où tout l'apport d'anticorps maternels a lieu via le vitellus, la mesure peut être directe. Chez les mammifères, où l'apport par le lait est continu, il serait par contre nécessaire de contrôler cette mesure par des mesures concomitantes des niveaux d'anticorps dans le lait pour mesurer l'apport continu au jeune et estimer la balance entre apport et décroissance. D'une manière plus générale, suivre la dynamique de décroissance des anticorps maternels permettra d'apporter des informations intéressantes sur les déterminants écologiques de ce mécanisme.

II. ECOLOGIE DU TRANSFERT D'ANTICORPS MATERNELS

1. Le transfert d'anticorps maternels : un trait d'histoire de vie négligé ?

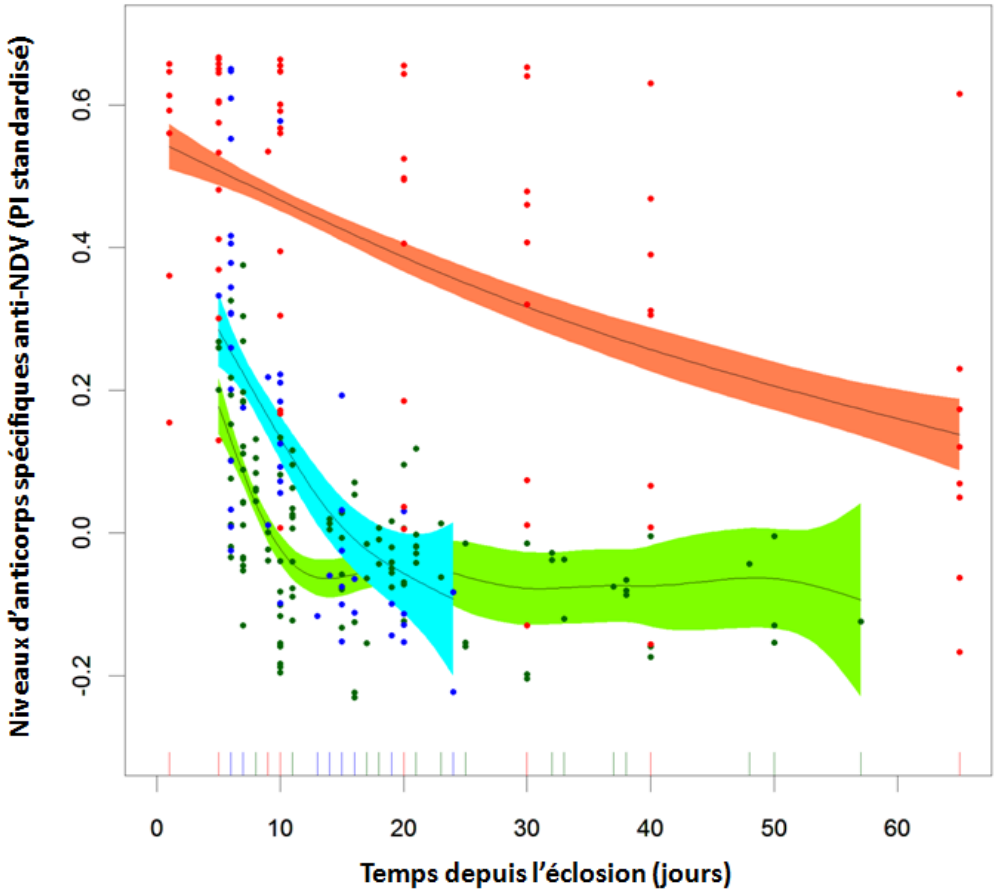
Si à la fois les résultats de modélisation et la théorie des traits d'histoire de vie prédisent un allongement de la persistance des anticorps maternels, les données pour corroborer empiriquement ces prédictions sont encore limitées. De manière surprenante, chez les oiseaux, il est généralement admis que les anticorps maternels disparaissent dans les premières semaines de vie (Davison *et al.* 2008). Il faut cependant remarquer que la plupart des études amenant à cette hypothèse a été obtenue chez les oiseaux d'élevage, et surtout le poulet (voir par exemple Nemeth & Bowen 2007). Quelques études montrent une possible variabilité entre différents groupes d'oiseaux (e.g. Graczyk *et al.* 1994; Gibbs *et al.* 2005; Chang *et al.* 2007; Nemeth *et al.* 2008; King *et al.* 2010), mais les protocoles ne permettent en général pas d'avoir des informations précises sur la décroissance des anticorps maternels. En particulier, ces études mesurent la persistance des anticorps maternels qui donne un poids particulièrement important à quelques points extrêmes où la mesure doit être réalisée à la limite de la zone de détection des tests immunologiques utilisés. La mesure de la demi-vie des anticorps (c'est-à-dire le temps nécessaire à la disparition du taux initial) permet en revanche d'obtenir des informations sur la décroissance des anticorps maternels en s'affranchissant de cette limitation technique. Explorer la variabilité de la demi-vie des anticorps maternels dans des espèces à cycles de vie contrastés pourrait ainsi permettre d'apporter des informations sur les déterminants écologiques et évolutifs du transfert d'anticorps maternels. C'est l'objectif de l'étude rapportée dans l'Annexe 3.

La première étape pour mesurer la décroissance des anticorps maternels chez les jeunes consiste à contrôler l'exposition des mères, ce qui peut être fait en utilisant la vaccination (Staszewski & Boulinier 2004). Un protocole utilisant un vaccin à virus tué contre la maladie de Newcastle avait déjà été utilisé chez des espèces présentant des histoires de vie contrastées, la mouette tridactyle (*Rissa tridactyla*) et la caille des blés (*Coturnix coturnix*), et des données de décroissance des anticorps maternels avait été obtenues (Staszewski *et al.* 2007a; Staszewski & Siitari 2010) sans que des différences aient pu être mises en évidence. Un protocole similaire de vaccination des mères a été mis en place chez une espèce de Procellariiforme, le puffin cendré (*Calonectris diomedea*). Les Procellariiformes sont en effet un groupe d'oiseaux au rythme de vie particulièrement lent et les puffins cendrés par exemple

incubent leur seul œuf (Warham 1983) pendant 54 jours et la période d'élevage du jeune se prolonge pendant approximativement 90 jours (Giudici *et al.* 2010). Etant donné les traits d'histoire de vie du puffin cendré, une décroissance lente des anticorps maternels qui pourrait permettre la mise en place lente d'une réponse immunitaire acquise forte (Ricklefs & Wikelski 2002; Lee 2006) était attendue.

Les mères ont été capturées et vaccinées à l'aide du même vaccin contre la maladie de Newcastle (Nobilis Paramyxo P201, Intervet, France) suffisamment tôt dans la saison de reproduction pour toutes avoir des taux d'anticorps circulants détectables au moment de la ponte. La naissance des jeunes a ensuite été suivie et ces jeunes ont été échantillonnés de manière répétée de J1 à J65 après l'éclosion. Les échantillons de plasma ainsi récupérés ont ensuite été analysés par une technique ELISA compétition (Enzyme-Linked ImmunSorbent Assay) spécifique du virus de la maladie de Newcastle (Svanovir-Ab, Svanova Biotech, Suède). De manière à être comparables entre espèces, les taux d'anticorps, présentés sous forme de pourcentage d'inhibition (PI), ont été standardisés en soustrayant à chaque valeur de PI la valeur seuil de négativité (calculée comme la moyenne des contrôles négatifs + 2 déviations standards ; caille : 0.22 ; mouette tridactyle : 0.30 ; puffin cendré : 0.31).

Les dynamiques de décroissance des anticorps maternels chez les poussins ont été modélisées par des modèles additifs mixtes généralisés (GAMMs) en prenant en compte leur statut et celui de leur mère (vaccinée/non vaccinée) à l'aide du package « mgcv » dans R (R Development Core Team 2009). Pour faciliter la comparaison entre les différentes espèces, une demi-vie des anticorps maternels (et l'intervalle de confiance à 95% associé) a été calculée en supposant une décroissance exponentielle des taux d'anticorps.



<u>Figure 6 :</u> Décroissance des anticorps anti-NDV chez les poussins de trois espèces : la caille (en vert), la mouette tridactyle (en bleu) et le puffin cendré (en rouge). Le pourcentage d'inhibition (PI) standardisé est utilisé sur l'axe des Y, et 0 représente le seuil au dessus duquel le prélèvement est considéré comme positif. Les courbes présentent pour chaque espèce la moyenne déterminée par un modèle additif mixte généralisé (GAMM) et les zones colorées encadrant la courbe représentent les intervalles de confiance à 95% de la valeur de la pente.

La décroissance des anticorps apparait plus lente chez le puffin cendré (figure 6) que ce qui est rapporté chez d'autres espèces : alors que les anticorps disparaissent de la circulation du jeune aux alentours de l'âge de 15 jours chez la caille (en vert) ou chez la mouette (en bleu), la plupart des poussins de puffin cendré (en rouge) présente encore des niveaux d'anticorps spécifiques anti-NDV plasmatiques détectables à 30 jours, 40 jours voire même 65 jours après l'éclosion. Cette différence de dynamique se retrouve au niveau des demi-vies qui sont clairement plus longues chez les puffins cendrés (24.75 jours [IC 95% : 18.07 - 39.24]) que chez la mouette tridactyle (5.43 jours [IC 95% : 3.46 – 11.55]) ou chez la caille (5.25 jours [IC 95% : 3.58 – 9.90]). De plus, chez le puffin cendré, à l'instar de ce qui est observé chez la caille ou chez la mouette tridactyle (Staszewski *et al.* 2007a; Staszewski & Siitari 2010), le niveau d'anticorps plasmatique du poussin est corrélé à celui de sa mère à 5

jours (r_{5j} = 0.81, N = 17 poussins, $p < 0.001$). De manière plus surprenante, cette corrélation perdure durant tout la durée du suivi (voir l'annexe 3) et reste significative à 65 jours après l'éclosion (r_{65j} = 0.72, N = 8 poussins, p = 0.042).

En relation avec le résultat de modélisation précédent (voir figure 5), la décroissance particulièrement lente des anticorps maternels chez le puffin cendré met en lumière l'importance du transfert d'anticorps maternel comme un effet maternel dont l'évolution pourrait être soumise à des compromis évolutifs avec d'autres traits d'histoire de vie. Les espèces longévives sont en particulier supposées investir plus fortement dans des réponses acquises que dans les réponses innées (Lee 2006). En effet, le système immunitaire inné est associé à une réponse inflammatoire forte et particulièrement coûteuse à long terme (Graham *et al.* 2005) alors que l'activation du système immunitaire acquis résulte en une réponse mémoire (Gatto *et al.* 2006) qui serait particulièrement bénéfique chez des espèces qui rencontreraient les mêmes parasites plusieurs fois au cours de leur vie. L'absence de décroissance avec l'âge de différents effecteurs de la réponse acquise chez une espèce d'albatros (Lecomte *et al.* 2010) ainsi que la corrélation négative entre une mesure de l'efficacité du système immunitaire inné et la survie annuelle (Tella *et al.* 2002) vont aussi dans le sens de cette hypothèse.

Pourtant, la seule étude qui a cherché à mettre en relation l'investissement maternel dans le transfert d'anticorps avec l'espérance de vie des espèces a montré que les espèces les plus longévives avaient tendance à déposer moins d'IgY dans le vitellus (Addison *et al.* 2009), ce qui semble supposer un investissement moindre dans le transfert d'anticorps maternels. Cependant, dans cette étude, les auteurs se sont limités à des analyses des anticorps contenus dans les œufs. Ils n'ont ainsi mesuré ni la dynamique de décroissance des anticorps chez les poussins ni le taux d'anticorps de la mère au moment de la ponte. Les résultats présentés dans la figure 6, et en particulier la dynamique des anticorps maternels chez le puffin cendré, montrent qu'une exploration plus complète de l'écologie du transfert d'anticorps maternels nécessite une approche comparative de la variabilité de la persistance des anticorps maternels entre espèces. Chez les espèces longévives, associée à une efficacité renforcée des défenses innées pendant la période d'élevage (Tella *et al.* 2002; Lee *et al.* 2008), la persistance prolongée des anticorps maternels pourrait au final faire partie intégrante d'une stratégie immunitaire nécessitant la mise en place lente d'un système immunitaire acquis particulièrement efficace.

2. Transfert d'anticorps maternels et socialité

La durée de la protection offerte par les anticorps maternels (voir ci-dessus et l'annexe 3) ainsi que le niveau d'anticorps maternels circulants sont des facteurs qui doivent déterminer directement les conséquences écologiques du transfert d'anticorps maternels. Le répertoire de parasites couvert par les anticorps maternels est aussi probablement important à prendre en compte. Ainsi, des mécanismes se traduisant par le renforcement du niveau d'anticorps maternels ou par l'acquisition d'anticorps dirigés contre de nouveaux parasites pourraient apporter de nouveaux bénéfices au transfert d'anticorps. Cet élargissement du répertoire d'anticorps du jeune pourrait être permis par l'acquisition postnatale d'anticorps contre de nouveaux parasites. Par exemple, dans le modèle à 2 souches de parasites présenté plus haut (figure 1), un mécanisme de transfert postnatal non lié à la mère biologique pourrait permettre l'occurrence d'individus protégés contre les deux souches (M_{12}, en violet). Biologiquement, chez les mammifères sociaux, un tel effet pourrait être obtenu par l'accès au lait ou au colostrum de plusieurs femelles allaitantes, l'allosuckling, un mécanisme largement répandu chez les mammifères sociaux (Packer *et al.* 1992). Si cette hypothèse de la fonction immunitaire de l'alloscukling a été émise verbalement (Roulin & Heeb 1999) et que les anticorps maternels peuvent être transférés et jouer un rôle protecteur entre une mère adoptive et un jeune (Gustafsson *et al.* 1994), elle n'a jamais été validée empiriquement par la démonstration d'un réel transfert d'anticorps entre une mère adoptive et un nouveau-né dans un contexte de groupe social.

Pour répondre à cette question, des femelles d'un petit mammifère, la gerbille de Mongolie (*Meriones unguiculatus*) ont été vaccinées en utilisant soit un vaccin porcin contre le sous type H1N1 du virus de la grippe (Gripovac, Merial, France) soit un vaccin canin contre la bactérie *Borrelia burgdorferi* (Merilym, Merial, France). Après fécondation, les femelles ont ensuite été placées par groupe de 2 femelles, une vaccinée contre chaque parasite, avant la mise bas. Du fait de problèmes d'agressivité des femelles envers les mâles, une phase supplémentaire de mise en contact entre les mâles et les femelles a dû être ajoutée. De manière à permettre des contacts limités et une habituation progressive, le mâle et la femelle ont été placés de chaque côté d'une séparation en plexiglas percée de trous. Après une semaine où les animaux étaient déplacés d'un côté à l'autre de la cage, la séparation a été

enlevée et les animaux mis en contact. La mise en contact des deux femelles de chaque paire a été faite en suivant le même protocole. Malheureusement, à cause d'un très fort de cannibalisme des femelles envers les nouveau-nés, seul un couple de femelles a pu élever quatre jeunes au cours de l'étude. Une prise de sang a été réalisée sur les jeunes à 1 jour et à 8 jours par ponction intracardiaque, puis toutes les semaines à partir de l'ouverture des yeux au sinus rétro-orbital jusqu'à 48 jours après la naissance. Les concentrations plasmatiques des anticorps contre le virus de la grippe ont été mesurées à l'aide d'un kit ELISA compétition (ID Screen Antibody Influenza A Competition, ID Vet, Montpellier, France) et ceux contre *Borrelia burgdorferi* avec un kit ELISA sandwich (Borrelia IgG+ VlsE ELISA, IBL International GMBH, Hambourg, Allemagne) modifié pour utiliser un anticorps secondaire spécifique de la gerbille (HRP Conjugated Rabbit anti-Gerbil IgG, Immunology Consultants Laboratory, Portland, OR, Etats Unis). Les taux d'anticorps sont exprimés par le pourcentage d'inhibition (influenza ; figure 7A) ou la densité optique (*Borrelia ;* figure 7B). Les détails du protocole expérimental se trouvent dans l'annexe 4 (voir en particulier la figure 1 de cette annexe).

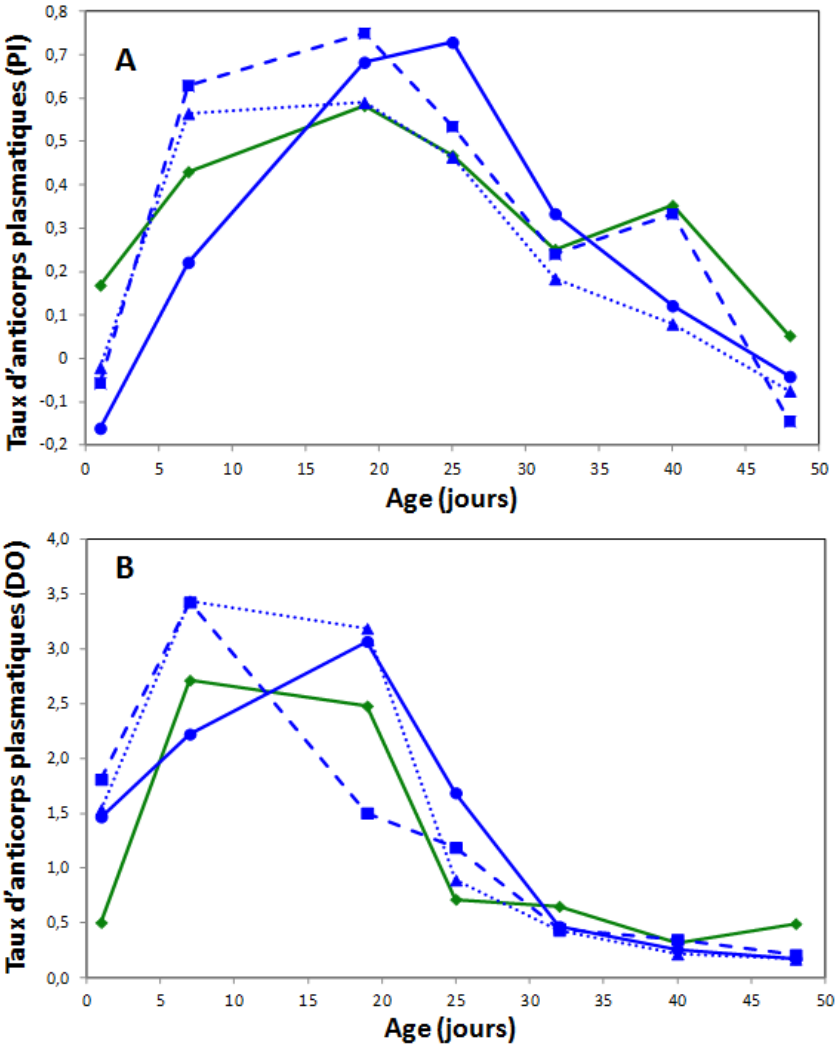


Figure 7 : Taux d'anticorps maternels spécifiques circulant dans le plasma des jeunes en fonction de leur âge. (A) Taux d'anticorps maternels spécifiques anti-grippe exprimé en pourcentage d'inhibition (PI) en fonction de l'âge. (B) Taux d'anticorps maternels spécifiques anti-*Borrelia burgdorferi* exprimé en densité optique (DO) en fonction de l'âge. Dans les deux graphiques, les couleurs font référence à la vaccination de la mère biologique contre *Borrelia burgdorferi* (en bleu) ou contre la souche H1N1 de grippe porcine (en vert). Un même format (pointillé, tiret, plein) de courbe fait référence à un individu identique entre A et B.

A la naissance, tous les nouveau-nés étaient négatifs pour les anticorps anti-influenza (figure 7A) alors que trois nouveau-nés sur les quatre présentaient des taux détectables d'anticorps anti-*Borrelia* (figure 7B). Les anticorps anti-*Borrelia* pouvant être transférés via le placenta chez les rongeurs (Morshed *et al.* 1993), ce résultat indique que ces trois nouveau-nés proviennent de la mère vaccinée contre *Borrelia* alors que le dernier provient de celle vaccinée contre influenza. Dans les deux cas, les dynamiques montrent une augmentation rapide des anticorps durant la première semaine de vie, probablement due à la consommation de colostrum et de lait. Les taux plasmatiques restent stables jusqu'à 19 jours, moment à partir duquel ils commencent à décroître. Ce moment correspond au sevrage qui intervient entre 21

et 30 jours chez la gerbille de Mongolie (Norris & Adams 1972), et aussi probablement à l'arrêt de l'expression du récepteur FcRn nécessaire au passage des anticorps maternels à travers la barrière intestinale (Roopenian & Akilesh 2007). Tous les individus sont négatifs 32 jours après la naissance pour influenza et 40 jours après la naissance pour *Borrelia*. Quoi qu'il en soit, des anticorps dirigés contre chacun des deux parasites utilisés ont pu être détectés dans le plasma de tous les nouveau-nés. Ce résultat démontre ainsi le potentiel de l'allosuckling comme source d'anticorps pour des nouveau-nés élevés par plusieurs mères.

L'étape suivante dans la compréhension de l'importance écologique de l'allosuckling serait de mettre en évidence le transfert croisé d'anticorps dans des populations naturelles de mammifères sociaux telles que le suricate (Clutton-Brock *et al.* 2001), la mangouste rayée (Cant 2000) ou encore des petits rongeurs (Hayes 2000) pour lesquelles des programmes de suivi à long terme existent. En utilisant des vaccins pour marquer les différentes femelles du groupe, une prise de sang pourrait alors suffire pour mettre en évidence un transfert croisé d'anticorps. De plus, l'impact d'autres facteurs tels que la synchronie des naissances, un facteur qui initialement aurait dû être exploré au cours de l'étude chez la gerbille, entre les différents individus du groupe social ou la structure hiérarchique du groupe pourraient être pris en compte. L'existence d'un allosuckling et la possibilité d'un transfert croisé d'anticorps pourrait par exemple participer à expliquer l'intérêt de la femelle dominante à autoriser la reproduction de ses subordonnés ou à forcer l'avortement d'une femelle subordonnée en fin de gestation (Gilchrist 2006; Cant *et al.* 2010).

III. IMPLICATIONS ECO-EPIDEMIOLOGIQUES DU TRANSFERT D'ANTICORPS MATERNELS

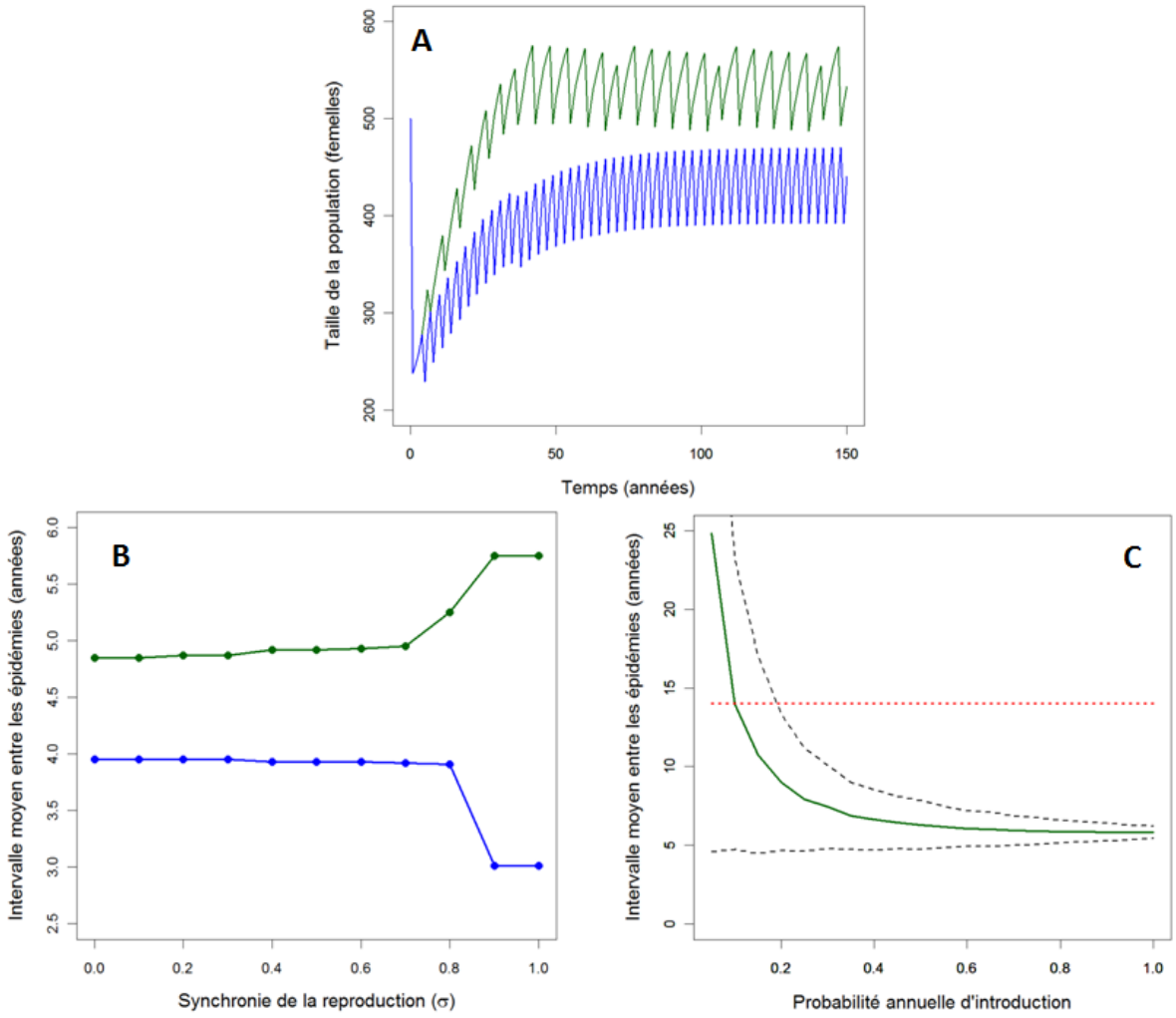
Le transfert d'anticorps maternels montre donc de la variabilité à la fois dans les sources d'anticorps et dans la durée de la protection susceptible d'être conférée. Ces deux effets combinés vont contribuer à modifier la structure de sensibilité de la population en protégeant un nombre variable de nouveau-nés et pour une durée plus ou moins étendue. Cette modification de la proportion d'individus sensibles par le transfert d'anticorps a été évoquée comme une source potentielle d'effets épidémiologiques liés directement au transfert d'anticorps maternels (Boulinier & Staszewski 2008). Il a ainsi été montré que la prise en compte du transfert d'anticorps maternels se traduit par une réduction des effets délétères d'un parasite plus importante dans des situations d'endémie que d'épidémie (Fouchet *et al.* 2006). Par ailleurs l'importance des effets épidémiologiques liés au transfert d'anticorps dépend de conditions écologiques telles que la fragmentation et la connectivité de la population d'hôtes (Fouchet *et al.* 2007) ou encore la durée de la période de reproduction et la persistance de l'immunité acquise (Fouchet *et al.* 2008). Ces derniers effets en particulier méritent une attention particulière dans des systèmes où la reproduction est synchronisée à la fois temporellement et spatialement et lorsque des épidémies suivies de l'extinction du parasite surviennent. Dans ces populations, la naissance d'un grand nombre de juvéniles sensibles sur une fenêtre temporelle et spatiale réduite est un moteur important des dynamiques épidémiologiques (Roberts & Kao 1998; Keeling & Rohani 2008) que la modification temporaire de l'immunité des jeunes par le transfert d'anticorps maternels est à même d'influencer fortement.

1. Allongement des intervalles inter-épidémiques

Cet effet potentiel pourrait être particulièrement important dans le cas de parasites provoquant des épidémies, notamment sur les zones de reproduction d'espèces coloniales comme certains mammifères et oiseaux marins. C'est par exemple le cas de la population de phoques veau-marins (*Phoca vitulina*) en Europe qui a subi deux épisodes épidémiques avec des mortalités sévères liées à la circulation du Morbillivirus de la maladie de Carré du phoque (Osterhaus & Vedder 1988; Jensen *et al.* 2002). En 1988 comme en 2002, l'épidémie a démarré sur la même colonie durant la période de reproduction (Härkönen *et al.* 2006) ce qui conduit à supposer soit une introduction locale rare (Grenfell *et al.* 1992; Harding *et al.* 2005),

soit une introduction via des contacts avec une autre espèce de phoque, le phoque gris (*Halichoerus grypus*)(Hall *et al.* 2006). Dans ce second cas, l'immunité de groupe forte après une épidémie pourrait empêcher la survenue d'une épidémie à chaque contact (Anderson & May 1985) et le transfert d'anticorps maternels pourrait venir renforcer cette immunité de groupe pendant la période cruciale de la reproduction où les individus sont particulièrement concentrés, ce qui favorise normalement la circulation des parasites (voir encadré 2).

J'ai donc modélisé une population isolée de phoques veau-marins par un modèle matriciel de Leslie (Caswell 2001) en utilisant les valeurs de paramètres de reproduction et de survie rapportés par Härkönen *et al.* (2002). La saison de reproduction est supposée durer 180 jours et la synchronisation des naissances est décrite par un paramètre σ. Quand $\sigma = 1$, toutes les naissances ont lieu le jour 90, et quand $\sigma < 1$ elles s'étalent sur $180 - 179\ \sigma$ jours centrés autour du jour 90. D'un point de vue épidémiologique, les individus sont considérés comme résistants au parasite quand ils ont survécu à une épidémie ou pendant la protection maternelle. Dans le cas contraire, ils sont considérés comme sensibles. La durée de protection par les anticorps maternels est fixée à 30 jours. Ainsi, suivant la longueur de la saison de reproduction, certains jeunes peuvent avoir perdu leur immunité au moment de l'introduction du virus. Le virus de la maladie de Carré du phoque est en effet introduit dans la colonie au jour 100 de chaque saison de reproduction, mais une épidémie ne se produit que quand l'immunité de groupe au moment de l'introduction est inférieure à $1 - 1/R_0$ (Hethcote 2000). Si l'épidémie se produit, les individus sensibles subissent une mortalité âge-dépendante (Heide-Jørgensen *et al.* 1992; Härkönen *et al.* 2007), qui est modélisée suivant Harding *et al.* (2005). L'ensemble des individus survivants est considéré comme résistant et l'immunité acquise est supposée persister et permettre un transfert d'anticorps pour le reste de la vie. Des détails sur le modèle peuvent être trouvés dans l'annexe 5.



<u>Figure 8 :</u> (A) Dynamique de la population (en nombre de femelles) prédite par un modèle structuré en âge paramétré pour la population suédoise de phoques veau-marins exposée à une introduction annuelle du virus de la maladie de Carré du phoque au 100ème jour de la saison de reproduction théorique. La dynamique montre des intervalles plus longs entre les épidémies lorsque le transfert d'anticorps maternels est pris en compte (courbe verte) par rapport à une simple protection immunitaire des survivants (courbe bleue). (B) Effet de la synchronie de la reproduction (σ) sur l'intervalle moyen entre deux épidémies quand le transfert maternel est pris en compte. La reproduction se répartit de manière homogène sur 180 jours quand la synchronie est nulle, et a lieu en une journée quand la synchronie est de 1. (C) Effet de la probabilité d'introduction sur l'intervalle moyen entre les épidémies. La courbe noire pleine représente l'intervalle moyen sur 10000 ans, et les lignes pointillées l'écart type associé. La ligne pointillée rouge représente l'intervalle de 14 ans, observé entre les deux épidémies ayant eu lieu en 1988 et 2002.

Le transfert d'anticorps maternels résulte en un allongement des intervalles entre épidémies (figure 8A). Un intervalle entre épidémies de 3 ans est obtenu lorsque seule l'immunité acquise après guérison est considérée (courbe bleue), et il est pratiquement doublé par la prise en compte de l'immunité passive temporaire (courbe verte). L'importance de cet allongement des intervalles entre épidémies dépend de la synchronie de la reproduction. Un accroissement de la synchronie se traduit par des intervalles plus longs lorsque le transfert

d'anticorps est pris en compte (figure 8B, courbe verte) alors que l'effet inverse est obtenu si l'immunité acquise lors de l'infection est considérée seule (figure 8B, courbe bleue). Ce résultat provient de l'effet contrasté d'un pic de naissances avec ou sans transfert d'anticorps. Lorsque le transfert est considéré, l'asynchronie des naissances conduit à une dilution de l'effet de renforcement de la protection, car seuls les individus nés suffisamment proches du moment de l'épidémie bénéficient effectivement de la protection maternelle. A l'inverse, quand seule l'immunité acquise est prise en compte, cette asynchronie diminue le nombre de juvéniles sensibles nés au moment de l'épidémie ce qui renforce indirectement l'immunité de groupe. Enfin, les résultats présentés jusqu'à présent représentent le cas extrême où une épidémie survient dès que l'immunité est trop basse pour être totalement efficace ce qui amène à prédire des intervalles beaucoup plus courts que les 14 ans du seul intervalle observé dans le système naturel. Cependant, notre modèle prédit qu'un tel intervalle est envisageable si la probabilité d'une infection efficace est inférieure à environ 20% chaque année (figure 8C), une valeur plus élevée que lorsque seule l'immunité acquise est considérée (voire annexe 5).

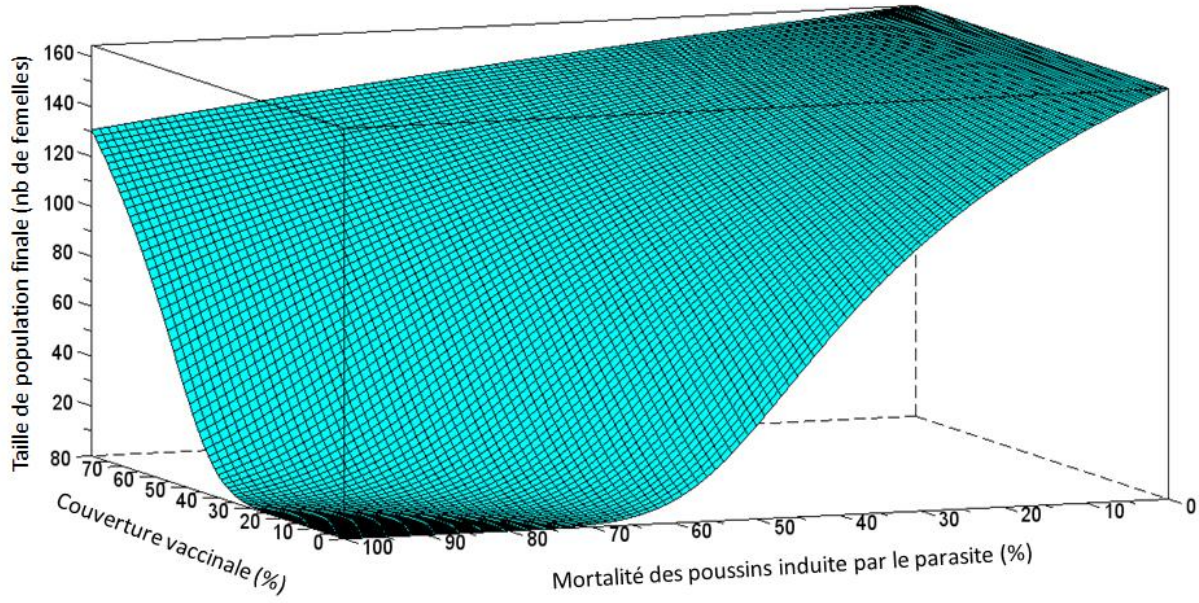
Dans les espèces coloniales, les effets du transfert d'anticorps maternels pourraient aussi être renforcés par des variations temporelles dans la composition des colonies. Chez de nombreuses espèces coloniales où l'entrée en reproduction est retardée, les subadultes sont peu présents sur les colonies (e.g. Reed *et al.* 1999) et interagissent moins que les adultes ou les jeunes avec leurs congénères (e.g. Härkönen & Harding 2001). En particulier, cet argument a été avancé pour expliquer un patron d'exposition différent des subadultes au virus de la maladie de Carré du phoque (Klepac *et al.* 2009) mais pourrait aussi participer au renforcement de l'immunité de groupe sur les colonies de reproduction et contribuer à l'allongement des intervalles entre épidémies.

2. La vaccination transgénérationnelle comme un outil de conservation

Les parasites, en particulier émergents, pourraient représenter un problème de conservation dans une espèce en danger (Daszak *et al.* 2000), en particulier si les changements climatiques augmentent les risques d'émergences (Harvell *et al.* 2002). La vaccination est ainsi régulièrement évoquée comme stratégie de conservation pour protéger des petites populations (Haydon *et al.* 2006; Boyce *et al.* 2011), mais l'évaluation de ses bénéfices s'est toujours cantonnée à la protection directe des adultes ou aux effets

épidémiologiques induits par cette protection. Pourtant, une protection prolongée telle que celle rencontrée chez le puffin cendré (figure 6) pourrait apporter des bénéfices importants directement aux jeunes, en particulier si le parasite est particulièrement virulent pour les jeunes sur les zones de reproduction.

Afin d'illustrer et d'explorer cet aspect, j'ai considéré la situation théorique d'un pathogène, pour lequel un vaccin sûr et efficace est disponible, responsable de mortalités de juvéniles à chaque saison de reproduction dans une petite population d'une espèce d'oiseau longévif (voir l'annexe 3 pour les détails du modèle). Une proportion donnée de la population de femelles adultes peut être vaccinée et elles peuvent alors transférer passivement leur protection à leur jeune. Le pathogène est supposé toucher une proportion variable des jeunes sensibles, les autres juvéniles restent alors sensibles et s'ils atteignent ultérieurement l'âge de se reproduire sont incapables de transférer des anticorps. La démographie de la population d'hôte est modélisée à l'aide d'un modèle matriciel structuré en âge (Caswell 2001) et de fécondités densité-dépendantes. Les valeurs de paramètres utilisées correspondent à une population d'une espèce de Procellariiforme, l'albatros à nez jaune (*Thalassarche chlorynchos*), soumise à des mortalités répétées de poussins pendant la phase d'élevage (Weimerskirch 2004; Rolland *et al.* 2009). Si cette espèce n'est pas directement menacée, elle niche à proximité immédiate de la seule colonie connue de l'albatros d'Amsterdam (*Diomedea amsterdamensis*), une espèce dont la taille de population est estimée à 170 individus (Rains *et al.* 2011) et qui pourrait donc particulièrement souffrir de l'introduction d'un pathogène.



<u>Figure 9 :</u> Effet de la couverture vaccinale sur la taille de population finale (après 300 ans de simulation) pour des niveaux variables de mortalité du parasite pour les poussins. Un effort de vaccination raisonnable permet d'éviter l'extinction de la population : la vaccination de 60-70 femelles la première année puis de 1-2 nouvelles femelles par an permet par exemple de maintenir une couverture vaccinale de 40%.

Le résultat des simulations suggère que la protection transgénérationnelle des nouveau-nés via la vaccination pourrait potentiellement permettre d'éviter l'extinction d'une population de taille réduite (figure 9) en protégeant les jeunes pendant une phase critique. Prendre en compte la possibilité d'une persistance étendue des anticorps maternels telle que rapportée chez le puffin cendré est aussi critique. En effet, dans ce modèle et en se basant sur les patrons de mortalité des poussins chez l'albatros à nez jaune (Weimerskirch 2004), l'exposition au parasite se produit après 4 semaines de vie, un âge auquel les anticorps maternels étaient supposés inefficaces chez les oiseaux jusqu'à aujourd'hui. L'importance des implications de ce résultat est parfaitement illustrée par le fait qu'un modèle paramétré avec une demi-vie des anticorps maternels d'une durée telle qu'anciennement connue (e.g. Davison *et al.* 2008) ou sans transfert d'anticorps maternels peut résulter, en fonction de la virulence du pathogène, en l'extinction de la population d'hôte.

La vaccination des mères pourrait donc représenter une stratégie de conservation particulièrement intéressante pour obtenir une protection passive des jeunes. De plus, en modifiant le profil de sensibilité des jeunes, une telle stratégie pourrait permettre potentiellement de provoquer l'extinction du parasite (Keeling & Rohani 2008), ou à tout le moins d'allonger les intervalles entre les épidémies (figure 8). De tels effets n'ont néanmoins

pas été considérés dans cette approche, et devront être considérés dans de futures analyses. L'emploi de vaccins dans des populations naturelles, en particulier dans des populations en danger, soulève néanmoins de nombreuses questions pratiques et surtout éthiques (Hudson *et al.* 2008). Une attention particulière doit toutefois être portée sur le choix du vaccin. Les vaccins reposant sur des parasites atténués devraient être évités en populations naturelles, de manière à éviter tout risque de recombinaison avec des souches virulentes circulant dans la population ou une évolution du parasite au cours de potentiels événements de transmission.

3. Utilisation des anticorps pour inférer la dispersion

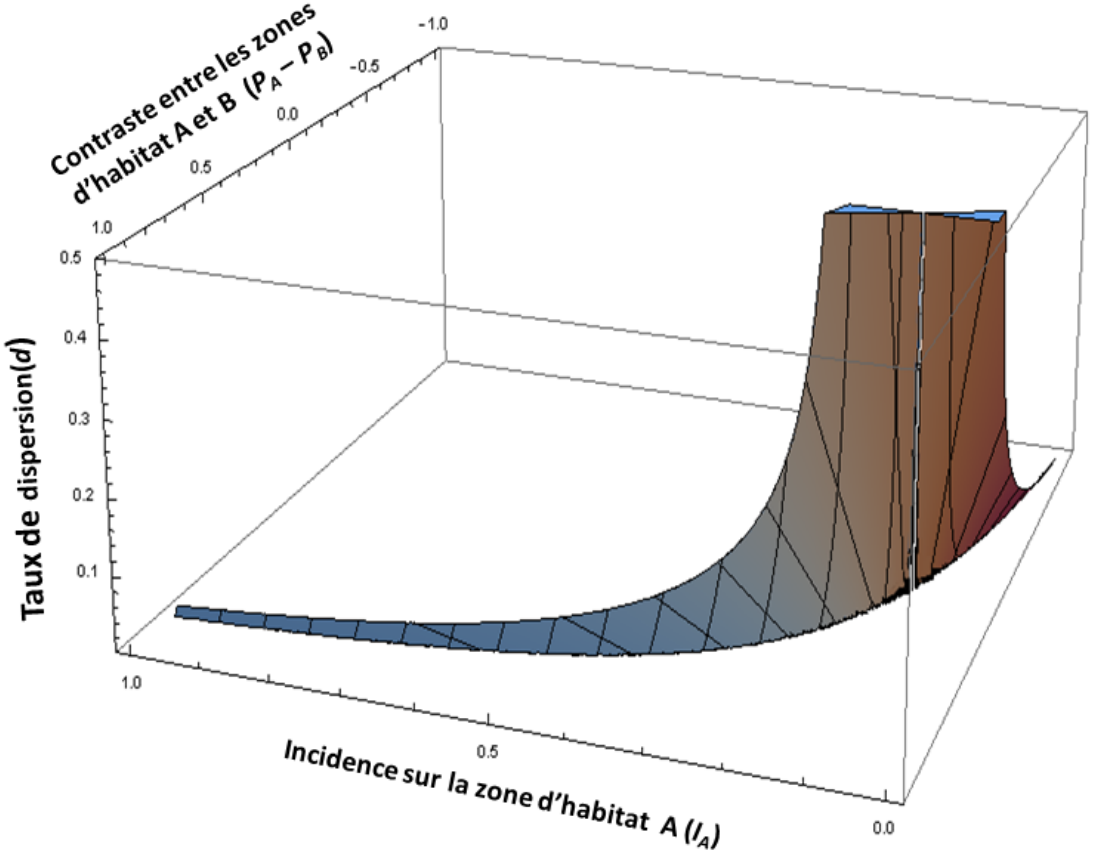
L'une des caractéristiques des anticorps acquis passivement par le jeune, c'est qu'ils reflètent les anticorps circulant chez la mère au moment de la gestation et de l'allaitement chez les mammifères ou au moment de l'oviposition chez les oiseaux. Dans ce dernier groupe, il a même été montré que les niveaux d'anticorps présent dans le vitellus d'une part et dans le système circulatoire du jeune d'autre part sont corrélés au niveau d'anticorps de la mère (Gasparini *et al.* 2002; Grindstaff 2010). Ainsi, récolter des données sur les anticorps maternels revient en quelque sorte à obtenir le profil d'exposition aux parasites de la mère. Ce profil est spécifique de la communauté de parasites rencontrée par la mère qui peut être variable dans l'espace (Holt & Boulinier 2005) et constituer une spécificité d'un environnement local (Poulin 1998). En particulier dans les espèces coloniales et sociales, les individus devraient présenter des histoires d'exposition à des parasites plus similaires à l'intérieur de leur colonie ou groupe social (Delahay *et al.* 2000; Gasparini *et al.* 2001) ce qui devrait se traduire par des profils d'anticorps plus similaires à l'intérieur de ces colonies et groupes sociaux. Il est donc probable que ces profils immunitaires, comme beaucoup d'autres informations sur les parasites (Boulinier *et al.* 2001), puissent apporter des informations sur la dispersion des hôtes. Enfin, étant donné l'abondance dans la littérature d'études rapportant des résultats de sérologie y compris dans des espèces sauvages, l'intérêt de ce type de données dans le cadre de l'estimation de la dispersion, un paramètre difficile à obtenir en populations naturelles, mérite d'être exploré.

Un certain nombre d'hypothèses est nécessaire pour cette première approche, et lever certaines d'entre elles devrait faire l'objet de développements ultérieurs. En particulier, nous allons considérer que la dispersion est limitée à deux zones d'habitat A et B, et qu'elle est identique de A vers B et de B vers A. Nous allons considérer que la dynamique du parasite est

influencée par un taux d'incidence locale qui traduit les nouvelles infections, qui est différent entre les deux zones d'habitat mais inconnu (comme cela est le cas dans la plupart des études rapportant des résultats de sérologie). Nous allons de plus considérer que le parasite circule de manière endémique et a atteint son équilibre (c'est-à-dire que sa prévalence ne varie pas au cours du temps). Enfin, le parasite ne modifie pas la propension de son hôte à disperser et n'a pas d'effet immédiat ou retardé sur la mortalité de l'hôte. Moyennant ces hypothèses, le modèle développé ici (et décrit dans l'Annexe 6) permet d'obtenir un intervalle de valeurs pour le taux de dispersion (d) :

$$d = \frac{(A^- + A^+)(I_A\, A^- - \lambda\, A^+)}{A^+\, B^- - A^-\, B^+}$$

avec A^-, B^-, A^+ et B^+ , le nombre d'individus respectivement négatifs et positifs pour la présence d'anticorps mesurés par une technique immunologique sur les zones d'habitat A et B ; I_A, l'incidence du parasite sur la zone d'habitat A, soit le nombre de nouvelles infections par unité de temps ; λ, le taux de recrutement d'individus reproducteurs dans la population (supposé identique entre A et B).



<u>Figure 10 :</u> Intervalle de taux de dispersion (*d*) plausibles entre les zones d'habitat A et B en fonction de l'incidence du parasite sur la zone d'habitat A (I_A) et du contraste de prévalence (P_A : prévalence du parasite sur la zone d'habitat A ; P_B : prévalence du parasite sur la zone d'habitat B) entre les zones d'habitat($c = P_A - P_B$). Quand les séroprévalences sont fortement contrastées, le taux de dispersion plausible se situe dans un intervalle de valeurs réduites. En revanche, quand ce contraste diminue, l'intervalle de taux de dispersion plausibles estimé est d'une précision moindre, voire impossible à estimer pour $c = 0$.

L'un des facteurs important influençant la précision de l'estimation du taux de dispersion est le contraste de prévalence entre les zones d'habitat A et B (figure 10). En effet, lorsque les prévalences sont peu différentes, il est compliqué de différencier la part de l'importance de la dynamique locale et de la dispersion dans le patron de prévalence observé : une forte dispersion associée à une faible incidence locale et une forte incidence locale associée à une faible dispersion ont en effet exactement la même signature en termes de prévalence. Dans ce cas, la précision de l'estimation des possibles est faible. Par exemple, si les séroprévalences estimées sur A et B sont respectivement de 80% et 20% (fort contraste), le modèle prédit un taux de dispersion compris entre 0 et 0.033. Si le contraste est plus faible avec des prévalences sur A et B de respectivement 60% et 40%, le modèle prédit alors un taux de dispersion compris entre 0 et 0.2. A l'extrême, en l'absence de contraste, l'estimation de la

dispersion est impossible : la prévalence peut en effet être expliquée soit entièrement par la dispersion, soit entièrement par l'incidence locale.

De manière à pouvoir utiliser l'exposition des individus aux parasites en système naturel il faudrait donc pouvoir vérifier que les prévalences vis-à-vis de parasites d'intérêt sont structurées entre différentes colonies ou groupes sociaux. Il est aussi nécessaire de montrer que les anticorps utilisés persistent suffisamment longtemps pour que la mesure de ces anticorps puisse fournir des informations sur une échelle de temps écologiquement pertinente. Par exemple dans le système mouette tridactyle, l'existence d'une structuration spatiale des prévalences entre 8 falaises (χ^2 = 23.79, ddl = 7, p = 0.001) ainsi que la persistance interannuelle des anticorps spécifiques (Staszewski *et al.* 2007b) font de *Borrelia burgdorferi sensu lato* un bon agent infectieux candidat. A l'inverse, dans le même système, les virus influenza ne sont pas de bons candidats. En effet, si leur corrélation entre années (N = 19, r^2 = 0.592, p < 0.001) semble indiquer une persistance interannuelle des taux d'anticorps, les prévalences des virus influenza, obtenues par une analyse ELISA dirigée contre une protéine commune aux différentes souches, ne sont pas structurées dans l'espace à petite échelle (χ^2 = 4.71, ddl = 7, p = 0.70). Au final, si cette approche ne permet pas de résoudre le difficile problème de l'estimation de la dispersion en population naturelle, le cadre développé ici peut ajouter un outil au panel de méthodes de mesure de la dispersion déjà disponibles (génétique des populations, capture – marquage – recapture,…). En particulier, même l'obtention d'un intervalle incluant la valeur réelle de dispersion est intéressante par exemple pour une utilisation ultérieure comme *a priori* dans un cadre d'analyses bayésiennes basées par exemple sur des jeux de données de capture – marquage – recapture à long terme.

IV. PERSPECTIVES

1. Perspectives théoriques

Si nous avons uniquement considéré l'évolution de l'hôte et particulièrement celle du transfert d'anticorps maternels, il est clair que les stratégies de défense chez les hôtes induisent aussi des changements évolutifs chez les parasites. En particulier, des traits d'histoire de vie importants des parasites comme la virulence ou la transmission sont connus pour coévoluer avec les défenses immunitaires des hôtes. La virulence comme la transmission de certains parasites présentent des patrons intéressants dépendant de l'âge de leur hôte et que l'on peut chercher à mettre en relation avec le transfert d'anticorps maternels. Ainsi, le virus de la varicelle se traduit plus souvent par des complications chez les adultes (Preblud 1981; Guess *et al.* 1986), ce qui traduit une virulence augmentant avec l'âge. La présence d'anticorps maternels aurait ainsi pu favoriser la sélection de souches peu virulentes pour les jeunes individus, de la même manière que des vaccins bloquant l'infection sélectionnent pour des souches moins virulentes (Gandon *et al.* 2001). La transmission de certains parasites dépend aussi fortement de l'âge de leur hôte. Par exemple, la rougeole présente un pic de transmission vers l'âge de 2 à 3 ans (Ferrari *et al.* 2010), avec de très bas niveau de transmission pour les individus plus jeunes. Bien que des facteurs extérieurs tels qu'une augmentation des contacts avec l'âge chez les jeunes individus puissent être évoqués, le transfert d'anticorps maternels pourrait aussi jouer un rôle dans ce type de patron. A l'inverse, la préexistence de patrons de virulence et de transmission provoquant des effets très délétères sur les nouveau-nés pourrait avoir contraint l'évolution d'un mécanisme de transfert transgénérationnel d'immunité. Des approches théoriques autorisant l'évolution de caractéristiques du parasite, et leur coévolution avec le transfert d'anticorps maternels, permettraient potentiellement d'apporter des réponses sur l'origine de ces patrons de virulence et de transmission. Cependant, il serait aussi nécessaire de modéliser plus finement la dynamique des anticorps maternels chez les jeunes via des modèles structurés en âge.

Une autre possibilité pour mieux décrire les dynamiques de l'immunité serait de coupler des modèles intra-hôtes avec des modèles épidémiologiques (Alizon & van Baalen 2008) tels que celui que j'ai utilisé. Cela permettrait notamment une meilleure description du transfert d'anticorps maternels dans son ensemble en prenant en compte les dynamiques de l'immunité des femelles et des jeunes. En effet, les taux des femelles et des jeunes sont

corrélés (annexe 3 ; Gasparini *et al.* 2002) et le taux initial des anticorps maternels des jeunes est important pour la persistance de ces anticorps (Grindstaff 2010). De tels modèles permettraient notamment l'étude de l'évolution non seulement de la persistance de la protection immunitaire chez les individus résistants et chez les nouveau-nés mais aussi de s'intéresser aux déterminants de l'évolution de ce que nous avons identifié comme un potentiel trait d'histoire de vie immunitaire relativement négligé, la demi-vie des anticorps.

Par ailleurs, hôtes et parasites sont naturellement rarement distribués de manière homogène dans l'espace (Holt & Boulinier 2005) et la prise en compte de cette dimension spatiale pourrait modifier fortement les bénéfices associés aux réponses acquises. En particulier, dans le cas du transfert d'anticorps, nous avons pu montrer l'importance d'une bonne adéquation entre l'environnement maternel et l'environnement du jeune en termes d'exposition au parasite. Des modèles spatialisés permettraient de prendre en compte les risques de mauvaises adéquations entre mère et jeune notamment en cas de mouvements de la mère entre les occasions de reproduction. La valeur sélective de ces femelles pourrait s'en trouver grandement impactée et les parasites, et particulièrement la nécessité d'un transfert d'anticorps maternels efficace pour permettre la survie du jeune qu'ils imposent, pourraient donc constituer une contrainte ayant conduit à l'évolution d'une certaine fidélité aux sites de reproduction.

Enfin, une autre question qui pourrait se poser concerne l'utilisation de la vaccination comme stratégie de conservation. La vaccination des mères semble pouvoir potentiellement apporter des bénéfices substantiels dans le cas d'un parasite circulant sur les colonies de reproduction et provoquant des mortalités chez les jeunes. Si ces bénéfices perdurent lorsque l'épidémie n'est pas limitée à une classe d'âge reste encore à déterminer. De plus, parallèlement à ce qui a pu être étudié concernant les effets démographiques de la réintroduction d'individus adultes ou juvéniles (Sarrazin & Legendre 2000), il serait intéressant d'évaluer l'intérêt comparé de la vaccination des adultes et des jeunes. Si l'immunité conférée persiste pendant toute la vie de l'individu, la vaccination des jeunes pourrait alors être totalement efficace dans un premier temps avant que des cas d'échec ne surviennent avec l'entrée en reproduction d'individus pouvant transférer des anticorps maternels. A long terme, ces échecs vaccinaux associés à la mortalité naturelle de certains juvéniles protégés avant l'âge reproducteur pourraient conduire à une diminution de la protection de la population et ne permettraient peut être pas d'atteindre un niveau suffisant. Une telle stratégie pourrait aussi poser des problèmes dans le cas d'un arrêt de la vaccination,

puisqu'une proportion moindre d'adultes seraient alors protégés du fait des échecs vaccinaux. Une analyse prenant en compte les coûts et les bénéfices à court et à long terme de la vaccination des jeunes et des adultes serait donc importante pour évaluer l'intérêt de ces deux stratégies dans un objectif de conservation. Pour qu'une telle approche puisse aussi être validée dans plusieurs espèces, il serait aussi nécessaire d'estimer dans quelle mesure le risque d'échec de la vaccination chez les jeunes serait dépendant de l'espèce considérée, notamment en s'intéressant à la variabilité de la persistance des anticorps maternels entre différentes espèces.

2. Perspectives empiriques

La décroissance particulièrement lente des anticorps maternels chez les puffins cendrés pose en effet la question de l'origine de cette variabilité d'un point de vue évolutif. En particulier, si au vue de la théorie des traits d'histoire de vie, un effet de la longévité parait probable, il ne faut pas perdre de vue que les Procellariiformes sont un groupe d'oiseaux particuliers du point de vue de leur physiologie. Ainsi, on pourrait imaginer que, de manière alternative ou additive, un effet phylogénétique sur la décroissance des anticorps puisse survenir. Une répétition du même protocole de vaccination des mères contre le virus de la maladie de Newcastle et de suivi de la décroissance des anticorps chez les jeunes dans de nombreuses espèces aux traits d'histoire de vie variés, incluant des Procellariiformes encore plus longévifs comme les albatros mais aussi d'autres oiseaux longévifs (perroquets, manchots, vautours…) pourrait permettre une approche comparative à même de répondre à cette question.

D'un point de vue plus mécanistique, l'explication de cette demi-vie très étendue est probablement à chercher du côté soit du catabolisme des Igs, et en particulier du FcRY, soit directement du côté de la structure des Igs. Une expérimentation relativement simple consistant à injecter des anticorps purifiés de puffins (dirigés par exemple contre le NDV) à des poules permettrait d'orienter la réponse. En effet, si les demi-vies des anticorps injectés sont allongées, alors l'hypothèse d'une modification de structure des immunoglobulines serait soutenue. Dans le cas contraire, il faudrait aller chercher plus du côté du catabolisme et du FcRY. Dans les deux cas cependant, une approche de génomique comparative ciblant pour différentes espèces les gènes soit des chaines lourdes des IgY, qui se lient au FcRY (He & Bjorkman 2011), soit directement le FcRY pourrait permettre d'identifier des facteurs

génétiques potentiellement impliqués dans une modification de cette interaction. Dans tous les cas, identifier le(s) mécanisme(s) déterminant la très longue persistance d'anticorps chez les puffins cendrés ainsi que les bases génétiques associées pourrait fournir des applications à la fois en biomédecine (Zalevsky *et al.* 2010) mais aussi dans l'industrie de l'élevage. Par exemple, des injections d'anticorps voire des anticorps donnés par voie orale dès l'éclosion pourraient, si des demi-vies de l'ordre de celle des puffins peuvent être obtenues par génie génétique, permettre de protéger les poussins pendant toute la durée de l'élevage et d'éviter des protocoles de vaccination fastidieux et relativement coûteux.

D'un point de vue immuno-écologique, il serait intéressant d'apporter plus d'informations sur les coûts et les bénéfices du transfert d'anticorps maternels en populations naturelles, et ce notamment dans des systèmes mammifères moins bien étudiés que les systèmes aviens. En particulier, s'il a été évoqué chez les oiseaux qu'il est difficile de séparer les coûts de la réponse immunitaire des coûts associés à la reproduction (Nilsson *et al.* 2007), les coûts de la reproduction sont bien étudiés dans des populations de mammifères suivies à long terme (e.g. Clutton-Brock *et al.* 1989; Tavecchia *et al.* 2005; Moyes *et al.* 2006; Bårdsen *et al.* 2008; Bårdsen *et al.* 2009; Bårdsen *et al.* 2011). Il pourrait alors être possible d'estimer les coûts liés au transfert d'anticorps maternels. D'autre part, il serait aussi intéressant de mettre en évidence un réel effet bénéfique sur la survie des jeunes en populations naturelles. Si cet effet n'a jamais été directement mis en évidence, des résultats récents ont montré que des femelles ayant une concentration plasmatique plus forte en ANA (acides anti nucléique) produisent moins de jeunes mais qui survivent mieux (Graham *et al.* 2010). Or les ANA sont supposés représenter un investissement général dans la réponse immunitaire acquise, et de fortes concentrations en ANA pourraient donc aussi signaler indirectement un fort niveau de transfert d'anticorps maternels. Dans ce cas, la meilleure survie des jeunes serait atteinte chez ceux ayant reçu le plus d'anticorps maternels d'une manière générale. Chez ces espèces de mammifères ongulés, le transfert d'anticorps présente aussi la spécificité de ne plus avoir qu'une action locale au niveau du tractus digestif. Si la mesure des ANAs chez les jeunes pourrait permettre d'en apprendre un peu plus dans ce système, une expérience de supplémentation des jeunes notamment en IgE spécifiques des parasites intestinaux rencontrés serait intéressante. Si les jeunes recevant la supplémentation performent mieux ou à l'identique que ceux ayant naturellement des anticorps, alors la survie augmentée serait directement le fait des anticorps maternels.

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ANNEXE 1

Manuscrit 1:

Coevolution between maternal transfer of immunity and other resistance strategies against pathogens

Etat du manuscrit : en révision après soumission

Coevolution between maternal transfer of immunity

and other resistance strategies against pathogens

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Abstract:

Among the wide variety of resistance mechanisms to parasitism, the transgenerational transfer of immunity from mother to offspring has largely been overlooked and never included in evolutionary or coevolutionary studies of resistance mechanisms. Here we study the evolution and coevolution of various resistance mechanisms with a special focus on maternal transfer of immunity. In particular we show that maternal transfer of immunity is only expected to evolve when cross immunity is high and when the pathogens have an intermediate virulence. We also show that the outcome of the coevolution between various resistance mechanisms depends critically on the life span of the host. We predict that short-lived species should invest in avoidance strategies, while long-lived species should invest in acquired resistance mechanisms. These results may help understanding the diversity of resistance strategies that have evolved in vertebrate species. Our framework also provides a general basis for the study of the evolution of other transgenerational resistance mechanisms.

1.Introduction

Parasites can impose important fitness costs on their hosts (Grenfell & Dobson 1995) and, in response, hosts have evolved different costly defense strategies that aim at reducing the deleterious effects of parasites (Sheldon & Verhulst 1996; Sandland & Minchella 2003). Defense strategies can include avoidance mechanisms that prevent infection by a parasite (e.g. Hart 1990) or different mechanisms that directly reduce the impact of a parasite when it has successfully infected the host. In particular, the immune system has evolved as a way to fight infection inside hosts, and in many cases eradicating the parasite. In vertebrates, the efficiency of the immune system relies on a combination of innate and acquired responses and on the ability of recovered hosts to remain protected for an extended period of time (Frank 2002). Moreover, an important arm of the acquired immune response of vertebrates induces the production of parasite specific immune compounds, the antibodies, which can be transferred by mothers to their offspring through the transfer of maternal antibodies (Carlier & Truyens 1995). Although decaying after birth, these antibodies have the potential to provide newborns with a protection early in life, a critical time at which their own immune system may not be fully mature (Grindstaff *et al.* 2003; Boulinier & Staszewski 2008; Hasselquist & Nilsson 2009).

It is thus apparent that defense against parasites relies on a combination of different resistance mechanisms (Schulenburg *et al.* 2009; Schmid-Hempel 2011). The generation and coexistence of such a large range of defense strategies has been the focus of several theoretical studies (see Boots *et al.* 2009 for a review). Overall, these studies show that the evolution and coevolution of resistance mechanisms depend critically on (i) the fitness costs imposed by the infection, (ii) the epidemiological feedbacks, and (iii) the shape of the trade-off between defense strategies and various fitness components of the host.

The evolution of resistance to parasites has indeed been extensively studied from a theoretical point of view using various methods ranging from population genetics to adaptive dynamics models (Gillespie 1975). Adaptive dynamics has been used to explore the evolution of a variety of resistance mechanisms including avoidance (Antonovics & Thrall 1994; Bowers *et al.* 1994; Boots & Haraguchi 1999), tolerance (Boots & Bowers 1999; Roy & Kirchner 2000), recovery (van Baalen 1998) and acquired immunity (Boots & Bowers 2004; Miller *et al.* 2007). Some of the studies focused on the coevolution of different resistance mechanisms (e.g. Boots & Bowers 1999, 2004; Carval & Ferriere 2010) while some others explicitly tackled the importance of other characteristics of either the host (Gandon *et al.* 2002; Zuk & Stoehr 2002; Miller *et al.* 2007) or the parasite (van Baalen 1998; Alizon & van Baalen 2008). Yet, and in spite of its impact on the fitness of juveniles and its potential influence on epidemiological dynamics, the evolution of the transgenerational transfer of immunity has been largely overlooked in previous studies.

In an attempt to fill this gap, we study here the evolution and the coevolution between different resistance mechanisms using a classical epidemiological model modified to include the transgenerational transfer of immunity. Resistance can thus be achieved at different steps of the interaction between the host and the parasite: (i) avoidance before the infection by the parasite, (ii) recovery and (iii) transgenerational transfer of immunity. Each of these resistance mechanisms has implications on the epidemiology which in turns feeds back on the intensity of the selection for resistance to the parasite. We derive a general invasion condition using the Next Generation Theorem (van den Driessche & Watmough 2002; Hurford *et al.* 2010) and use this host fitness function to study the evolution of the transfer of maternal immunity. We first consider the force of infection as a constant in a model with one or two strains of parasite. Then, focusing on the one strain model, we add epidemiological feedbacks to study the effect of host and parasite traits on the evolution of the maternal transfer of immunity. We

also study the coevolution between the transfer of maternal immunity and recovery rate, and finally add avoidance as another coevolving resistance mechanism. We discuss how particular traits of the host life cycle may mold the allocation between different resistance strategies.

2. Epidemiological model

We model a population of asexually reproducing hosts exposed to several parasites, each of which being characterized by its force of infection, h, and its virulence, α (i.e. additional mortality). We assume no vertical transmission and no effect of parasites on host fecundity. Four different types of hosts are considered: susceptible, infected, recovered and protected by maternal immunity. The density of hosts in these four different compartments is noted S, I, R and M, respectively. The following system of differential equations (the dot notation refers to differentiation with respect to time) describes the change in these different compartments when a single parasite is considered (see also figure 1):

$$\begin{aligned}
\dot{M} &= \lambda\theta R - (\delta_M + \mu)M \\
\dot{S} &= \lambda(M + S + I + (1-\theta)R) + \delta_M M + \delta_R R - (\mu + (1-\pi)h)S \\
\dot{I} &= (1-\pi)hS - (\mu + \alpha + \gamma)I \\
\dot{R} &= \gamma I - (\mu + \delta_R)R
\end{aligned} \qquad (1)$$

We assume that all hosts can reproduce with a rate $\lambda = r - \kappa N$ which depends on host fecundity, r, the total host density, $N = M + S + I + R$, and a crowding parameter κ (which is related to the carrying capacity by the relation $K = r / \kappa$). Newly produced offspring are all susceptible except a fraction θ of the offspring of recovered hosts which are assumed to be fully protected by maternal immunity. The protection of those individuals is temporary and depends on δ_M, the rate of loss of maternal immunity. Susceptible hosts can become infected with a rate that depends on the force of infection of the parasite, h, and the ability π of the host

to reduce the probability of infection by avoiding infection. The natural mortality rate of the host is μ, but infected individuals suffer an additional mortality due to parasite virulence, α. Infected individuals can recover with a rate, γ, and they remain in the recovered compartment until they lose immunity, which occurs with a rate δ_R. In the absence of the parasite, the host population reaches a stable equilibrium with a density $\hat{S}$ of only susceptible hosts. The parasite will invade this fully naïve host population if its basic reproductive ratio is higher than one (see appendix): $R_0 = \frac{(1-\pi)h}{\mu+\alpha+\gamma}\hat{S} > 1$. After this invasion, the system reaches a new stable endemic equilibrium where all the four different types of hosts coexist.

In this model, the host may thus defend itself through three different, yet not mutually exclusive, ways. First, it may try to limit early on the risk of being infected (avoidance, host defense trait π). Second, upon infection, it may recover from the infection (recovery rate, host defense trait γ), and transmit this protection via maternal immunity (transfer of maternal immunity, host defense trait θ). In the following, we study the evolution of the maternal transfer of immunity and its coevolution with other resistance mechanisms.

3. Evolutionary model

To study host evolution we derive a general invasion criteria for a rare mutant host that could affect any of the host resistance traits (see appendix 1). This invasion condition depends on the costs of resistance on both fecundity and survival. In the following, however, we will restrict our study to a simple cost function that will be assumed to affect only fecundity (see Appendix 1 for the full definition of the trade-off). There is empirical evidence to support the idea of a cost of resistance to parasites in terms of reduced birth rate (e.g. through an increase of developmental period; Boots & Begon 1993). Maternal transfer of antibodies in particular is likely to be a costly mechanism for the mother given the amount of

antibodies transferred (Kowalczyk *et al.* 1985; Coe *et al.* 1994) and the set up and maintenance of the specific receptors required for the transfer (West *et al.* 2004; Roopenian & Akilesh 2007). We further assume a slightly accelerating trade-off shape which has been shown to prevent evolutionary branching (Boots & Haraguchi 1999; Hoyle *et al.* 2008) because it simplifies the evaluation of the optimal investment in the different resistance strategies.

The general invasion condition we derive in the appendix can be understood as a quantity that relates to the average number of offspring produced in one generation by a mutant host in a resident host population (see appendix 1 and figure S1):

$$R_m = \left(\tau^m_{S\to S} + \sqrt{4\tau^m_{M\to S}\tau^m_{S\to M} + {\tau^m_{S\to S}}^2}\right)/2$$

where $\tau^m_{S\to S}$, $\tau^m_{M\to S}$ and $\tau^m_{S\to M}$ refer to the different transitions leading to the production of two types of newborns mutants: the ones that are susceptible, S, and the ones that are protected by maternal antibodies, M. Under the hypothesis of adaptive dynamics (i.e. rare mutations), this invasion condition can be used to find singular points and to characterize their evolutionary properties in order to determine the optimal investment in the different resistance traits. In all the scenarios we study below the evolutionary equilibria we found are both convergence and evolutionary stable and thus correspond to continuously stable strategies (Geritz *et al.* 1998).

4. Evolution of maternal transfer of immunity with a constant force of infection

The evolutionary stable investment in the maternal transfer of immunity is an increasing and saturating function of the force of infection, *h* (see supplementary figure S1A). In other words, and not surprisingly, higher levels of resistance are evolving when the rate of acquisition of a new infection is increasing. Perhaps more surprisingly we find that an increase of pathogen virulence has a non monotonous effect on the evolution of maternal

transfer (see supplementary figure S1B). This is because when virulence becomes very high it is not worth investing in a resistance mechanism that will never be expressed as most individuals die from the infection and never recover (recall that only recovered individuals can transfer immunity). In addition, we also find a non monotonous effect of recovery on the evolution of maternal transfer of immunity (see supplementary figure S1C). Indeed when recovery becomes very high, the best strategy is to let the offspring encounter the parasite as they will recover very fast from the infection anyway. We will come back to these effects in the following section where we will allow the force of infection to be a dynamical variable that depends on parasite density and transmission rate.

The model with a constant force of infection can also be used to illustrate the potential effects of being exposed to multiple parasites on the evolution of maternal transfer of immunity. We explain in the appendix how to modify the above model to account for the circulation of two different strains (described by their force of infection h_1 and h_2). We assume that a host that has recovered from an infection by one strain can transfer immunity to this strain, but the cross immunity against the other strain is imperfect and measured by the parameter χ. When $\chi = 0$ there is no cross immunity and, in contrast, when $\chi = 1$ cross immunity is perfect and the two parasites model reduces to the previously described model with $h = h_1 + h_2$. We further introduce a parameter η describing the potential bias towards the transmission of one strain over the other. More precisely, holding the total force of infection constant and equal to $h = h1 + h2$, we assume the force of infection of each strain to be equal to $h_1 = \eta h$ and $h_2 = (1 - \eta)h$. A value of η of 0.5 maximizes the symmetry of the force of infection ($h_1 = h_2$), while any deviation from 0.5 increases the bias towards one strain.

Figure 2 shows that lower levels of cross immunity select for decreased levels of maternal transfer of immunity. When cross immunity is high, the own protection of mothers is a good predictor of the efficiency of the protection they can provide to their newborns and

they are thus expected to transfer their immune protection. A bias in the force of infection towards one strain increases also the predictability of the environment and always selects for higher levels of maternal transfer of immunity.

As outlined by Boots et al. (2009), one important force driving the evolution of resistance mechanisms is how epidemiology feeds back on the selection gradients. In the following section, we investigate how such epidemiological feedbacks will affect the evolution of the transfer of maternal immunity.

5. Evolution of maternal transfer of immunity with epidemiological feedbacks

Here, we focus on the evolution of the ability to transfer maternal immunity when the force of infection includes the effects of epidemiology, $h = \beta I$. For the sake of simplicity, we will restrict our analysis to the one strain model. The duration of transgenerational protection and the lifespan of the host have monotonous effects on the evolutionary stable ability to transfer immunity to offspring. The optimal transfer of immunity is an increasing function of the host lifespan (figure 3A) because, as outlined by Miller et al. (2007), increasing host life span modifies the prevalence of the parasite and increases the force of infection. Thus it increasingly pays off to invest in transgenerational protection with longer life expectancy. In contrast, the evolutionary stable level of transfer is a decreasing function of the rate of loss of maternal protection (figure 3B): the longer the maternal protection, the higher the benefits of this mechanism. Ultimately, when maternal protection is lost sufficiently quickly, there is no more investment in maternal transfer of immunity and the model reduces to a classical SIRS model.

Increasing the virulence of the parasite has a non monotonous effect on the evolution of the maternal transfer of immunity (figure 3C). At first, the investment in the transfer of maternal immunity increases towards a maximum and then decreases until eventually

reaching a point when there is no more investment in the transgenerational transfer. This drop is due to two effects. First, as mentioned earlier (see above section), higher virulence reduces the number of recovered individuals, and thus the efficacy of the transfer of immunity. Second, when virulence reaches high values, infected hosts die rapidly which causes a rapid drop in the force of infection. As a consequence, the transfer of maternal immunity is only expected to evolve for intermediate levels of parasite virulence.

Optimal investment in the transfer of maternal immunity is also a non monotonous function of the recovery rate (figure 3D). Because only recovered individuals can transfer immunity, low recovery rates do not offer the possibility for an efficient transfer of immunity. Evolutionary stable maternal transfer increases with the rate of recovery and reaches a maximum for intermediate recovery rates. Beyond this point, larger recovery rates reduce the fitness cost of infection (see above section with constant force of infection) and select for lower levels of transfer. This effect is amplified by the epidemiological feedback as higher recovery rates decrease the force of infection. Increasing the rate of loss of immunity in adults decreases the maximal level of transfer but allows the selection for this mechanism on a wider and higher range of recovery rates.

6. Coevolution between maternal transfer of immunity and other traits

The invasion criteria derived in the appendix can be used to determine the coevolutionary stable set of strategies (CoESS, see also van Baalen 1998). We first consider the coevolutionary dynamics between maternal transfer of immunity and recovery. The CoESS of both traits is an increasing saturating function of the baseline duration of the lifespan (figure 4A) with maternal transfer of immunity (black curve) starting to increase after recovery rate (gray curve) and reaching lower coevolutionary stable levels. These levels are maximized for intermediate values of the rate of loss of immunity (figure 4B). When

immunity decays quickly enough, there is no more transfer of maternal immunity and acquired resistance only relies on recovery. Similarly, virulence has a non monotonous effect on the coevolutionary outcome. First, an increase in virulence selects for higher investment in both recovery rate and maternal transfer of immunity (figure 4C). Second, for higher virulence levels, both recovery and maternal transfer decrease with virulence. As outlined earlier, the effects of all those parameters are mediated by different epidemiological feedbacks acting on the prevalence of the parasite. Finally, it is interesting to note that the transfer of immunity is consistently selected for on a smaller range of parameter values than recovery rate. This is because the evolution of the transfer of maternal immunity is constrained by the requirement of a prior evolution of recovery (only recovered individuals can transmit).

Recovery and maternal transfer of immunity are both acquired responses to parasites that represent only part of possible responses to parasites. Avoidance is another resistance mechanism relying on an ability of the host to directly reduce the infection probability. Not surprisingly, we find that an increase in the ability to avoid infection by the parasite results in decreased coevolutionary levels of both recovery and maternal transfer of immunity (figure 4D). When avoidance is sufficiently high there is no more investment in acquired resistance mechanisms. Yet, these results rely on the assumption that avoidance mechanisms are fixed quantities. This alternative resistance mechanism may, however, coevolve with the other strategies.

When we allow the three different resistance mechanisms to evolve jointly we find that the coevolutionary outcome is very sensitive to the host lifespan (figure 5). When the life span is short, evolution leads to investment into the ability to avoid the infection by the parasite. When the life span increases above a threshold value, a bistability emerges where two coevolutionary outcomes may occur. Depending on the initial conditions, the host may continue to invest only in avoidance or may invest only in a combination of recovery and

maternal transfer of immunity. This is because increasing the host lifespan increases the probability of encountering the parasite again and thus increases the benefits of developing an acquired resistance. To summarize, short lived individuals are expected to invest in innate (i.e. avoidance) rather than acquired (i.e. recovery rate and maternal transfer of immunity) defense mechanisms whereas longer lived individuals are expected to display the opposite pattern.

7. Discussion

In spite of an abundant literature on the evolution of various host defense mechanisms against parasites (van Baalen 1998; Miller *et al.* 2007; Boots *et al.* 2009), the evolution of the transgenerational transfer of immunity has been largely overlooked in previous studies. This is probably due to the intrinsic complexity which arises when one wants to model this effect as it becomes necessary to add a class of individuals which are temporary protected by a vertically transmitted immunity. Here we provide a general theoretical framework to study the evolution of this trait, as well as more classical resistance mechanisms against parasites (i.e. avoidance of infection, recovery).

We first analyze the evolution of the transgenerational transfer of immunity when the other traits remain fixed. This allows us to show that several parameters like parasite virulence and host recovery rates have a non monotonous effect on the evolution of this trait. This effect is partly mediated by the feedback of epidemiology on this evolution via the force of infection (see Boots *et al.* 2009 for a general discussion of these feedbacks on other resistance mechanisms). Another important result of our study is that, when two strains of parasite are circulating, the evolutionary outcome depends on the level of cross immunity provided by the protection acquired vertically. Less cross immunity hinders the evolution of this resistance mechanism. This result illustrates that what matters for the evolution of the transfer of immunity is the correlation between the environment of the mother and the

environment of the offspring (see also Mousseau & Fox 1998 for a discussion of the importance of these correlations for the evolution of maternal effects). Considering the variations in time and space of the distribution of parasites and their hosts could bring more insight into our understanding of the evolution of passive protection by transfer of maternal immunity.

We also studied the coevolution between maternal transfer of immunity and other resistance mechanisms. In particular, our model shows that parasite virulence can mould the investment in both recovery and transfer of immunity, with maternal transfer of immunity being selected for on a more restricted range of values. We also show the host lifespan has a massive impact on the allocation between avoidance and acquired resistance strategies like recovery and maternal transfer of immunity: short-lived hosts always invest in avoidance, while long-lived hosts display a bistability between an investment in innate or acquired immunity. Lifespan has indeed been suggested as one of the life history traits that could modify the evolution of physiological processes such as resistance to parasites (Ricklefs & Wikelski 2002). Because they are more likely to encounter the same parasites on multiple occasions during their lifetime, long-lived species are expected to favor investment in acquired responses (Lee 2006). The results of our theoretical analyses support this hypothesis and highlight the need for more empirical studies of the variability of (maternal) immunity in species with contrasted life histories.

Perspectives

In our model, transgenerational immune protection fully protects newborns with maternal antibodies (*M*) from an infection by a parasite their mother encountered. Such protective transgenerational immunity is notably used in the poultry industry to protect chickens against some pathogens (Davison *et al.* 2008) and it likely occurs in natural host-

parasite systems, although little evidence exists yet (Boulinier & Staszewski 2008). High maternal antibody levels early after birth have been shown to prevent newborns from mounting a costly immune response (Staszewski *et al.* 2007). Later in early life, decaying levels of maternal antibodies could provide a partial protection resulting in an activation of the newborns immune system at reduced costs (Zinkernagel 2003; Navarini *et al.* 2010). This would increase the infectious period and the prevalence of the parasite, and the effect on the CoESS would be very similar to a reduction in the virulence of the parasite (see figure 4C). Even after complete disappearance of the passive protection, newborns having received antibodies could benefit from higher recovery rates when later exposed to the parasite (Lemke *et al.* 2003; Grindstaff *et al.* 2006).Accounting for that sort of effect would add a direct link between maternal transfer of immunity and recovery rate and would certainly modify the CoESS between those acquired resistance traits. Our modeling framework could be readily modified to explore the quantitative consequences of this new scenario.

Our model does not account for the age structure of the host population. Age structure may indeed modify the evolution of the transgenerational transfer of immunity as newborns may face less virulent parasites. Several disease agents indeed show a strong age dependent virulence. For instance, varicella zoster virus leads more often to complications and death in adults than in children (Preblud 1981; Guess et al. 1986). However, as mentioned above, the immune system requires time to achieve its full efficiency and newborns only display part of their future adult ability to recover from the infection. Accounting for age structure in our framework may thus give interesting insights on how the occurrence of such variable patterns of virulence and ability to recover may modify the evolution of acquired resistance mechanisms.

Our model focused on the evolution of host defense strategies but host resistance feeds back on the evolution of the parasite. In particular, increase in recovery rate has been shown

to result in an increase in parasite virulence (van Baalen 1998). As maternal transfer of immunity is associated with recovery and results in the protection of another part of the population, accounting for this mechanism should thus lead to decreased levels of virulence. We also showed that considering several strains of parasite modified the evolution of the transfer of maternal immunity. How maternal transfer of immunity would affect the coexistence and the characteristics of different parasite strains remains to be determined. Our theoretical framework, in particular when accounting for two strains, could be readily modified to provide insight on that question.

Concluding remark

Although we primarily built this framework with vertebrate models in mind as the transfer of maternal antibodies is a mechanism specific to this group, there is no reason to exclude the possibility of specific acquired resistance mechanisms in invertebrates (Schmid-Hempel 2005). There is also increasing evidence for specific transgenerational immune priming in invertebrates (Little & Kraaijeveld 2004; Sadd & Schmid-Hempel 2007; Tidbury *et al.* 2011) and our model could thus be readily extended to that situation. It could also be developed to explore the evolution of genetic resistance mechanisms such as CRISPR (Clustered Regularly Interspaced Short Palyndromic Repeats) in bacteria (Sorek et al. 2008) or epigenetic resistance mechanisms like herbivory in plants (Agrawal 2002). By inserting some more specific biological features and including various resistance mechanisms, the general modeling framework we develop here could thus contribute to a better understanding of the evolution and coevolution of transgenerational resistance mechanisms.

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Figure legends

Figure 1: Flow diagram of the epidemiological model including the maternal transfer of antibodies to offspring. The model distinguish four categories of individuals: maternally protected (*M*), susceptible (*S*), infected (*I*) and recovered and immune (*R*). The ability of immune individuals to transfer antibodies to their newborns is described by the parameter θ. Other parameters: λ, birth rate; δ_R, rate of loss of immune protection; δ_M, rate of loss of the maternal protection; *h*, force of infection, equates to βI when epidemiological feedbacks are considered; γ, recovery rate; α, virulence of the parasite (resulting in an increase in the mortality of infected individuals); μ, natural mortality rate.

Figure 2: Evolutionary stable investment in the ability to transfer maternal immunity (θ) as a function of the cross protection of newborns (χ) in a model with two strains of parasites described by their force of infection, respectively h_1 and h_2. The plain curve is obtained when the parasites are identical with regard to their force of infection ($h_1 = h_2 = 1$) and the dashed curve when the force of infection is skewed towards one parasite ($h_1 = 0.2$; $h_2 = 1.8$). Other parameters: $r_0 = 1.5$; $\mu = 0.05$; $\kappa = 0.1$; $c_{r,\theta} = 0.075$; $k_\theta = 1/0.9$; $\alpha = 5$; $\pi = 0$; $\gamma = 0.8$; $\delta_R = 1$; $\delta_M = 1$.

Figure 3: Evolutionary stable investment in transgenerational transfer of immunity ability (θ) as a function of different parameters. (A) Effect of the host life span ($1/\mu$) on the evolutionary stable ability to transfer maternal immunity. (B) Effect of the rate of loss of maternal protection (δ_M) on the evolutionary stable ability to transfer maternal immunity. (C) Effect of the virulence of the parasite (α) on the evolutionary stable ability to transfer maternal

immunity. (D) Effect of the recovery rate (γ) on the evolutionary stable ability to transfer maternal immunity for different rates of loss of immune protection (δ_R). Default parameter values used in the figures: $r_0 = 1.5$; $\mu = 0.1$; $\kappa = 0.1$; $c_{r,\theta} = 0.075$; $k_\theta = 1/0.9$; $\alpha = 2.75$; $\beta = 2$; $\delta_R = 1$; $\delta_M = 1$; $\gamma = 0.6$.

Figure 4: Coevolution between recovery rate (γ, gray line) and transgenerational transfer of immunity ability (θ, black line). (A) Effect of the host life span ($1/\mu$) on the evolutionary stable values of recovery rate (γ) and transgenerational transfer of immunity ability (θ). (B) Effect of the rate of loss of immunity (δ_R) on the evolutionary stable values of recovery rate (γ) and transgenerational transfer of immunity ability (θ). (C) Effect of the virulence of the parasite (α) evolutionary stable values of recovery rate (γ) and transgenerational transfer of immunity ability (θ). (D) Effect of the avoidance ability (π) on the evolutionary stable values of recovery rate (γ) and transgenerational transfer of immunity ability (θ) Default parameter values used in the figures: $r_0 = 1.5$; $\mu = 0.1$; $\kappa = 0.1$; $c_{r,\theta} = 0.075$; $c_{r,\gamma} = 0.2$; $k_\theta = 1/0.9$; $k_\gamma = 1/0.9$; $\alpha = 2.75$; $\beta = 2$; $\pi = 0$; $\delta_R = 1$; $\delta_M = 1$.

Figure 5: Effect of the host life span ($1/\mu$) on the coevolutionary stable investment in recovery rate (γ, dark grey lines), maternal transfer ability (θ, light grey lines) and avoidance ability (π, black lines) depending on initial conditions (plain curves: $\pi_{start} = 0.7$; $\gamma_{start} = 0.01$; $\theta_{start} = 0.01$; dashed curves: $\pi_{start} = 0.01$; $\gamma_{start} = 0.7$; $\theta_{start} = 0.7$). When lifespan is short, investment is always solely in avoidance ability (grey background). When lifespan increases (white background), investment shifts from avoidance to a combination of recovery and maternal transfer depending on initial conditions. Default parameter values: $r_0 = 1.5$; $\mu = 0.1$; $\kappa = 0.1$;

$c_{r,\pi} = 0.3$; $c_{r,y} = 0.2$; $c_{r,\theta} = 0.08$; $k_{r,\pi} = 0.5$; $k_{r,y} = 1/0.9$; $k_{r,\theta} = 1/0.9$; $\alpha = 2.75$; $\beta = 2$; $\delta_R = 0.5$; $\delta_M = 0.75$.

Figures

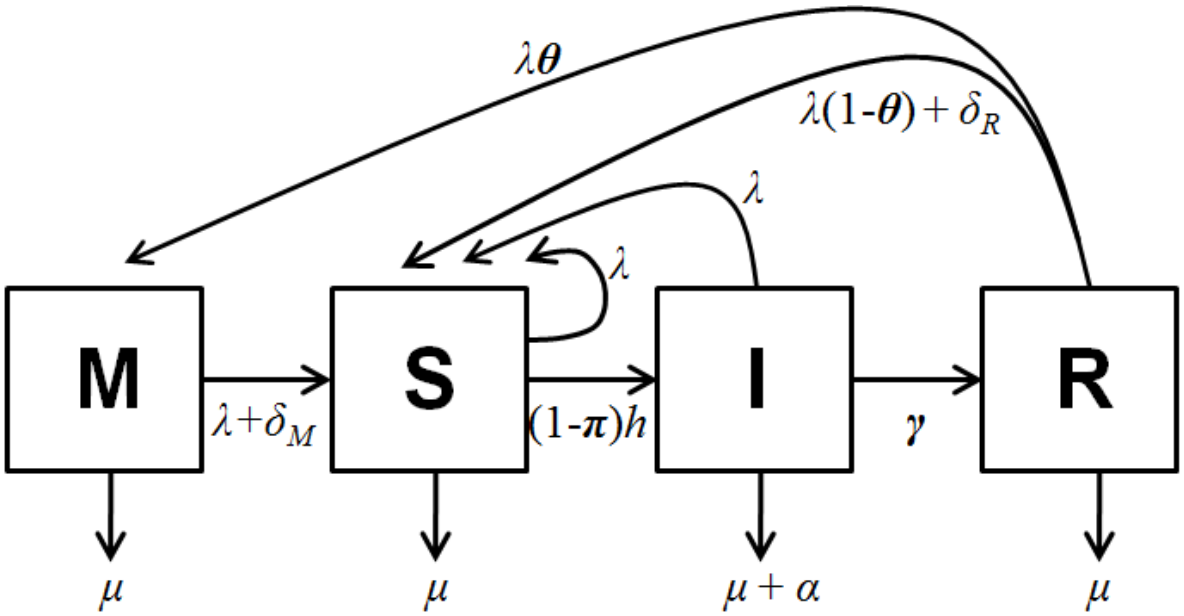


Figure 1

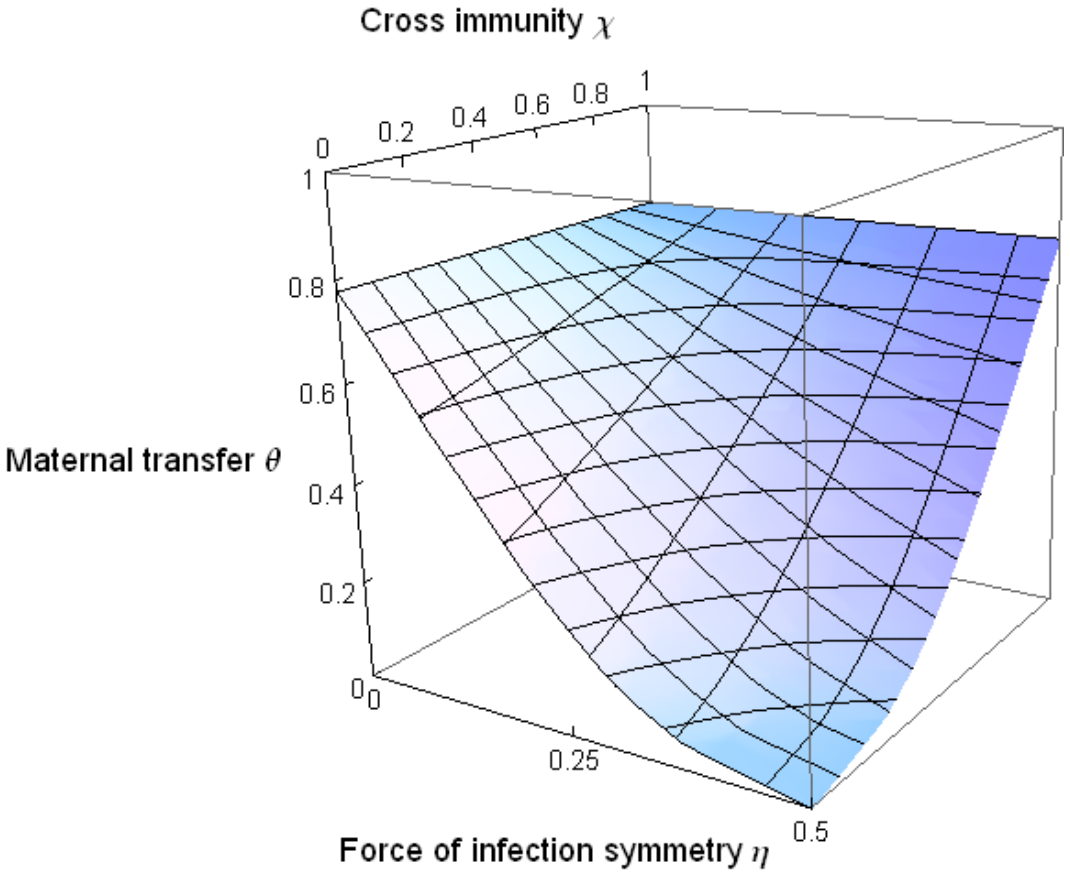

Cross immunity χ
0
0.2
0.4
0.6
0.8
1
Maternal transfer θ
0
0.2
0.4
0.6
0.8
1
0
0.25
0.5
Force of infection symmetry η

Figure 2

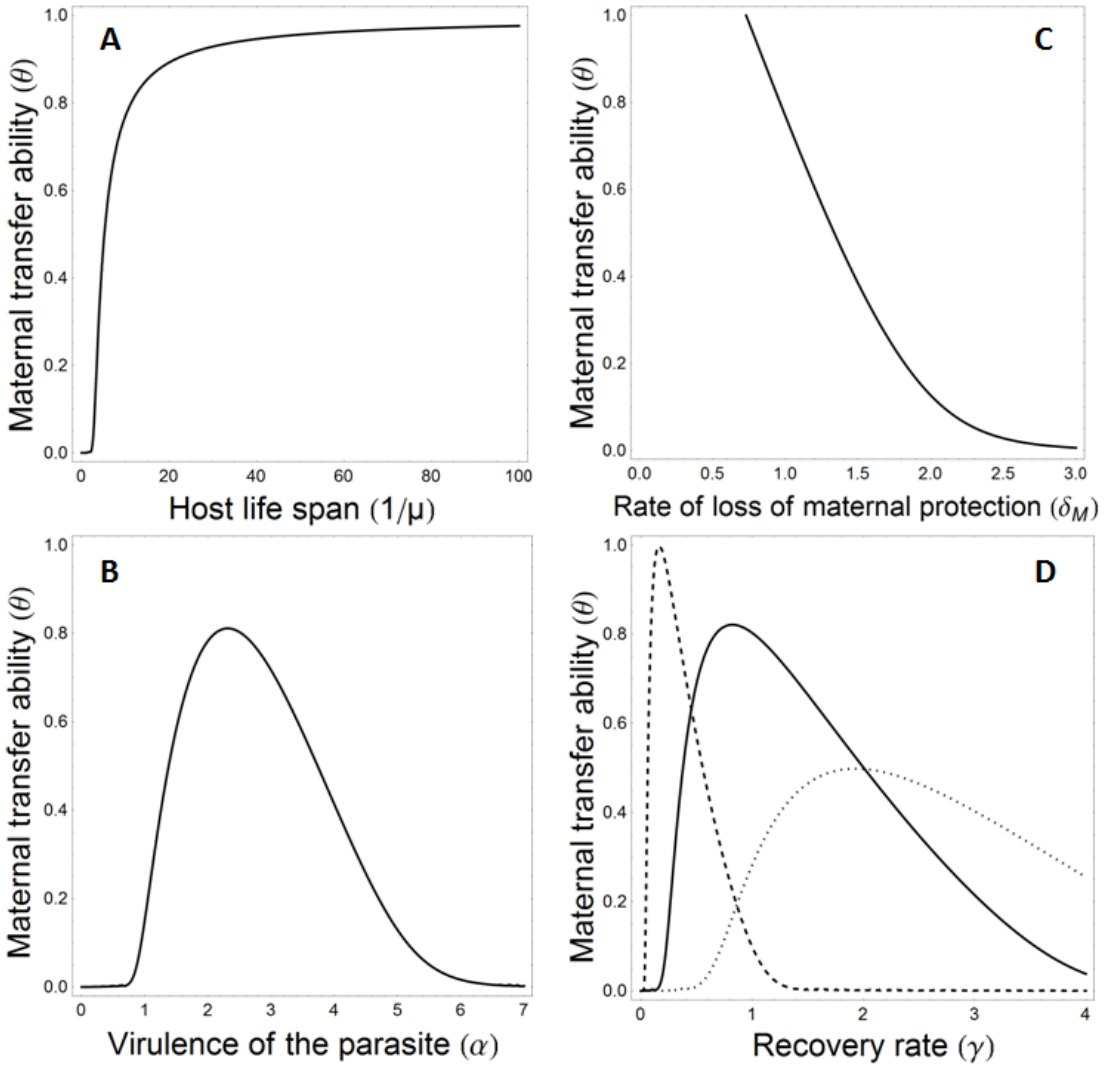

A
Maternal transfer ability (θ)
Host life span (1/μ)
C
Rate of loss of maternal protection (δ_M)
B
Virulence of the parasite (α)
D
Recovery rate (γ)

Figure 3

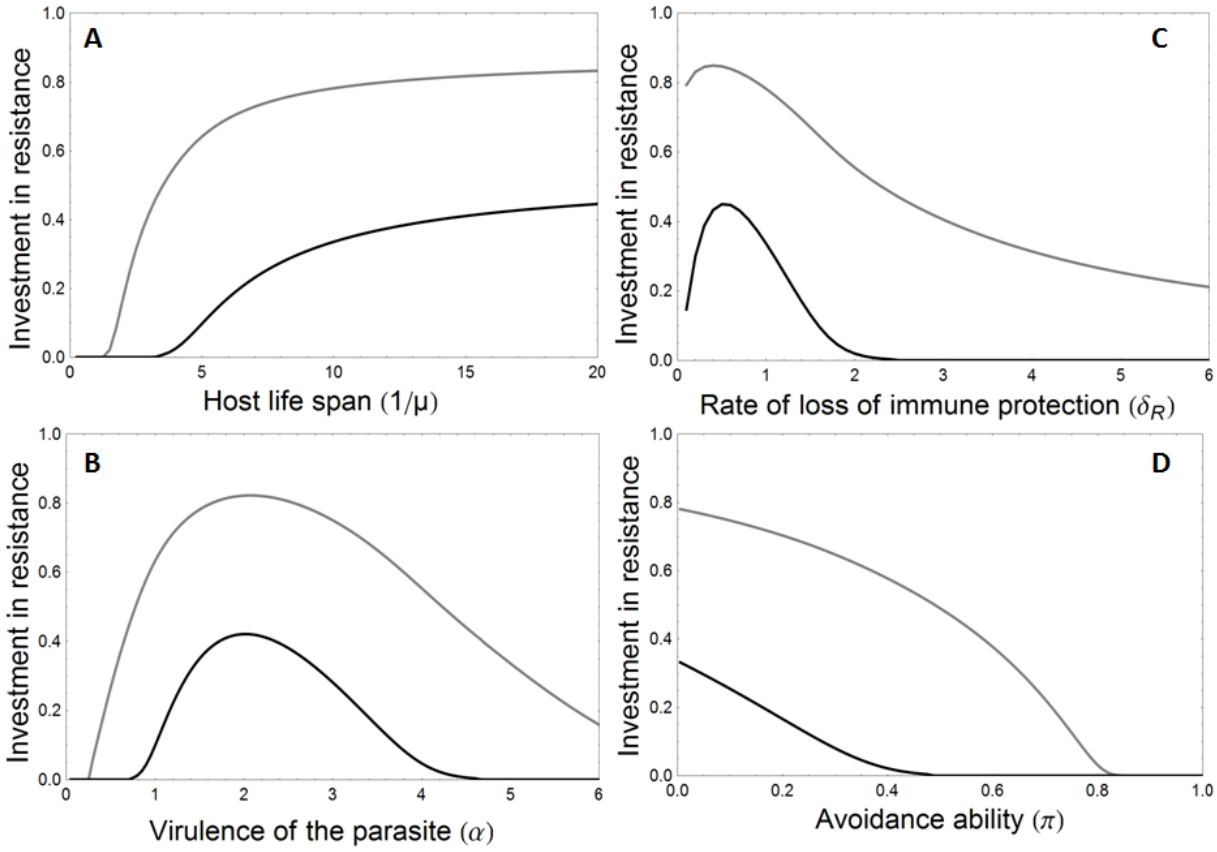

A
Investment in resistance
Host life span (1/μ)
C
Rate of loss of immune protection (δ_R)
B
Virulence of the parasite (α)
D
Avoidance ability (π)

Figure 4

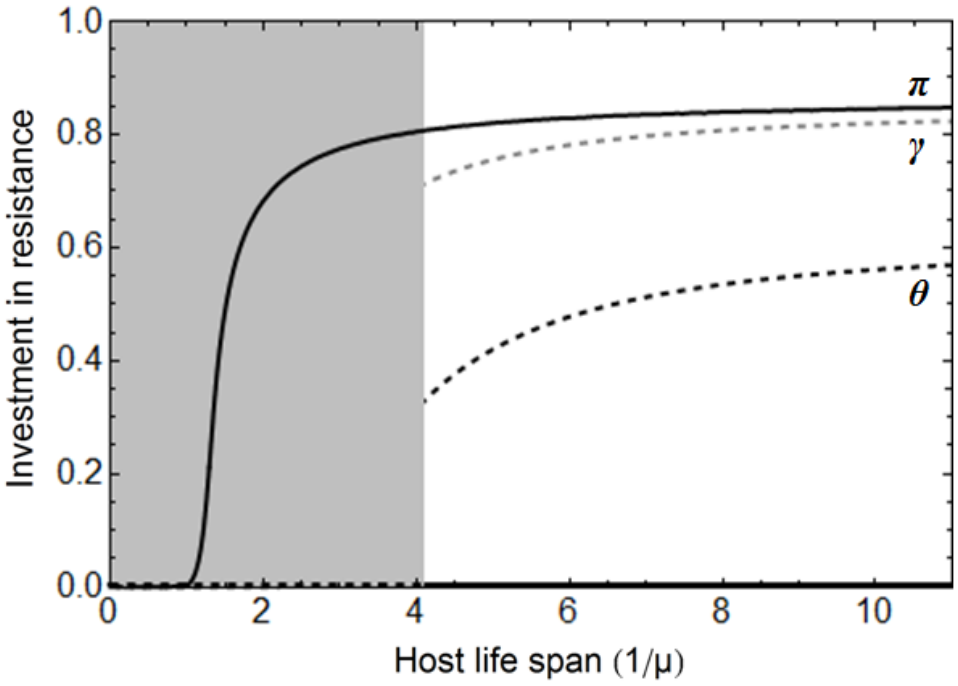

1.0
0.8
0.6
0.4
0.2
0.0
Investment in resistance
0
2
4
6
8
10
Host life span (1/μ)
π
γ
θ

Figure 5

Appendix

1. Epidemiology

In the absence of the parasite the dynamics of the host is determined by the balance between density dependent reproduction and mortality of susceptible individuals in the following way:

$$\dot{S} = (r - \kappa S - \mu)S \qquad \text{(A1)}$$

with κ being a crowding parameter, related to the carrying capacity for the host population by the relation $K = r / \kappa$. At the parasite-free equilibrium the host density is thus: $\hat{S} = (r - \mu)/\kappa$. The ability of a few parasites to invade the host population can be determined from the dynamics of the parasite when it is rare. The parasite will invade when its basic reproductive ratio, R_0, is above 1:

$$R_0 = \frac{(1-\pi)h\hat{S}}{\alpha+\gamma+\mu} > 1 \qquad \text{(A2)}$$

When the above condition is fulfilled the system (see equation 1 in the main text) reaches a new endemic equilibrium with different types of hosts coexisting ($\bar{M}$, $\bar{S}$,$\bar{I}$ and $\bar{R}$). The overbar refers to the endemic equilibrium. In particular, the endemic density of susceptible hosts is:

$$\bar{S} = \frac{\alpha+\gamma+\mu}{(1-\pi)h} \qquad \text{(A3)}$$

For the other types of hosts there is no simple analytical expression but they can easily be obtained numerically.

2. Evolution of the host with a single parasite

Let us now assume that the above system has reached an endemic equilibrium and that a new mutant host appears (i.e. its frequency is initially rare). Will this mutant invade the host population? Because we assume that the resident system has reached an endemic equilibrium and that the mutant is initially rare, the invasion condition can be derived from the linearization of the system (equation 1 in the main text) near the endemic equilibrium of the resident (the superscript *m* refers to the mutant):

$$\dot{\boldsymbol{H}}^m = \boldsymbol{A^m}.\boldsymbol{H^m} \qquad \text{(A4)}$$

where $\boldsymbol{H^m} = (M^m \;\; S^m \;\; I^m \;\; R^m)^T$ is the vector of the densities of the mutant in the different states of the hosts, and $\boldsymbol{A^m}$ describes the growth of the mutant in these different states :

$$\boldsymbol{A^m} = \begin{pmatrix} -(\mu^m + \delta_M) & 0 & 0 & \theta^m\lambda^m \\ \lambda^m + \delta_M & \lambda^m - (\mu^m + (1-\pi^m)h) & \lambda^m & (1-\theta^m)\lambda^m + \delta_R \\ 0 & (1-\pi^m)h & -(\mu^m + \gamma^m + \alpha) & 0 \\ 0 & 0 & \gamma^m & -\mu^m - \delta_R \end{pmatrix} \text{(A5)}$$

We assume that the mutation may act on various host defense traits and that this mutation is supposed to be associated with costs on reproduction or survival. More specifically we assume:

$\lambda^m = r_0(1-C_r)(1-\bar{N}/K)$ and $\mu^m = \mu_0(1+C_s)$ where $\bar{N} = \bar{M} + \bar{S} + \bar{I} + \bar{R}$ is the equilibrium density of the total host population, r_0 and μ_0 are the intrinsic birth and death rates of the host, respectively. The parameters C_r and C_s refer to the costs on reproduction and on survival, respectively; and are of the form:

$$C_r = \sum\nolimits_x c_{r,x}\, x^{k_x}$$

$$C_s = \sum\nolimits_x c_{s,x}\, x^{k_x}$$

The coefficients $c_{r,x}$ and $c_{s,x}$ measure how an increase in host trait x affects reproduction and survival, and the coefficients k_x measure the shape of the relationship between the trait and the cost. In the present paper we consider the evolution of three different host defense strategies: $x \in \{\pi^m, \gamma^m, \theta^m\}$. Moreover, for the sake of simplity, we only assume host defense affects fecundity (a cost on survival does not modify qualitatively the results). In other words we assume throughout the paper that $C_s = 0$ and $\mu^m = \mu = \mu_0$.

The above matrix can be written as $\boldsymbol{A}^m = \boldsymbol{F}^m - \boldsymbol{V}^m$, where $\boldsymbol{F}^m$ refers to fecundity (how many mutant hosts are created in each of the four different types) and $\boldsymbol{V}^m$ refers to the transition between the different types:

$$\boldsymbol{F}^m = \begin{pmatrix} 0 & 0 & 0 & \theta^m\lambda^m \\ \lambda^m + \delta_M & \lambda^m & \lambda^m & (1-\theta^m)\lambda^m + \delta_R \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix} \quad \text{(A6)}$$

$$\boldsymbol{V}^m = \begin{pmatrix} \mu + \delta_M & 0 & 0 & 0 \\ 0 & \mu + (1-\pi^m)h & 0 & 0 \\ 0 & -(1-\pi^m)h & \mu + \gamma^m + \alpha & 0 \\ 0 & 0 & -\gamma^* & \mu + \delta_R \end{pmatrix} \quad \text{(A7)}$$

Note however that the gain of *S* individivuals through the loss of immunity endured by *M* and *R* individuals described by the parameters δ_M and δ_R are included in the $\boldsymbol{F}^m$ matrix. Although they do not correspond to reproduction events, including those transitions in $\boldsymbol{F}^m$ greatly simplifies the expression of $\boldsymbol{V}^{m-1}$ which in turn allows for the derivation of a simpler invasion condition for the mutant (see below). As λ^m, *1 -* θ^m, δ_M and δ_R are all positive quantities, considering δ_M and δ_R in F^m instead of V^m does not modify the signs of F^m and V^m and the conditions of the NGT are thus fulfilled in any case.

The mutant host will invade the resident equilibrium when the dominant eigenvalue of $\boldsymbol{A}^m$ is positive or, equivalently, when the dominant eigenvalue of $\boldsymbol{B}^m = \boldsymbol{F}^m.\boldsymbol{V}^{m^{-1}}$ is higher than one (Diekmann et al. 1990, Hurford et al. 2010). We focus on the latter criteria because it yields a per-generation measure of parasite population growth rate. This matrix $\boldsymbol{B}^m$ is of the form:

$$\boldsymbol{B}^m = \begin{pmatrix} 0 & \tau^m_{S\to M} & \tau^m_{I\to M} & \tau^m_{R\to M} \\ \tau^m_{M\to S} & \tau^m_{S\to S} & \tau^m_{I\to S} & \tau^m_{R\to S} \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix} \quad \text{(A8)}$$

where the coefficients $\tau^m_{i\to j}$ are the rates of production of host type j by a host type i. The dominant eigenvalue of this matrix is:

$$R_m = \left(\tau^m_{S\to S} + \sqrt{4\tau^m_{M\to S}\tau^m_{S\to M} + {\tau^m_{S\to S}}^2}\right)/2 \quad \text{(A9)}$$

Hence, this expression depends only on three elements of $\boldsymbol{B}^m$.

$$\tau^m_{S\to M} = \frac{(1-\pi^m)h}{((1-\pi^m)h+\mu)}\frac{\gamma^m}{(\alpha^m+\gamma^m+\mu)}\frac{\theta^m\lambda^m}{(\mu+\delta_R)} \quad \text{(A10)}$$

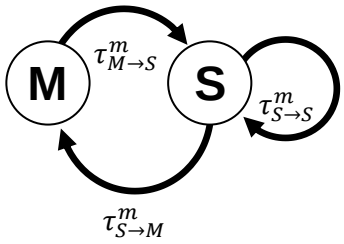
$$\tau^m_{M\to S} = \frac{\lambda^m+\delta_M}{\mu+\delta_M} \quad \text{(A11)}$$

$$\tau^m_{S\to S} = \frac{\lambda^m}{(1-\pi^m)h+\mu} + \frac{\lambda^m(1-\pi^m)h}{\big((1-\pi^m)h+b\big)(\alpha^m+\gamma^m+\mu)} + \frac{(1-\pi^m)h\big((1-\theta^*)\lambda^m+\delta_R\big)\gamma^m}{\big((1-\pi^m)h+\mu\big)(\alpha^m+\gamma^m+\mu)(\mu+\delta_R)} \quad \text{(A12)}$$

These three coefficients correspond to the different transitions between M and S hosts (see figure S1). For instance, focusing on the expression of $\tau^m_{S\to M}$, there are three steps for a susceptible individual to produce maternally protected newborns: the individual has first to get infected (S to I), then to recover to become resistant (I to R) and finally to reproduce depending on its immunity transfer strategy (R to M). Equation A10 is the product of these three terms. The same reasoning applies to the expression of $\tau^m_{M\to S}$, which includes both

reproduction of M individuals and loss of immunity. Finally, in $\tau^m_{S\to S}$, each term of the sum describes three different ways in which a susceptible individual can produce new susceptible individuals (see figure S1).

Figure S1: Diagram showing the transitions represented by $\tau^m_{M\to S}$, $\tau^m_{S\to M}$ and $\tau^m_{S\to S}$.



3. Evolution of the host with two parasites

Here we expand the previous model to a situation where two strains of parasites (1 and 2) are circulating in the host population. With regard to each parasite, individuals can thus be sensitive (S), infected by either strain 1 (I_1) or strain 2 (I_2), recovered from infection (R_1 or R_2) and protected by maternal immunity (M_1 or M_2). For the sake of simplicity, we consider that when individuals have recovered from an infection by a given strain (R individuals), they cannot be infected by the other strain. Individuals can thus only develop their immunity against one of the strains. Maternally protected (M) individuals for a given strainmay, in contrast, be infected by the other strain. How maternal transfer of immunity against one strain efficiently prevents infection by the other strain is described by an additional parameter, χ, which measures the level of cross immunity of the maternal transfer.

As for the one strain model , the invasion of a mutant host requires a description of the dynamics of the system near the endemic equilibrium of the resident (the superscript *m* refers

to the mutant) using equation A4 with $\boldsymbol{H^m} = ({M_1}^m\ {M_2}^m\ S^m\ {I_1}^m\ {I_2}^m\ {R_1}^m\ {R_2}^m)^T$ and where $\boldsymbol{A}^m$ can be decomposed as $\boldsymbol{A}^m = \boldsymbol{F}^m - \boldsymbol{V}^m$ with $\boldsymbol{F}^m$ and $\boldsymbol{V}^m$:

$$\boldsymbol{F}^m = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & \theta^m\lambda^m & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \theta^m\lambda^m \\ \lambda^m + \delta_M & \lambda^m + \delta_M & \lambda^m & \lambda^m & \lambda^m & (1-\theta^m)\lambda^m + \delta_R & (1-\theta^m)\lambda^m + \delta_R \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & \gamma & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \gamma & 0 & 0 \end{pmatrix}$$

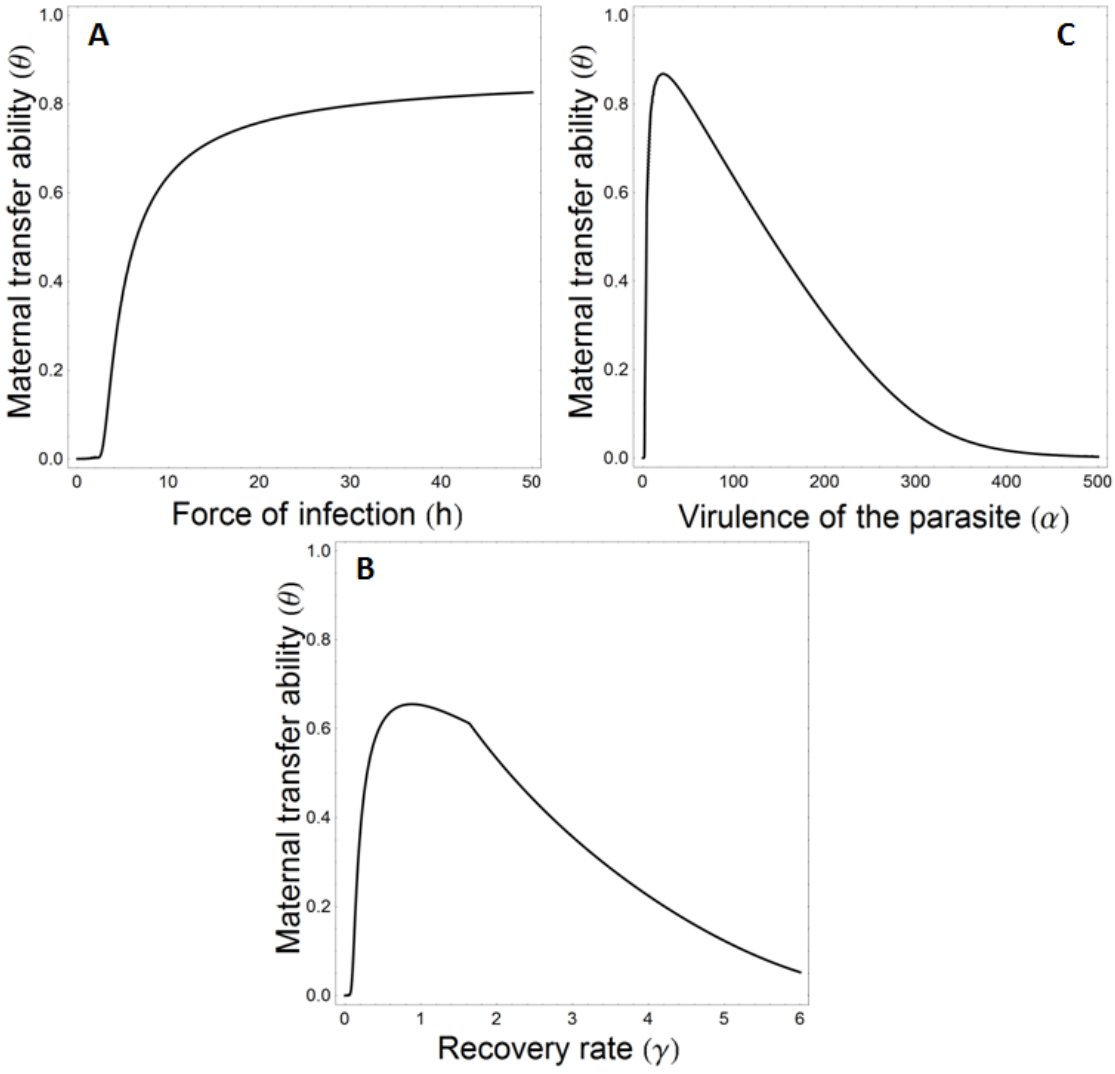
$\boldsymbol{V}^m$

$$= \begin{pmatrix} \mu + \delta_M + (1-\chi)h_2 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & \mu + \delta_M + (1-\chi)h_1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \mu + (1-\pi^m)(h_1+h_2) & 0 & 0 & 0 & 0 \\ 0 & -(1-\chi)h_1 & -(1-\pi^m)h_1 & \mu + \gamma^m + \alpha & 0 & 0 & 0 \\ -(1-\chi)h_2 & 0 & -(1-\pi^m)h_2 & 0 & \mu + \gamma^m + \alpha & 0 & 0 \\ 0 & 0 & 0 & -\gamma^m & 0 & \mu + \delta_R & 0 \\ 0 & 0 & 0 & 0 & -\gamma^m & 0 & \mu + \delta_R \end{pmatrix}$$

The same calculation using the Next Generation Theorem can be applied to obtain an expression of the invasion criterion R_m that determines whether a mutant can invade the resident host population. This expression, however, is a bit more complicated and we only use it to derive the evolutionary stable level of maternal transfer of immunity in figure 2.

Supplementary material

Figure S1: Evolutionary stable investment in transgenerational transfer of immunity ability (θ) as a function of different parameters. (A) Effect of the force of infection (h) on the evolutionary stable ability to transfer maternal immunity. (B) Effect of the virulence of the parasite (α) on the evolutionary stable ability to transfer maternal immunity. (C) Effect of the recovery rate (γ) on the evolutionary stable ability to transfer maternal immunity. Default parameter values used in the figures: $r_0 = 1.5$; $\mu = 0.1$; $\kappa = 0.1$; $c_{r,\theta} = 0.25$; $k_\theta = 1/0.9$; $\alpha = 5$; $h = 10$; $\delta_R = 1$; $\delta_M = 1$; $\gamma = 0.6$.



ANNEXE 2

Manuscrit 2:

Evolution of the persistence of immune protection

Etat du manuscrit : en préparation

Evolution of the persistence of immune protection

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Word count: 2483

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Abstract

The evolution of resistance to parasites has been the focus of numerous theoretical studies and numerous mechanisms, ranging from innate to acquired responses, have been considered. Yet, the persistence of the protection provided by acquired responses is usually considered as a parameter and not a resistance trait. Here, we study the evolution of the persistence of protection acquired passively or upon recovery from an infection. We find that several key features of the host and the parasite influence the evolution of both traits. We also show that protection acquired upon recovery is always longer than passive protection. We discuss these results in the light of empirical data available on the persistence of immunity in vertebrates.

Introduction

Among the wide variety of resistance mechanisms to parasites [1], acquired responses have evolved as adaptations to produce phenotypic changes specifically aiming at reducing the deleterious effects of parasites [2]. In some cases, such as the immune response of vertebrates, this resistance may result in a long term protection against a parasite. In that case, the modification of the density of susceptible individuals by the gradual loss of immunity may be relevant from an epidemiological point of view [3]. Thus, the rate at which the protection provided by acquired responses decays can be seen as part of a global resistance strategy to parasites. Yet, despite an abundant literature on the evolution of resistance to parasites [see 4 for a review], the loss of acquired protection by recovered individuals is usually treated as a parameter of evolutionary models and no studies have focused on the evolution of this mechanism.

In addition to the protection acquired upon recovery, vertebrate mothers can transmit effectors of the acquired immune response, and in particular antibodies, to the next generation [5]. This temporary protection has been shown to be critical for early life survival [e.g. 6] and its decay shows some variation between species [7]. This indicates that, together with the loss of protection after recovery, the loss of passive transgenerational protection may well be part of the resistance strategy of individuals against parasites.

Building on a previously described framework [8] that accounts for the protection of individuals after clearing the infection and by passive transgenerational protection, we study the evolution of the rates of loss of passive and acquired protections. We particularly focus on how different traits of the host and of the parasite can modify the evolution of the persistence provided by acquired and passive protection.

The model

We model a host population that can display different status with regard to a parasite circulating in the population: sensitive (*S*), infected (*I*), recovered (R) or passively protected by maternal immunity (*M*). The parasite is characterized by its transmission rate β and its virulence α, and infects sensitive individuals depending on the force of infection βI. Recovery occurs at a rate γ, and subsequently recovered individuals can transfer their immunity passively to their offspring at a rate θ. Recovered individuals lose their protection at a rate δ_R and maternally protected individuals at a rate δ_M. All hosts are assumed to reproduce equally at a density dependent rate $\lambda = r\,(1 - \kappa N)$ (with $N = M + S + I + R$, the total population size) and to die from natural causes at a rate μ. These transitions correspond to the following system of differential equations:

$$\frac{dM}{dt} = \lambda\theta R - (\delta_M + \mu)M$$

$$\frac{dS}{dt} = \lambda(M + S + I + (1 - \theta)R) + \delta_M M + \delta_R R - (\mu + (1 - \pi)h)S$$

$$\frac{dI}{dt} = (1 - \pi)hS - (\mu + \alpha + \gamma)I$$

$$\frac{dR}{dt} = \gamma I - (\mu + \delta_R)R$$

The epidemiology of this system has been described elsewhere [8] and we will consider here that the parasite has reached an endemic equilibrium in the host population. Using the Next Generation Theorem [9] to linearize and analyze the system 1, a general invasion criterion can be obtained and corresponds to the average number a rare mutant host will produce in the resident population at the following generation [see 8 for the mathematical derivation of the invasion criterion].

The invasion condition depends on the cost of the mechanisms considered. In our case, to avoid evolutionary branching [10], we assume a slightly accelerating cost of the persistence of the protection (δ_R and δ_M) on the reproductive rate of its host. Specifically, we will assume that $r = r_0(1 - C_{\delta_M} - C_{\delta_R})$ with $C_{\delta_M} = (1/\delta_M)^{k_M}$ and $C_{\delta_R} = (1/\delta_R)^{k_R}$. There is indeed evidence supporting a cost of resistance to parasites on the reproductive rate of their hosts [e.g. 11] and the persistence of immunity is likely to be a costly mechanism because it requires in vertebrates the maintenance of a specific receptor that will prevent circulating antibodies from entering the normal catabolic pathway [12-13].

In an adaptive dynamics framework [14], this allows us to determine the evolutionary properties of the system. All the results we report here proved to be convergence and evolutionary stable, and correspond to continuously stable strategies and thus represent the expected outcome of the evolution.

Results

The evolutionary stable investment in both rates of loss of immune protection follows the same pattern of decay with regard to the life span of the host species (figure 1A). In other words, long-lived species are expected to invest in longer-lived protections, either acquired passively through the mother or upon recovery. This is because increasing the host lifespan also increases the evolutionary stable density of infected individuals in the population, which in turn increases the force of infection (figure 1B). This increases the risk for longer-lived species to encounter the parasite again during their lifetime, and increases the selection for lower rates of loss of immune protection. It should be noted however that this longer-lived protection also results in an increase in the proportion of the recovered fraction of the population and overall selects for lower evolutionary stable prevalence at high life spans.

Transgenerational protection (plain curve) is also expected to be lost consistently faster than self-acquired protection (dashed curve) although this difference tends to be reduced at low life spans. This is because the effect on the density of infected individuals of maternally protected individuals is less important, which selects for lower evolutionary investment in this protection mechanism.

In accordance with the previous argument, increasing the transmission rate of the parasite results in reduced rates of loss of protection by recovered or maternally protected individuals (figure 2A). This is again because increasing the transmission directly increases the force of infection and selects for longer protective mechanisms.

Finally, similarly to what can be found for other resistance to the parasite such as recovery [15] or the ability to transfer antibodies [8], the virulence of the parasite has a non-monotonous effect on the evolutionary stable investment in the rate of loss of immune protection (figure 2B). Persistence of transgenerational and self immune protection is maximal for intermediate values of virulence. Low virulence implies a weak cost of infection, which does not make it worth paying a cost to remain protected after infection. High virulence result in a rapid death of infected individuals and cause a rapid drop in the probability of infection, which does not make it worth investing in any resistance mechanism.

Discussion

Here we investigated the evolution of the duration of the immune protection acquired upon recovery or through the transfer of maternal immunity. We show that protection acquired upon recovery is always expected to last longer than protection obtained passively through the mother. Adults indeed have the potential to synthesize antibodies even long after encountering the parasite [16] leading to half-life of protection against common antigens of

more than 11 years in humans [17]. In contrast, maternal antibodies decay within months in infants within 12 months of birth [18]. In birds, antibodies against Newcastle Disease Virus decay in about 2 weeks in kittiwake newborns [19] while adults from the same species retain comparable levels of antibodies against *Borrelia burgdorferi* year-round [20]. Another characteristic of the immune system we did not consider in our model is the possibility of immune memory [21]. Even after immune compounds produced during the course of an acquired immune response have decayed, sensitized immune cells produce a secondary immune response upon re-infection which is more efficient at clearing the parasite. This corresponds to an increased recovery rate upon secondary infection, leading to a decreased immune period which may in turn reduce the benefits of long-lived protection.

Our analysis revealed that different parasites are expected to result in the production of responses that will display variability in their persistence. Empirically, various antigens can lead to various half-lives of protection which is in accordance with this prediction [17]. We also show that a critical parameter for the evolution of the immune persistence is the life span of the host. Longer-lived hosts are expected to invest in longer-lived persistence of both acquired maternally or upon recovery. Immune response is considered as a physiologically demanding process [22] and as such its evolution has been suggested to be constrained by life history traits such as life span [23]. As part of an immune strategy favoring acquired over innate responses [24], long-lived species are expected to invest in recovery and maternal transfer of antibodies [8] and may thus benefit from an extended protection. Moreover, this prediction is also in accordance with the variability observed in the half-life of antibodies against a common vaccine in several bird species [7].

Overall our study suggests that how transgenerational and self immune protection persist represent an integral part of defense strategies against parasites. Another step to achieve better biological reality would be to combine a framework like ours to describe the

dynamics of inter-host events and a within host dynamics model [e.g. 25] to describe more closely the immune response. In particular such a within host model would allow a better description of antibody levels in recovered and maternally protected individuals and focus on the evolution on a trait such as half-life of antibodies. Such theoretical studies focusing directly on antibody half lives may provide interesting insights into the evolution of a trait of great interest for fields ranging from life history evolution [e.g. 7] to biomedicine [e.g. 26].

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Figure legends

Figure 1: Effect of the host life span on: (A) the evolutionary stable investment in the rate of loss of protection by recovered individuals (δ_R, dashed curve) and maternally protected individuals (δ_M, plain curve); (B) the evolutionary stable force of infection (βI) related to the loss of protection by recovered individuals. Default parameters: $r_0 = 1.5$; $\kappa = 0.1$; $c_{\delta R} = 0.02$; $c_{\delta M} = 0.02$; $k_{\delta R} = 1/0.9$; $k_{\delta M} = 1/0.9$; $\alpha = 2.75$; $\beta = 2$; $\delta_R = 0.5$; $\delta_M = 0.5$.

Figure 2: Evolutionary stable investment in the rate of loss of protection by maternally protected (δ_M, plain curve) and recovered individuals (δ_R, dashed curve) as a function of: (A) the transmission rate of the parasite (β); (B) the virulence of the parasite (α). Default parameters: $r_0 = 1.5$; $\mu = 0.1$; $\kappa = 0.1$; $c_{\delta R} = 0.02$; $c_{\delta M} = 0.02$; $k_{\delta R} = 1/0.9$; $k_{\delta M} = 1/0.9$; $\alpha = 2.75$; $\beta = 2$; $\delta_R = 0.5$; $\delta_M = 0.5$.

Figures

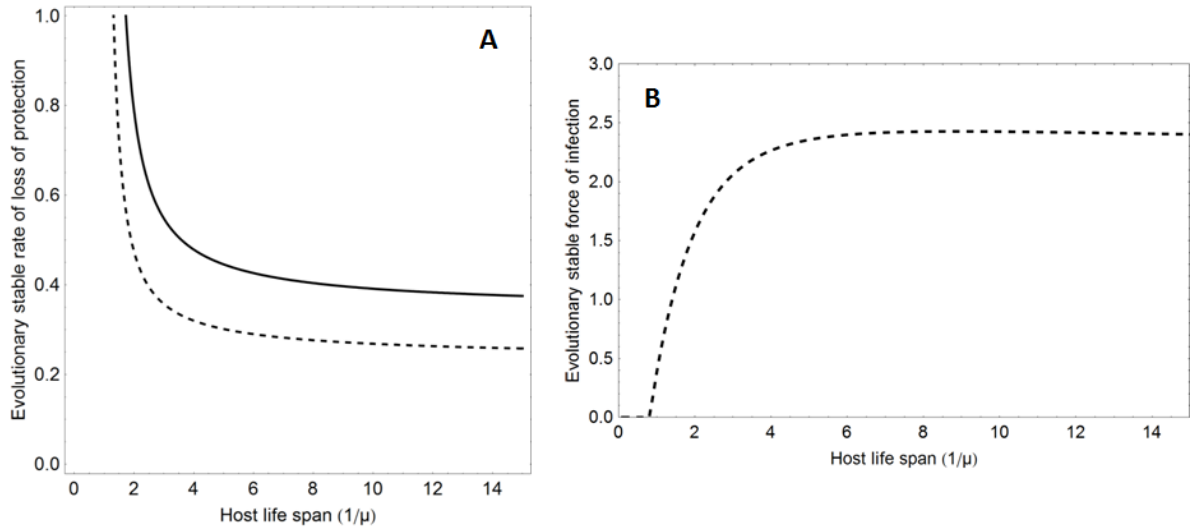

Figure 1

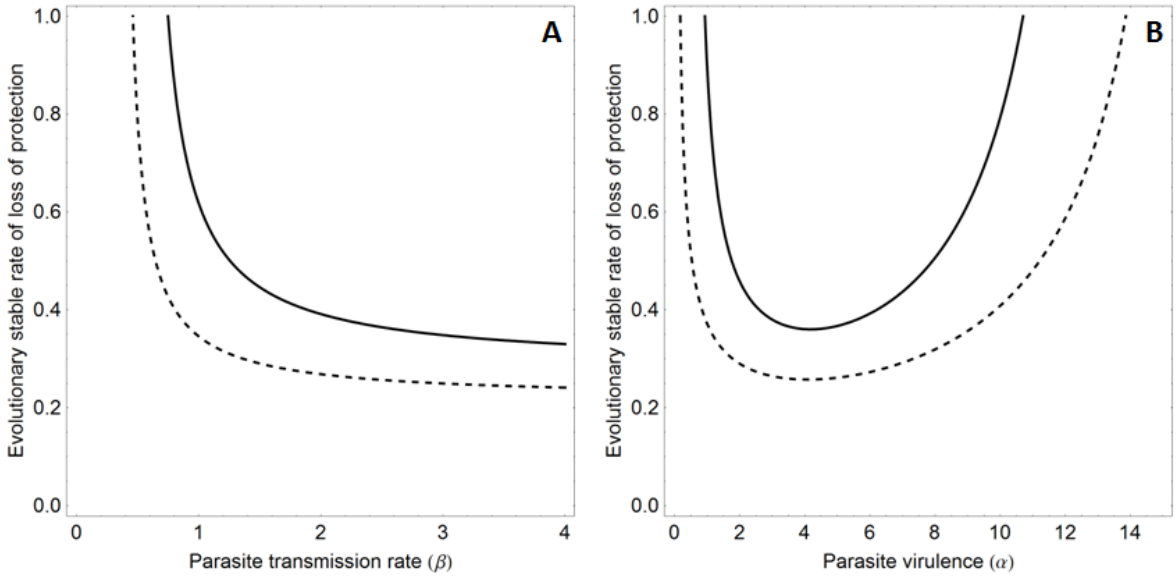
A
B
Evolutionary stable rate of loss of protection
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
Parasite transmission rate (β)
0
2
4
6
8
10
12
14
Parasite virulence (α)

Figure 2

ANNEXE 3

Manuscrit 3:

Maternal antibody persistence: a neglected life history trait with implications from albatross conservation to comparative immunology

Etat du manuscrit : en révision après soumission

Maternal antibody persistence: a neglected life history trait with implications from albatross conservation to comparative immunology

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Abstract

The evolution of different life history strategies has been suggested as a major force constraining physiological mechanisms such as immunity. In some long-lived oviparous species, a prolonged persistence of maternal antibodies in offspring could thus be expected in order to protect them over their long growth period. Here, using an intergenerational vaccination design, we show that specific maternal antibodies can display an estimated half-life of 25 days post hatching in the nestlings of a long-lived bird. This temporal persistence is much longer than previously known for birds and it suggests specific properties in the regulation of IgY immunoglobulin catabolism in such a species. We also show that maternal antibodies in the considered procellariiform species are functional as late as 20 days of age. Using a modelling approach, we highlight that the potential impact of such effects on population viability could be important, notably when using vaccination for conservation. These results have broad implications, from comparative immunology to evolutionary eco-epidemiology and conservation biology.

INTRODUCTION

The evolution of different paces of life has been suggested as a major force constraining physiological mechanisms [1]. In particular, species with a slow pace of life are expected to trade high adult survival rates with slow developmental rates [2]. As expected under this hypothesis, long-lived bird species display longer incubation periods [3] and longer nestling periods. Immune responses should exhibit the same trend, with a stronger allocation to responses favoring adult survival but requiring time to be fully effective in long-lived species [4]. However, the sophisticated response of the host immune system against parasites relies on different components involving various timing and allocation issues during their development [5]. In particular, the activation of acquired humoral immunity is a critical process to prevent the deleterious effects of exposure to a wide variety of parasites in vertebrates, but this response is not active at birth. Passive acquisition of immune compounds from the mother nevertheless occurs, notably via the egg yolk, placenta or colostrum [6] and can prevent negative effects of parasites [7-8]. The ability of mothers to transfer antibodies of their own acquired immunity to their young is thus probably an essential pathway to enhance offspring survival early in life [9-10]. Critical features of this transgenerational transfer of immunity could thus represent important life history traits that have coevolved with other life history traits and components of the immune system [4, 9]. Although the evolutionary ecology of this transgenerational induced response has recently attracted increasing attention [9-11], little is known to date about ecological and life history factors affecting its variability among species [12], despite wide potential implications.

In birds, maternal antibodies accumulate in the oocyte during egg yolk formation and receptor-mediated absorption by the chick begins shortly before hatching [13]. These

antibodies are usually considered to decay within a few days or weeks after hatching in classical model species, such as quails and chickens [14-15], although variability may exist among individuals and species and the protection conferred by those antibodies can last after they reached undetectable levels [8, 16]. The temporal persistence of those maternal antibodies has been shown to be positively related to the level of antibodies initially transferred in the egg yolk [17], but little is known about the potential role of other factors. In particular, within and among-species variability in the propensity to transfer immune protection has been largely overlooked in vertebrates [9, 12] and investigations with non-model species in natural settings have the potential to lead to important findings. The few studies conducted so far with species other than classical avian models have consistently reported a relatively short temporal persistence of those antibodies [17-23] and some evidence of variability among species [17, 23-24]. In particular, in species with a slow pace of life, one could expect maternal antibodies to persist for a prolonged time to provide hatchlings a protection over the long rearing period, thus allowing for the slow development of a strong immune system. If maternal antibodies were to persist much longer in offspring of species with slow growth, this could have implications for the ecology and evolution and host-parasite interactions, but also with regards to allocation issues related to offspring growth.

Much interest has been developing on the relationship between immunocompetence and its costs during development. Different studies have demonstrated in birds that an immune challenge during development was responsible for a reduction in the growth rate [25-26], which is known to influence the fitness of nestlings [27]. However, those studies have mostly focused on innate immune mechanisms and the potential role of the transfer of maternal antibodies as a mechanism favoring chick growth in the face of parasitic challenge has often been neglected [but see 28]. This is perhaps because experimentally assessing such a role

requires a specific challenge of mothers before breeding and the monitoring of the dynamics of specific maternal antibodies in offspring via repeated sampling during the subsequent rearing period. Although such studies would greatly benefit from considering species with contrasted life histories, to date, data are mostly available for relatively fast-living species, precocial or altricial [e.g. 14, 16, 21-23, 29-30]. Some studies on longer-lived species, such as the Californian condor (*Gymnogyps californianus*), suggest a longer maternal protection [24], but design limitations do not permit a clear interpretation of the dynamics of maternal antibodies decay. Very little information is also available for other important groups of slow-developing birds displaying long rearing period, such as Procellariforms. Using a transgenerational vaccination experimental design, we explored the temporal persistence and functionality of maternal antibodies in such species.

Procellariform species are long-lived colonial seabirds that display a slow pace of life with among the longest chick rearing periods in birds [31]. For instance, the Cory's shearwater (*Calonectris diomedea*) develops very slowly with chicks spending about 90 days at the nest. Despite such extreme and specific life histories, little information exists on the ecology of immunity in these species [32-34], notably on specific humoral immunity. Prior to egg laying, and using a vaccine to mimic a microparasitic infection, we experimentally manipulated exposure to Newcastle Disease Virus (NDV) in female Cory's shearwaters in order to explore properties of the maternal transfer of antibodies in this species, notably by monitoring the dynamics of specific anti-NDV antibody levels in chicks throughout the rearing period. Here, we report a long temporal persistence of maternal antibodies in nestlings of this long-lived colonial procellariiform species. Data gathered using a similar design in the black-legged kittiwake (*Rissa tridactyla*) and in the quail (*Coturnix coturnix*) allow a comparison of the temporal persistence among species and highlight a previously unsuspected strong variability

of this important trait that deserves more attention. We also show that maternal antibodies in the considered procellariiform species are functional as late as 20 days of age. A modelling approach exploring a realistic but hypothetical situation allowed us to illustrate that the potential impact of such effects on population viability could be important, notably if vaccination was used for conservation of populations threatened by pathogens affecting nestling survival. These results, which have potential implications in eco-epidemiology and wildlife conservation, but also in biomedicine, highlight how basic approaches in ecological immunology and the evolution of life histories can lead to findings that can have far-reaching implications, well beyond these already vast fields.

MATERIAL AND METHODS

Model species and study populations

The Cory's shearwater has a long lifespan of over 30 years and a long annual breeding season (8 months). As do all Procellariformes, Cory's shearwater lays one large single egg [35] and incubation is quite long (54 days in this species). The hatched chick takes about 90 days to complete its development and to fledge [36]. In contrast, incubation lasts 17 days in quails and 27 days in black-legged kittiwakes. Quail chicks leave the nest just after hatching, while kittiwake chicks fledge at about 35 days of age [37]. Fieldwork with Cory's shearwaters was conducted in Gran Canaria (15°47′ 18″ N; 27°50′41″ E, Canary Archipelago, Spain) in 2010. The experiment with kittiwakes took place in Northern Norway [21]. Quails were bred in Konnevesi Research Station [University of Jyväskylä, Finland; 29].

Vaccination design and quantification of NDV antibodies

In all species, vaccines were used to mimic the exposure of mothers to microparasitic antigens prior to egg laying [38]. At the time of their first capture, females either received a

subcutaneous injection with a killed NDV vaccine (Nobivac Paramyxo P201, Intervet, France) or a subcutaneous injection of saline solution. We checked that all females did not have anti-NDV antibodies prior to vaccination. When sampled at the time of egg-laying, all vaccinated females subsequently displayed detectable levels of anti-NDV antibodies while all control females remained negative throughout the study. Chicks were sampled repeatedly throughout the rearing period, starting at one day post hatch in shearwaters and five days post hatch in kittiwakes and quails (see figure 1 for the ages), to allow the determination of the dynamics of maternal antibodies in their plasma.

On each occasion, blood was collected from the ulnar vein (kittiwake, quail) or the tarsal vein (shearwater) using 1mL syringes and stored at 4°C. Within a few hours, samples were centrifuged and plasma was stored at -20°C pending serological analyses. Measures of specific anti-NDV antibody levels in females and chicks were performed once for each sample using a competitive ELISA test (Svanovir NDV-Ab, Svanova Biotech, Sweden) and are expressed as percentage of inhibition (*PI*). Analyses conducted on sub-samples allowed us to check the high repeatability of the measures. Antibody levels were standardized across species by subtracting the negativity threshold to the inhibition percentage (negativity threshold: mean of negative controls + 2 standard deviations; shearwater: 0.31, kittiwake: 0.30, quail: 0.22). In addition, in shearwaters, a subset of chicks was vaccinated when 20 days old (n= 11 chicks from control mothers, n = 5 chicks from vaccinated mothers) in order to investigate whether persistent anti-NDV maternal antibodies might block a response to vaccination.

Permits for the experiments were granted respectively by the Consejería de Medio Ambiente del Cabildo de Gran Canaria for shearwaters and the Norwegian Animal Research Authority for kittiwakes. The experiment with quails conformed to legal requirements in Finland. The

intergenerational vaccination design was approved by the Ethical committee of the French Polar Institute.

Statistical analyses

We modeled the dynamics of anti-NDV antibody levels in chicks according to their treatment and the treatment of their mothers using generalized additive mixed models (GAMMs), using the library mgcv in R [39], based on penalized regression splines and generalized cross-validation to select the appropriate smoothing parameters. GAMMs combine the utilities of linear mixed models [40] and generalized additive models [41] so that random factors, fixed factors and nonlinear predictor variables can all be estimated in the same statistical model. To compare dynamics of decay of maternal antibodies accounting for their level at hatching, we also calculated the half-lives of these antibodies for each species. To do so, we determined the curve of exponential decrease in concentration using mixed models with chick nested within species as a random effect. We then calculated the half-life for each species using the equation $t_{1/2} = \ln(1/2)/a$ with $\ln(PI) = a(\ln(\text{age})) + b$. Results present half-lives and associated 95% confidence intervals.

Modeling effects on population viability

In order to illustrate the effect of the temporal persistence of maternal antibodies on population viability in such long-lived species and its potentially strong conservation implications, we built an age-structured matrix population model [42] that allowed comparing contrasted scenarios with regard to the protective effect of maternally acquired antibodies. To consider a simple and demonstrative situation, we hypothesized that a vaccine could be available against a pathogen negatively affecting young offspring survival and circulating in a small population of a long-lived wild bird species. Such a situation is highly plausible if we

consider a wild bird species threatened by a pathogen originating from domesticated animals [43]. The model enabled us to address the importance of the length of the protection offered by maternally acquired antibodies as a result of the vaccination. The model was parameterized for a small population of an endangered procellariiform species exposed to annual epidemics of a pathogenic microparasite greatly impairing newborn survival during the rearing period. For this modeling approach, we focused on the realistic case of the endangered Amsterdam albatross (*Diomedea amsterdamensis*) on Amsterdam Island, breeding close to a population of yellow-nosed albatross (*Thalassarche chlororhynchos*) which is exposed to recurrent epizootics during the breeding season, possibly due to the avian cholera agent [44-45]. Realistic parameter values for survival and reproductive rates are available for this yellow-nosed albatross population [44]. Reproduction happens once a year and births are synchronous in the population. The annual interbreeding life cycle of individuals can be described by a Leslie matrix so that population at time $t+1$ can be obtained from the equation

$$N_{t+1} = A.N_t$$

with A designating the Leslie matrix, and N_t and N_{t+1} describing the population respectively at time t and $t+1$.

We assumed a density-dependent decrease of reproductive rate in order to keep the maximum population sizes below a certain threshold (fecundity = $f_0(1 - N / K)$ with f_0 the maximal fecundity, K the carrying capacity and N the population size). Massive die-offs have been reported in the first weeks after hatching in yellow-nosed albatross chicks of this population, with mortalities up to 74%, while adults remained mostly unaffected [45]. We considered in the model that a fraction of the sensitive breeding female population could be vaccinated each year against the disease-causing agent and thus transmit a temporary passive protection to their chicks. We assumed that a safe and efficient vaccine is available [46] and that protection

given by vaccination is lifelong. Females are individually marked when vaccinated (*e.g.*, using leg rings), thus sensitive and vaccinated females could be distinguished at any time, and a protocol relying on the vaccination of only sensitive females could be implemented.

Following vaccination, the lifelong protection of adult females is supposed to come with lifelong detectable levels of specific antibodies. The transmission of maternal antibodies by adult females is thus assumed to be persistent over the rest of their lives. Maternal antibodies are assumed to be protective for chicks during a time equivalent to 2 half-lives after hatching. Assuming an exponential decay, when this time point is reached, chicks have antibody levels corresponding to a quarter of their initial level. We set that each year newborns from sensitive mothers suffered an additional mortality of 70% due to an annual epidemic of the parasite occurring when they are a few weeks of age, while offspring born to vaccinated mothers suffered no additional mortality. All surviving offspring and subsequent adults are considered not to transmit protective antibodies against the parasite until they may be vaccinated (this is either because they have not been exposed to the parasite, given that 100% of exposed chicks without maternal antibodies are assumed to die, or because they have lost their maternal protection due to the natural decay of maternal antibodies). For simplicity, no further heterogeneities among individuals are considered. Also, the dynamics of pathogen circulation is not modeled as a function of the proportion of susceptible individuals although maternal protection decreases the pulse of sensitive newborns and is thus likely to affect the persistence of the pathogen [46]. This other potential benefit of the vaccination design is not explored in the current paper. Projections of the long term impact of the maternal effect on population viability is then evaluated by comparing scenarios with no (or short term) maternal protection of newborns to scenarios with a long term maternal protection of nestlings on the breeding ground (outside the breeding season, no transmission occurs as the birds are out at sea). All

evaluations begin with a population displaying the stable age structure of the population and are run with the software Scilab (the code is provided as supplemental online material).

RESULTS

Temporal persistence of maternal antibodies

A total of 314 blood samples from females and chicks of various ages, obtained during a period of 6 months of fieldwork, allowed us to explore in detail the dynamics of maternal antibodies in nestlings of a natural population of Cory's shearwater. All chicks from control mothers (n = 37) were negative at hatching while immunization of mothers (n = 19) resulted in detectable levels of anti-NDV antibody in early offspring life. Maternal antibodies decayed at a much slower rate in shearwaters than in the two other species (figure 1). While maternal antibodies waned before 15 days in kittiwake and quail chicks, most shearwater chicks still had detectable antibodies at 30 and 40 days of age. The half-life of maternal anti-NDV antibodies was indeed much higher in shearwaters (24.75 days [95% CI: 18.07-39.24]) than in kittiwakes (5.43 days [95% CI: 3.46-11.55]) or quails (5.25 days [95% CI: 3.58-9.90]).

As in quails and kittiwakes [21, 29], the antibody levels of females of Cory's shearwaters showed a positive correlation with chick levels soon after hatching (Pearson's correlation coefficient at 5 days of age: $r_{5d} = 0.81$, n = 17 chicks from vaccinated mothers, $P < 0.001$). Importantly, this correlation between females and chicks antibody levels lasted throughout the rearing period in shearwaters despite decreasing numbers of chicks due to natural mortality during rearing and the use of a sub-sample of chicks for testing a late blocking effect (figure 2; at 10 days of age, $r_{10d} = 0.95$, n = 17; at 20 days of age, $r_{20d} = 0.91$, n = 12; at 30 days of age, $r_{30d} = 0.91$, n = 12, at 40 days of age, $r_{40d} = 0.91$, n = 9, $P < 0.001$ and at 65 days of age, $r_{65d} = 0.72$, n = 8, $P = 0.042$).

Late blocking effect of the maternal antibodies

To investigate a potential blocking effect of maternal antibodies, we exposed a subgroup of nestlings to the NDV vaccine at 20 days of age and found that nestlings which had received maternal anti-NDV antibodies did not show an increase in antibody levels by 65 days, while nestlings from control mothers did (65 days mean level of nestlings from vaccinated mothers: 0.49 ± 0.03, $n = 3$; 65 days mean level of nestlings from non-vaccinated mothers: 0.70 ± 0.16, $n = 9$; figure 3). This blocking effect suggests that maternal antibodies are functional as late as 20 days after hatching in shearwaters. In contrast, quail chicks that received maternal antibodies mounted an immune response after vaccination at 20 days of age [29].

Population projections

The long persistence of maternal protection suggested by the empirical results could prevent the local extinction of an endangered albatross population hypothetically facing a recurrent epidemic affecting newborns at the end of their 4^{th} week of rearing (figure 4, green curve, corresponding to maintaining 40 % of the females vaccinated). The effect of an assumed fast decay of maternal antibodies is not different from an absence of protection if newborns are facing an epidemic at the end of their 4^{th} week of rearing. In the considered situation, it leads to a dramatic reduction of population size over a few decades and likely extinction (figure 4, red curve). By simulating different scenarios of vaccination coverage of females and parasite-induced chick mortality, we further show that when a transfer of temporally persistent maternal antibodies is accounted for, a realistic range of vaccination coverage can lead to important population rescue effects (figure 5).

DISCUSSION

The results obtained show a predicted but surprisingly long persistence of maternal antibodies in nestlings of a long-lived colonial procellariiform species, an order of birds with among the longest chick rearing periods. By implementing a field experiment using an intergenerational vaccination design, we indeed found that specific maternal antibodies can persist more than 40 days in the plasma of Cory's shearwater nestlings, with an estimated half-life of 25 days post hatching. Further, we provide evidence that this temporal persistence is much longer than previously known for birds by comparing the results with comparable data obtained from two shorter-lived species (the quail and the kittiwake). We also show that maternal antibodies in Cory's shearwater are functional as late as 20 days of age and that the impact of such effects on population viability could be important, notably when using vaccination for conservation.

Life history theories predict that investment in resource-demanding processes should depend on the individual pace of life [1]. In particular, this implies a stronger investment in acquired rather than innate immunity in long lived species [4]. This is because innate immunity is associated with a highly costly response while acquired responses in vertebrates include memory mechanisms that reduce the costs of multiple encounters of the same parasite, an event more likely to happen in long-lived species. Although evidence is scarce, the negative correlation between the response to injection of phytohemagglutinin (a test commonly used to assess innate cellular immune responsiveness) and survival in adult birds [47] and the absence of a decay in acquired immunity with age in a long lived procellariiform species [34] tend to support this hypothesis. The results we report here further show that the transfer and temporal persistence of maternal antibodies in offspring are key maternal effects that may relate tightly with the evolution of other life history characteristics.

Procellariformes are particular in many aspects, notably with regards to the precocity of chick development [35], and key perspectives of our results are whether the high temporal

persistence of maternal antibodies we found in the Cory's shearwater (1) is a feature common to other Procellariforms and (2) is also found in other long lived bird species with long chick growing period at the nest. In addition to California condors [24], candidate species to be considered are notably Griffon vultures (*Gyps fulvus*), King penguins (*Aptenodytes patagonicus*) and parrots. Complementary data on various species would allow a more formal comparative approach to test whether the species half-life of maternal antibodies is positively related to the length of the chick rearing period when accounting for phylogenetic constraints and other ecological characteristics, such as general exposure to parasites or colonial breeding habits [9-11, 48]. In any case, obtaining such data would allow further investigations of the implications and underlying causes of the variability of this neglected trait. It should also be noted that any data obtained to assess the persistence of maternal antibodies would also provide an efficient approach to explore the inter-species variability in immunoglobulin persistence in adults of oviparous species [15]. This would however be more complicated for mammals as immunoglobulins can also be transferred after birth via the colostrum and the milk [6].

Events occurring early in life can have long-lasting implications [27], and thus the discovery of a very slow decay of maternal antibodies in a long-lived species has implications with regards to the interpretation of the optimization of life histories and potential phylologenetic effects on the dynamics of host-parasite interactions. The very long persistence of immunoglobulins in the Cory's shearwater could thus be an adaptation favoring chick survival in early life and allowing the development, and potentially the shaping, of the humoral immune repertoire in procellariiform species.

Humoral immunity based on antibodies does not provide efficient protection against all parasites, but it clearly provides a powerful mechanism of protection against many [5]. The

maternal transfer of antibodies could thus have strong effects on the dynamics of infectious diseases. Despite being temporary, the protection potentially provided by specific maternal antibodies may indeed dramatically change the susceptibility landscape of parasites as it is effective at a time thought to favour the spread of diseases agents as temporal aggregation of breeding individuals increases contact rates [49] while reproduction results in the production of a pulse of naïve individuals [46]. This could be especially the case for long lived colonial birds such as Procellariforms, which can breed in aggregates of thousands of pairs. In addition to protective effects against pathogens, maternal antibodies have been suggested to be responsible for an educational effect by acting on the ongoing process of lymphocytes maturation to select clones that display a higher reaction to selected parasites [50]. Maternal antibodies could also allow the occurrence of a "natural vaccination" by attenuating the effects of an infection by a parasite, thus producing optimal condition for the immunization of the newborn [51], a mechanism that might be particularly valuable in long lived species. Our results thus highlight that the maternal transfer of antibodies may well be a key example of an adaptive transgenerational induced response with far reaching implications [52-53].

The only study that investigated the relation between the transfer of maternal antibodies and the pace of life suggested that slow living species deposit less IgY in the yolk of their eggs [12]. However, this study is based on the quantification of levels of antibodies in the egg yolk and did not consider the persistence of the transferred antibodies in the newborn, nor accounted for the level of circulating antibodies in mother plasma at the time of egg laying. Moreover, species may vary in their propensity for taking up antibodies from the egg yolk (see below). Finally, the innate immune response of chicks [47] and levels of natural antibodies [54], which are important components of innate humoral immunity [55], have been found to be positively correlated with the length of the incubation period. Together with

persistent levels of maternal antibodies, higher levels of both cellular and innate humoral immunity after hatching could thus be part of a strategy allowing for the slow development of a fully functional acquired immune system in long-lived species.

Understanding the factors affecting the persistence of immunoglobulins is important to gain further insight into the evolution of the vertebrate immune system, but also into the dynamics of immunity in natural populations and its consequences on the ecology of host-parasite interactions [56]. Infectious diseases are thought to be a major threat for endangered species [43] and the results we obtained could thus have relatively direct implications for the conservation of some species. For instance, if the current finding about maternal antibody persistence extends to other Procellariiformes, our modeling results suggest that the use of efficient vaccines on breeding females of species such as albatrosses may be a possible way of protecting populations against the risk of extinction due to pathogens affecting offspring during the rearing period in the colony. The modeling results highlight that despite a strong classical dependence of the rate of population change on adult survival in long-lived species, manipulating the levels of protections of endangered long-lived Procellariiformes could dramatically change population projections in the case of a highly virulent nestling pathogen, in particular when disease agents are possibly locally novel pathogens introduced through human activities. Of course, such a conservation application would require more specific research work, notably in terms of detailed understanding of the host-pathogen system involved, and would need to account for ethical and practical issues regarding the use of vaccines in wild populations and especially in endangered species. Further modeling work could also be valuable to optimize potential vaccination coverage and management interventions [57]. Vaccination has been considered a relevant part of conservation programs of endangered species [58] and our results suggest that considering the prolonged persistence

of some protective antibodies in nestlings of long lived colonial species could greatly increase the potential usefulness of specific vaccines as management tools.

Functionally, the strong temporal persistence of maternal antibodies could be a by-product of the evolution of a decreased catabolism of proteins in these species with a slow pace of life. Immunoglobulins (Ig) Y, the avian equivalent of the mammalian IgG, are protected from the normal catabolic pathway by an intracellular recycling mechanism that relies on a receptor, FcRY [59]. Interestingly, this receptor is also responsible for the uptake of antibodies from the egg yolk in the hatchling [60]. An increase in the expression of the FcRY or in the strength of the FcRY-IgY interaction could thus not only contribute to greater half-lives of IgY but would also help hatchlings achieving high levels of circulating IgY despite a possible lesser investment of females in egg yolk deposition of IgY in longer-lived birds [12]. Evidence for the possibility of such mechanisms comes from studies of the mammalian counterpart of FcRY, the neonatal Fc receptor (FcRn), which is implicated in both the transfer of antibodies from mother to young and the recycling of IgG [see 61 for a review]. Both the level of expression of FcRn [62] and the affinity of the receptor for IgG [e.g. 63] are important factors governing the half-lives and the serum levels of antibodies in mammals. In parallel to comparative genomic approaches, investigations on the functionality and density of FcRY receptors in Procellariforms and other bird species could thus yield important complementary findings. Understanding mechanisms underlying the prolonged half-life of IgY might also have direct relevance for the development and the engineering of more effective therapeutic antibodies [63], a fast developing and important way of treatment of autoimmune and inflammatory diseases.

In conclusion, using an intergenerational vaccination design, we showed in a long-lived species that specific maternal antibodies can persist in offspring much longer than previously known for birds. Using a modelling approach, we show that this could importantly affect the dynamics of host-parasite interactions, and can have strong conservation implications. The results also raise important questions about the underlying mechanisms involved in the temporal persistence of antibodies in species with contrasted life histories. Overall, our study underlines how current interest in ecological immunology, provided that it is based on sound comparative and experimental approaches, has the potential to lead to further important new findings at the interface between fields such as evolutionary ecology, biomedicine, conservation biology and eco-epidemiology.

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Figure Legends:

Figure 1. Decay of specific anti-NDV antibody levels in chicks from mothers exposed to NDV for three different bird species, the Quail (green), the Black-legged kittiwake (blue) and the Cory's shearwater (red). The standardized percentage of inhibition (PI) is presented on the Y axis; 0 represents the negative threshold. The lines correspond to the mean for each species estimaded using generalized additive mixed models (GAMM) and the colored regions around the means represent the associated 95% confidence intervals of the slopes.

Figure 2. Specific anti-NDV antibody levels in 20 days post-hatch chicks from vaccinated mothers as a function of their mother anti-NDV antibody level at the time of laying (r_{20d} = 0.91, n = 12, $P < 0.001$).

Figure 3. Specific anti-NDV antibody levels in Cory's shearwater chicks for 4 treatment groups: chicks from control females, non-vaccinated (blue) and vaccinated when 20 days old (green); chicks from vaccinated females, non-vaccinated (red) and vaccinated when 20 days old (orange). General additive mixed models are used to control for individual effect and non-linear dynamics. Means and 95% CI of the slopes of the models are presented.

Figure 4. Dynamics of a hypothetical albatross population under scenarios of exposure to a disease agent deleterious to nestlings and against which an antibody-based vaccine would exist. Black curve: when there is no exposure to the disease agent in the population, the population is maintained stable by the density dependent reproduction. Red curve: when there is no maternal protection or when maternal protection vanishes before an annual epidemic of the disease agent (e.g., maternal antibodies half-life of 5 days), the population is driven

towards extinction on a short time scale even if adult females are vaccinated. Green curve: a vaccination coverage of 40 % of the sensitive breeding females associated with a 25 days half-life of maternal antibodies in offspring can dramatically dampen the effects of the annual epidemic and prevent local extinction. Parameters: adult annual survival rate: 0.95; subadult annual survival rate: 0.87; juvenile annual survival rate: 0.7; parasite induced chick mortality : 0.7; fecundity rate: 0.3 female/female. Initial population size corresponds to the equilibrium population size and age structure at carrying capacity.

Figure 5. Effect of the vaccination coverage on the population size at the end of the evaluation (300 years) for various levels of parasite induced chick mortality. Reasonable vaccination efforts have a strong rescue effect on the population: after a first year of high vaccination effort (60-70 females), the vaccination of only a few more (generally 1 or 2) sensitive adult females is needed each year to maintain a vaccination coverage of e.g., 40 % of the adult females. Parameters: adult annual survival rate: 0.95; subadult annual survival rate: 0.87; juvenile annual survival rate: 0.7; fecundity rate: 0.3 female/female; half-life of maternal antibodies = 25 days. Initial population size corresponds to the equilibrium population size and age structure at carrying capacity.

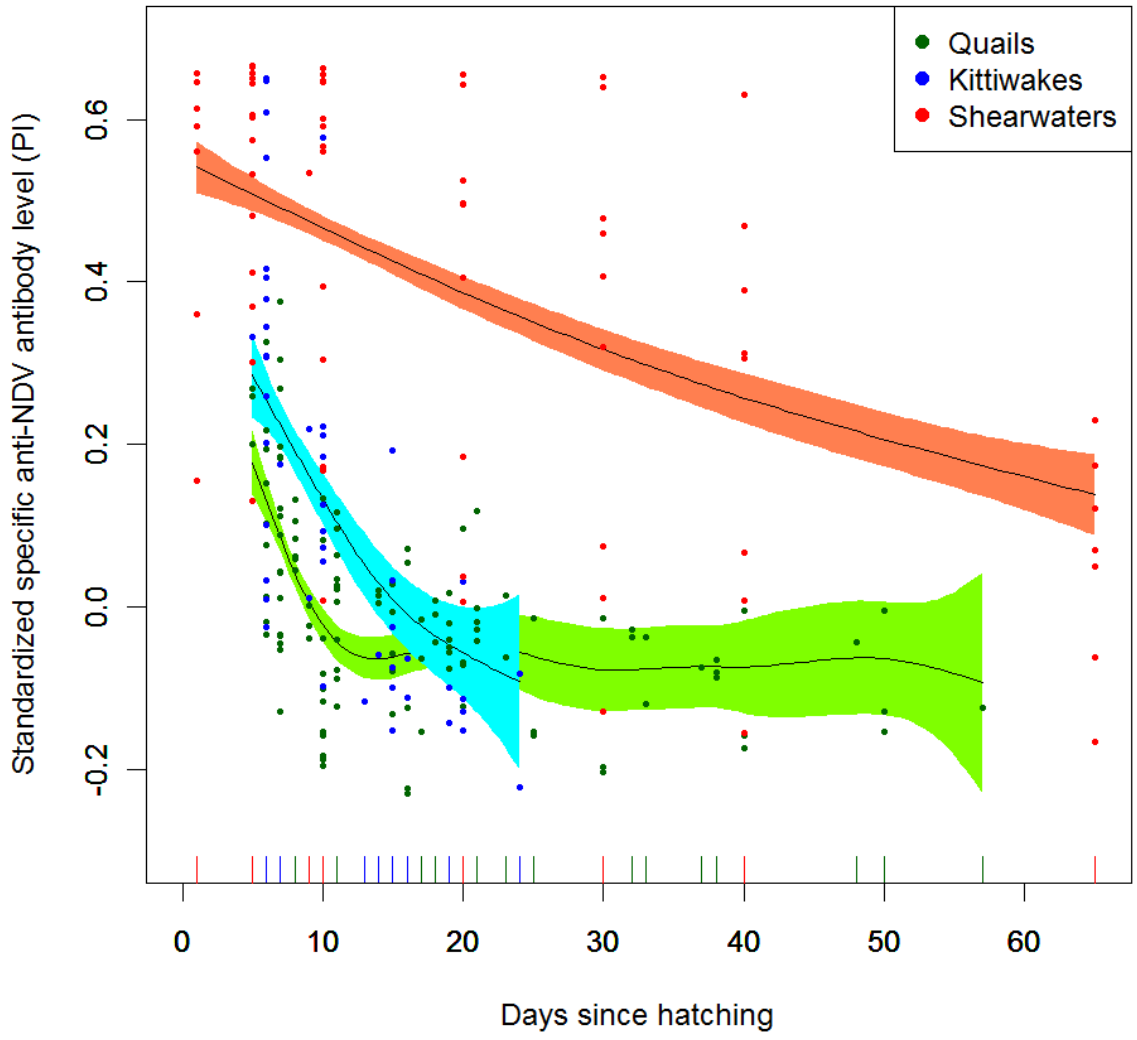
Quails
Kittiwakes
Shearwaters
Standardized specific anti-NDV antibody level (PI)
-0.2
0.0
0.2
0.4
0.6
0
10
20
30
40
50
60
Days since hatching

Figure 1

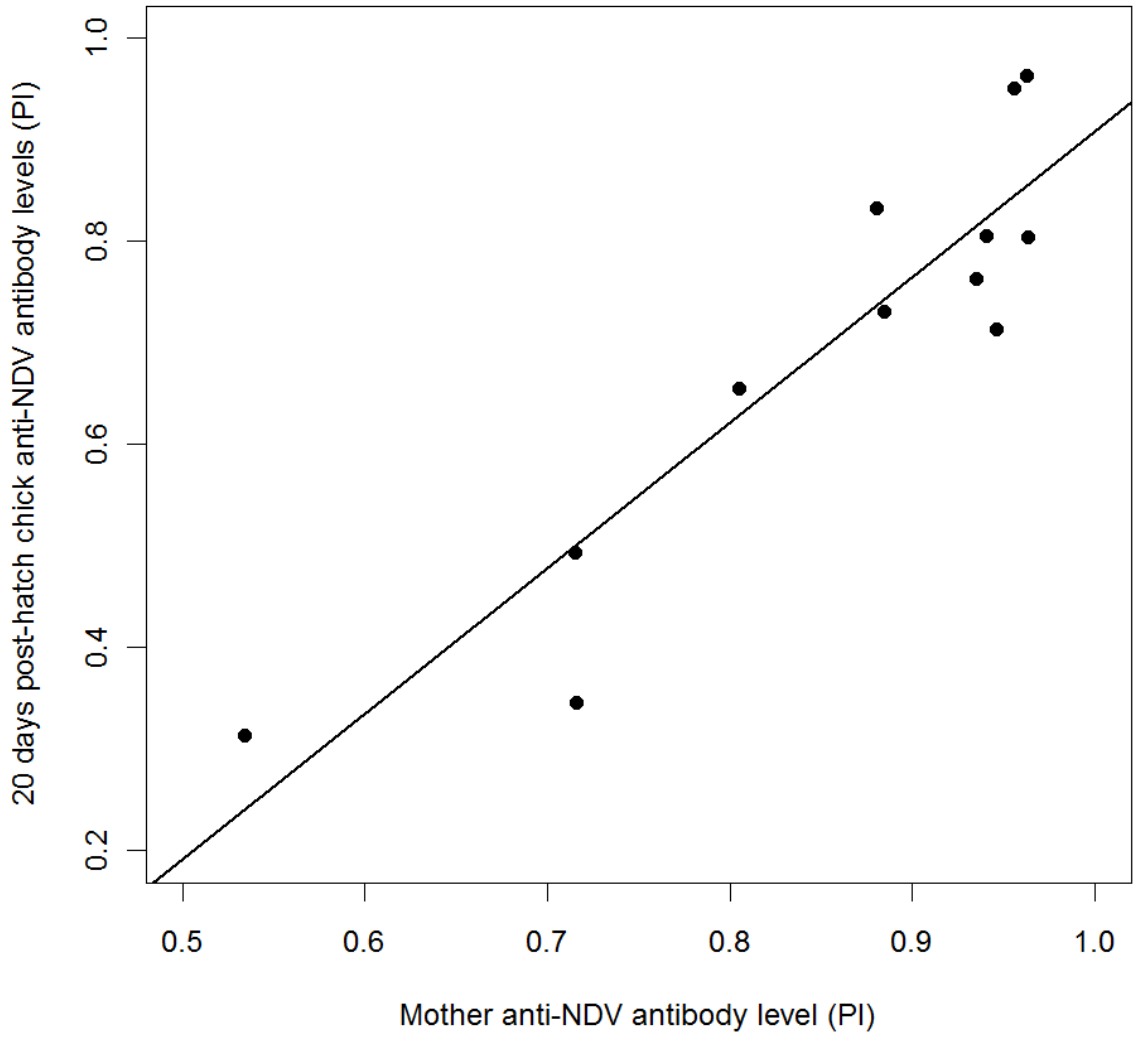
20 days post-hatch chick anti-NDV antibody levels (PI)
0.2
0.4
0.6
0.8
1.0
0.5
0.6
0.7
0.8
0.9
1.0
Mother anti-NDV antibody level (PI)

Figure 2

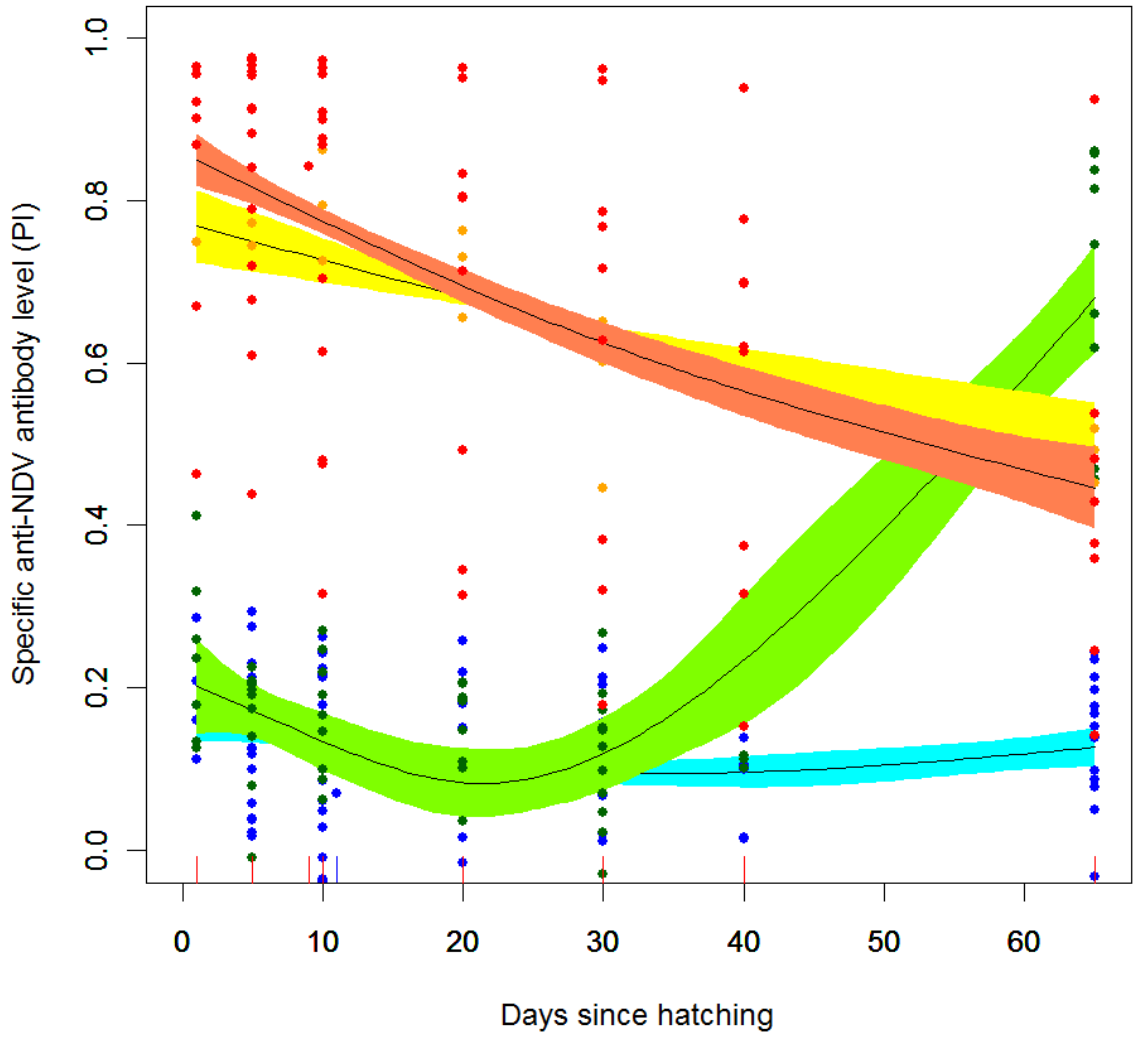
Specific anti-NDV antibody level (PI)
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
50
60
Days since hatching

Figure 3

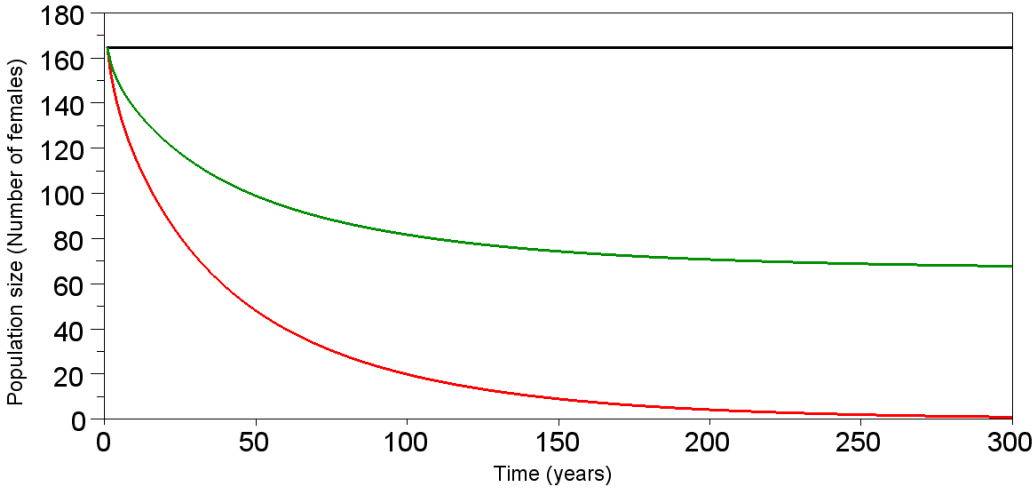
Population size (Number of females)
180
160
140
120
100
80
60
40
20
0
0
50
100
150
200
250
300
Time (years)

Figure 4

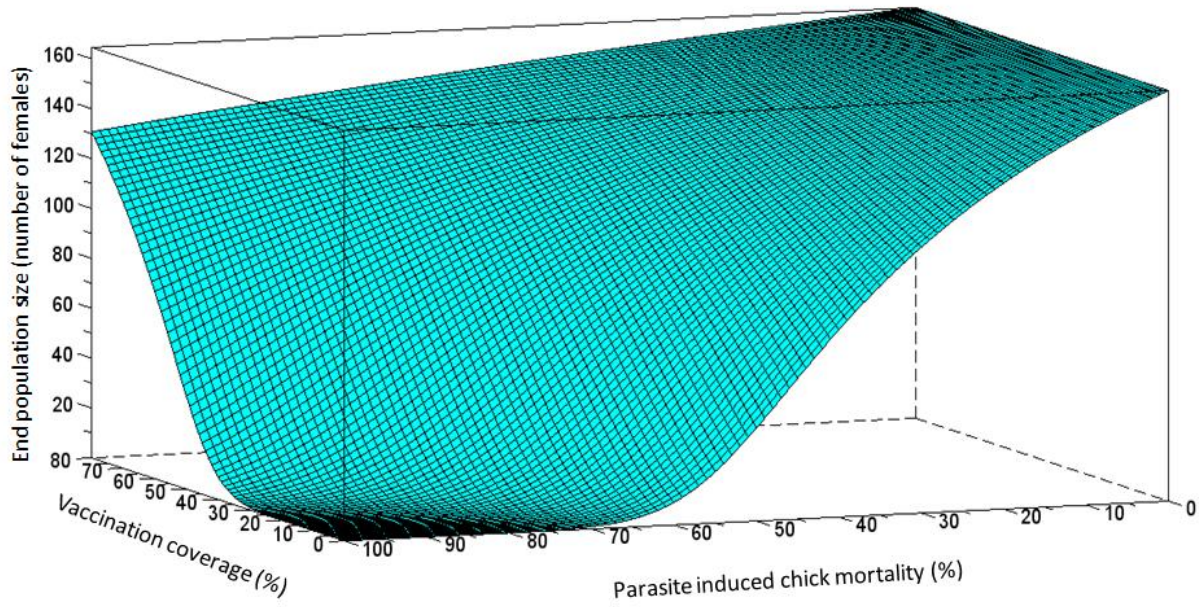
End population size (number of females)
160
140
120
100
80
60
40
20
80
70
60
50
40
30
20
10
0
Vaccination coverage (%)
100
90
80
70
60
50
40
30
20
10
0
Parasite induced chick mortality (%)

Figure 5

Supplemental material

Script used for the modeling approach

```
clear
//Parameters used in the function:
//sj: juvenile annual survival (disease free)
//ssub: subadult annual survival
//sad: adult annual survival
//rep: reproductive rate
//CC: carrying capacity
//nbysub: number of years spent as subadults
//VC: vaccination coverage (percentage of sensitive females vaccinated annually)
//MR: parasite induced mortality rate of juveniles
//HL: half life of maternal antibodies
//wkepiz: number of the week (after birth) at the end of which the epizootic takes
place
//years: duration of the simulation, in years
function [albipop]=albi(sj, ssub, sad, rep, CC, nbysub, VC, MR, HL, wkepid,
years)

//Obtain a stable age structured population to begin the simulation
//10 000 years simulation without parasite and vaccination
//lesliemat: Leslie matrix for the population
//vecpop: vector describing the age structured population (Nt in the main text)
    vecpop=[0;0;100]
    for i=1:10000
        pop=sum(vecpop,"r")
        lesliemat=[0 0 ((CC-pop)/CC)*rep ; sj ssub*((nbysub-1)/nbysub) 0 ; 0
ssub/nbysub sad]
        vecpop=lesliemat*vecpop
    end

//albipop: vector describing the population of albatross with
//row 1-3: sensitive females
//row 4-6: protected females (either vaccinated or temporarily protected by maternal
antibodies)
    albipop=[vecpop;zeros(3,1)]
    albimat=[sum(albipop,"r")]

//protec: lengths in days of the protection by maternal antibodies, 2 half lives
    protec=2*HL
//Determine at which date the epizootic will take place
    dayepid=wkepid*7

//Interannual cycle
    for i=1:years,
        pop=sum(albipop,"r")
        lesliemat=[0 0 ((CC-pop)/CC)*rep ; sj ssub*((nbysub-1)/nbysub) 0 ; 0
ssub/nbysub sad]
```

```
        lesliem=[lesliemat zeros(3,3);zeros(3,3) lesliemat]

//Calculation of the number of new vaccination required to achieve the vaccination
coverage
needf=VC*(albipop(3,:)+albipop(6,:))-albipop(6,:)
if needf<0,
   needf=0
end

//Vaccination of the required number of sensitive females
//Vaccinated females are subsequently added to the resistant population, and remain
there for the remainder of their life
        albipop(6,:)=albipop(6,:)+needf
        albipop(3,:)=albipop(3,:)-needf

//Reproduction of vaccinated and non vaccinated females
        albipop=lesliem*albipop

//Tackle the special case of non persistent maternal antibodies
        if protec==0,
           albipop(1,:)=albipop(1,:)+albipop(4,:)
           albipop(4,:)=0
        end

//Intra-annual cycle : decay of maternal antibodies and annual epizootic
//duration of the intra-annual cycle : 365 days
        for j=1:365
//Epizootic: sensitive newborns die given the parasite induced mortality rate
//Protected newborns do not suffer any increased mortality
           if j==dayepid,
              albipop(1,:)=albipop(1,:)*(1-MR)
           end

//Loss of maternal antibodies after 2 half lives
           if j==protec,
              albipop(1,:)=albipop(1,:)+albipop(4,:)
              albipop(4,:)=0
           end
//End of the intra-annual cycle
        end
        albimat=[albimat sum(albipop, "r")]
//End of the interannual cycle
    end
    plot(albimat)
endfunction


//Epidemic after 4 weeks, half life of antibodies of 25 days, vaccination coverage of
//40%
albi(0.7,0.87,0.95,0.3,300,6,0.4,0.7,25,4,300)
```

//Epidemic after 4 weeks, half life of antibodies of 5 days, vaccination coverage of //40%
albi(0.7,0.87,0.95,0.3,300,6,0.4,0.7,5,4,300)

//Epidemic after 4 weeks, half life of antibodies of 25 days, no vaccination coverage
albi(0.7,0.87,0.95,0.3,300,6,0,0.7,25,4,300)

//No parasite
albi(0.7,0.87,0.95,0.3,300,6,0,0,25,4,300)

ANNEXE 4

Manuscrit 4:

Evidence for maternal cross transfer of antibodies through allosuckling in a mammal

Etat du manuscrit : en préparation

Evidence for maternal cross transfer of antibodies through allosuckling in a mammal

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Abstract

The transfer of maternal antibodies is a critical mechanism for the early life survival of vertebrate newborns. In mammals, passive transfer of immune compounds can occur prenatally through the placenta and postnatally through the consumption of colostrums and milk and have various beneficial effects for the newborn. To what extent pups will access colostrum and milk might be important for the repertoire of passively acquired antibodies they will display and may influence their resistance to parasite early as well as in late life. In social mammals, it has been hypothesized that allosuckling may be a way for pups to broaden and strengthen their access to maternal antibodies, but empirical evidence for this mechanism is still lacking. Here we report the dynamics of antibody levels in Mongolian gerbil pups from two mothers, each vaccinated with a different vaccine, showing that pups acquired antibodies

from both of them. Our result provides the first experimental evidence of a cross transfer between litters of passively acquired antibodies. We discuss how such evidence opens perspectives for exploring the potential importance of horizontal transfer of immunity in natural host-parasite systems.

Introduction

Environmental conditions encountered by a newborn during early life are important for its fitness later in life (Lindström, 1999). Parasites, in particular, represent a major pressure on the population dynamics and evolution of their host species (Grenfell and Dobson, 1995; Tompkins et al., 2002), and their effect may potentially be even reinforced in young vertebrates whose immune response is not completely functional at birth (Frank, 2002). Although resistance to parasites relies on a combination of different mechanisms (reviewed in Schmid-Hempel, 2011), the immune system and in particular the delayed activation of the specific acquired response plays an important role in the clearance of infections by vertebrates(Wakelin, 1996). As part of this acquired immune response, infection by a parasite results in the delayed production of specific immunoactive compounds, the antibodies or immunoglobulins (Ig). In vertebrates, mothers have the ability to transfer some antibodies to their offspring either prenatally or postnatally (Brambell, 1970) and provide the newborn with an efficient protection (Zinkernagel, 2001). This protection is particularly critical because newborn vertebrates lack a fully efficient immune system and the transfer of maternal antibodies thus represents a critical mechanism for early life survival (Boulinier and Staszewski, 2008; Grindstaff et al., 2003).

In mammals, this transfer occurs through the placenta before birth and/or via the colostrum and the milk after birth (Baintner, 2007). Various factors are known to affect the transfer of antibodies from mother to the young. Differences in Ig subtypes are important to consider as only IgG can reach the newborns bloodstream in mammals (Pastoret, 1998), and further differences have been shown between IgG subclasses (Mix et al., 2006; Simister, 2003). Variations in the anatomical structure of the placenta and in particular in the number of tissue layers between maternal and fetal blood (Chucri et al., 2010) lead to contrasted situations in terms of prenatal transfer between mammalian species (Baintner, 2007). Conversely, postnatal

absorption of antibodies by newborns also reveals variations across mammalian species. For instance, in artiodactyls, no placental transfer of Igs is possible and colostrum absorption is critical for early life survival. In contrast, placental transfer seem to be the dominant route in primates while Igs in the colostrums and the milk may mostly play a role in the local protection of the gut (Sadeharju et al., 2007). In rodents, both prenatal and postnatal absorption of antibodies appear to be important factors to ensure the survival of the newborn (Gustafsson et al., 1994).

How newborns will gain access to colostrum and milk may thus be important in terms of passive transfer of immunity in many mammalian species. An interesting way for newborns to get milk is through allosuckling, when young individuals feed on a different female than their biological mother. Allonursing is indeed widely reported in mammals (Packer et al., 1992) and various hypothesis have been proposed to explain the evolution of allosuckling (Roulin, 2002). In particular, offspring could gain important immunological benefits by acquiring antibodies from various lactating females (immunological function of allosuckling hypothesis; Roulin and Heeb, 1999). This effect might be particularly important in social mammals, as sociality typically leads to an increase in the exposure to parasites and to an increase in the prevalence of common parasites (Loehle, 1995). Animals living in the same group also experience more similar parasitic environments (Delahay et al., 2000). Increased levels of passively acquired antibodies may increase the direct protective effect for the newborn and thus potentially reduce the cost of immune activation during the growth period. Moreover, diversifying the source of the milk may also allow the acquisition by the newborn of antibodies against rare pathogens, only encountered by a few females in the group. This broadening of the immune repertoire may also be particularly interesting considering the potential educational effect that passively acquired antibodies can have on the newborns immune system (Fink et al., 2008; Lemke et al., 2004; Lemke et al., 2009).

The potential for an immunological function of allosuckling has been suggested through the efficient transfer of antibodies from a foster mother to her non biological offspring (e.g. Gustafsson et al., 1994) and detailed classical studies on the transfer of maternal antibodies have used a fostering approach (Halliday, 1955). However, adoption in laboratory conditions does not reflect natural conditions as both mothers and newborns are not exposed to biological and foster mother/pups at the same time. In order to investigate the potential for the occurrence of a cross-transfer of antibodies between pups exposed to several females, we bred groups of 2 females Mongolian gerbils (*Meriones unguiculatus*), each injected with a different vaccine in a common environment. We subsequently followed the dynamics of the specific passively acquired antibodies in the newborns sera. We predicted that if allosuckling indeed has an immunological function, offspring from both females would have detectable antibodies specific of each vaccine.

Materials and methods

The mongolian gerbil (*Meriones unguiculatus*) is a small mammal that in the wild usually form groups made of a breeding pair associated with a number of subordinates. In the lab however, it is nevertheless possible to breed them successfully in groups, with several females giving birth together (French 1994). For the purpose of our study, two groups of female Mongolian gerbils (obtained from Janvier SAS, Le Genet-St-Isle, France) were vaccinated respectively against porcine influenza (0.1 mL, intramuscular injection; Gripovac, Merial SAS, France) or *Borrelia burgdorferi* (0.1 mL, subcutaneous injection; Merilym, Merial SAS, France) at 8 weeks of age. They also received a booster vaccination 4 weeks later using the same route and dose. They were then housed separately during 2 weeks with a male, before being randomly matched by pair of females (1 female vaccinated against influenza and the

other against *Borrelia)*. Due to aggressive behavior of females towards the male, holed Plexiglas walls were used to allow for limited contacts at first. Both individuals were switched between each side of the wall on a daily basis during a week to get used to each other's odor. At the end of the week, the separation was opened and contact between male and female was rendered possible (only one male died in the process). The same procedure was used with females, about a week before expected birth. Birth then took place in this common environment. However, due to high rates of cannibalism on pups, only one pair of females among 12 successfully raised 4 pups to aldulthood. We expected pups to gain access to milk from both females and to subsequently display detectable levels of antibodies specific of *Borrelia* and influenza. A schematic of the design used is given in figure 1.

At birth, newborns were marked subcutaneously with an individual combination of tattoo ink dots. At 3 weeks of age, before the first marking started fading, an additional numbered ear tag (Monel 1005-1, National Band & Tag Co., Newport, KY, USA) was used to ensure a long lasting individual marking of individuals. The same ear tags were also used for the marking of females throughout the experiment.

To assess passive antibody acquisition, blood was regularly obtained from the newborns during the rearing period. At day 1 and day 8, a blood sample (20-30 µL) was obtained by cardiac puncture using an insulin syringe with 30G needle. After eyes were opened, blood sampling consisted of a puncture each week (starting day 19) in the retro-orbital venal sinus with a heparinized capillary (75 µL), alternatively from the left and right eye. Blood was then stored in dry tubes and centrifugated within the hour. Plasma was collected and kept frozen at -20°C pending analyses.

Antibody levels in newborns were measured using specific commercial Enzyme Linked Immuno-Sorbent Assays (ELISA). An ELISA competition was used for Influenza (ID Screen

Antibody Influenza A Competition, ID Vet, Montpellier, France) and the percentage of inhibition (PI) relative to a negative control was used as a measure of antibody level. High PI values indicate high plasma concentrations of specific antibodies against influenza. A direct sandwich ELISA kit was used for *Borrelia* analyses (Borrelia IgG+ VlsE ELISA, IBL International GMBH, Hamburg, Germany). As this kit is primarily designed for humans, we replaced the secondary antibody by a peroxidase conjugated rabbit anti-gerbil IgG (Immunology Consultants Laboratory, Portland, OR, USA) in order to detect gerbil IgG. In this analysis, optical density (OD) was used as a measure of antibody levels in the newborns and high OD values reflected high serum levels for *Borrelia* specific antibodies.

Approval of the protocol, and in particular of the repeated sampling of newborns, was granted by the Languedoc-Roussillon Regional Ethics Committee (project number CEEA – LR – 1003).

Results & Discussion

At birth, three of the pups had high anti-*Borrelia* antibodies levels while the remaining one had a much lower level (figure 2). IgG against *Borrelia* can be transmitted through the placenta in rodents (in particular IgG3, Morshed et al. 1993) and thus this result indicates that those three pups were born to the mother that had received the anti-*Borrelia* vaccine. In turn, the female vaccinated against influenza gave birth to the last one. The dynamics of anti-*Borrelia* antibodies showed a steep increase in antibody levels during the first week of age due to the acquisition of maternal antibodies from the milk and all individuals reached very high levels by 7 days of age. The antibody levels were relatively stable between 7 and 19 days of age, a period during which newborns fed on milk. Afterwards, antibody levels decayed rapidly and all individuals were negative by 40 days of age.

The dynamics was similar for anti-influenza antibodies throughout the rearing period of newborns (figure 3). However, at birth, all individuals had no detectable antibodies indicating that antibodies against the nucleoprotein of influenza viruses were not transmitted through the placenta. Antibody levels afterwards increased and reached high levels between 7 and 19 days of age. Levels then decayed rapidly and undetectable antibody levels were reached in newborns by 32 days of age.

All newborns displayed at some point during the rearing period detectable antibody levels specifically directed against both *Borrelia* and influenza. This result thus clearly demonstrates the efficiency of allosuckling as a source of antibodies in newborns and thus provides evidence for a potential immunological function of allosuckling. Although the transmission of antibodies from a mother to foster offspring has already been described, this is to our knowledge the first time that a cross transfer of maternal antibodies is reported in a "true" social context where both females and newborns are really given the choice of whom they give milk to or receive milk from.

Moreover, the persistence of antibodies in the serum of the newborns does not seem different whether the antibodies had been acquired from the biological mother or not as all newborns became negative at the same time point. The rapid decay starting after 20 days of age is associated with weaning, which usually occurs between 21 and 30 days in Mongolian gerbils (Norris and Adams, 1972). At that time, newborns stop feeding on milk and do not acquire passive immunity from their mothers anymore. Moreover, the transfer of antibodies from the intestinal lumen to the bloodstream of the newborn relies on a receptor, the neonatal Fc receptor (FcRn) and it has been shown in mice that this receptor is only expressed during the neonatal period (Roopenian and Akilesh, 2007). How this receptor is expressed in the intestine of newborn gerbils is not known, and likely to influence the acquisition of passive antibodies.

In social groups, newborns could efficiently receive antibodies from all the lactating females. However, how newborns will have access to antibodies is likely to vary with the synchronicity of birth events among females. Pups from the first litter may for instance be able to get large amount of antibodies via the colostrums of non-biological mothers while the mother of this first litter will provide less antibodies to younger pups because they will only be able to suckle milk. This effect might be particularly important when dominance structure is considered. Indeed, dominant females are expected to give birth first and be followed by subordinates. Pups from the dominant may thus be able to broaden and strengthen their repertoire of antibodies with the colostrum of foster mothers. The existence of an efficient cross transfer of antibodies may also participate in the decision of dominant females to allow subordinate breeding. In some social mammals, such as Banded mongooses (*Mungos mungo*), subordinate females can also be forced out of the group during the course of the gestation (Cant et al., 2010; Gilchrist, 2006). Excluded females can subsequently be accepted in the group again after having had an abortion. Should this abortion occur late in gestation, it has the potential to result spontaneous lactation which may further reduce the amount of maternal care required from the dominant female. In addition, subordinate females may experience a rather different parasitic environment during the course of exclusion and broaden and strengthen the repertoire of the pups of the dominant female through cross transfer of antibodies.

Here we provide experimental evidence for the efficiency of cross transfer of antibodies in laboratory conditions. The next step would be to check whether this cross transfer also happens in wild social mammals. Social mammals such as meerkats (Clutton-Brock et al., 2001), banded mongooses (Cant, 2000) or rodents (Hayes, 2000) for which long term monitoring programs may already be implemented might prove particularly suitable for such studies. As in social systems all individuals are likely to have similar exposure history to

parasites, it might however be difficult to find parasites that will be easy to track. An interesting option would be to use vaccines to tag females (Staszewski and Boulinier, 2004): each female from a social group would receive a different vaccine and antibodies against each vaccine would be searched for in newborns. Vaccines should be carefully chosen to ensure their safety for the indivual but also to come along with ready-to-use ELISA kits to measure antibody levels in the pups. Although it would be best to obtain several blood samples during the rearing period to assess the dynamics of antibodies in newborns, a single blood sample is sufficient to obtain evidence for the cross transfer of antibodies. One could also expect difference in the synchronicity of births between social groups, and thus repeating the same design in several may also provide insight on the role of this particular mechanism on the efficiency of the cross transfer of antibodies.

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Figure legends

Figure 1: Schematic of the experimental protocol implemented on the Mongolian gerbils. Females were vaccinated with either Borrelia (blue) or Influenza (green) vaccine and are subsequently expected to transmit specific antibodies to their biological pups. Whether this transfer will also occur between litters is the main question of our study.

Figure 2: Borrelia specific antibody levels in the serum of the pups at different age. Antibody levels are expressed as optical densities (OD) measured by ELISA: a high value of OD corresponds to high antibody levels.

Figure 3: Influenza specific antibody levels in the serum of the pups at different age. Antibody levels are expressed as percentage of inhibition (PI) by comparison with a control sample: a high value of PI corresponds to high antibody levels.

Figures

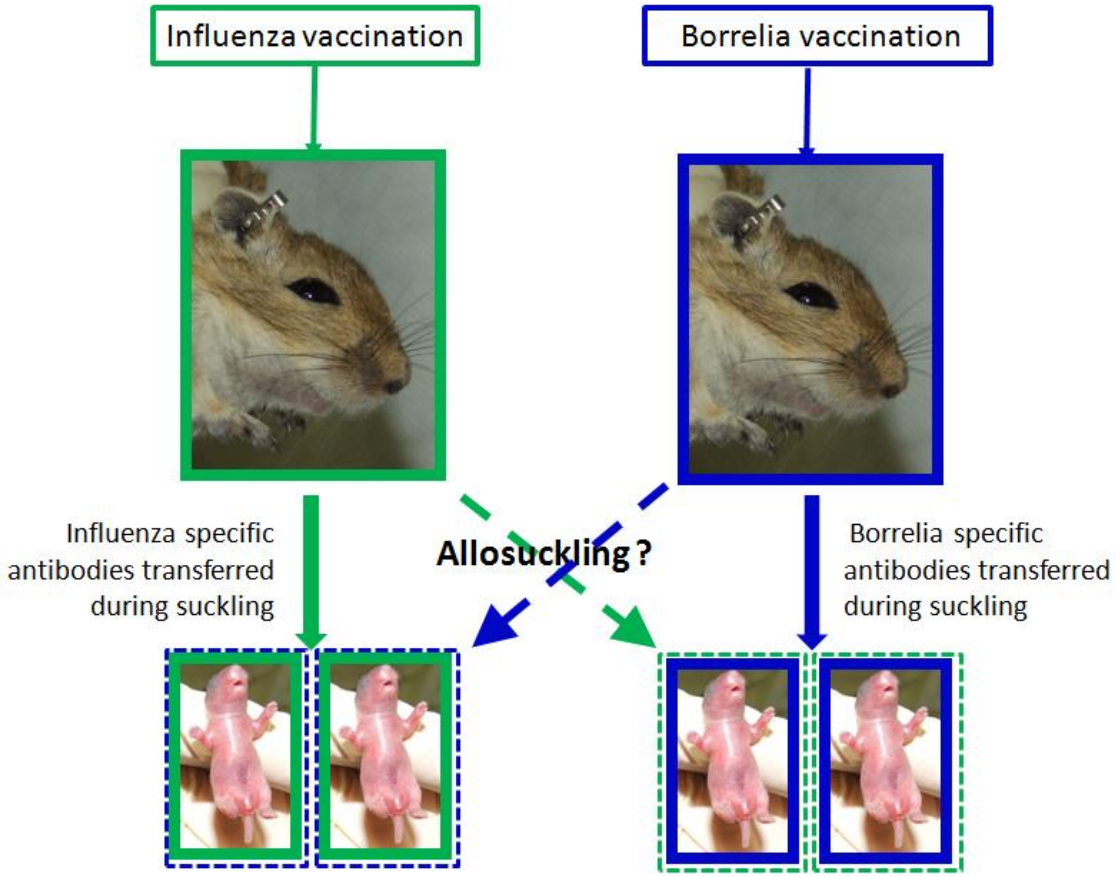

Figure 1

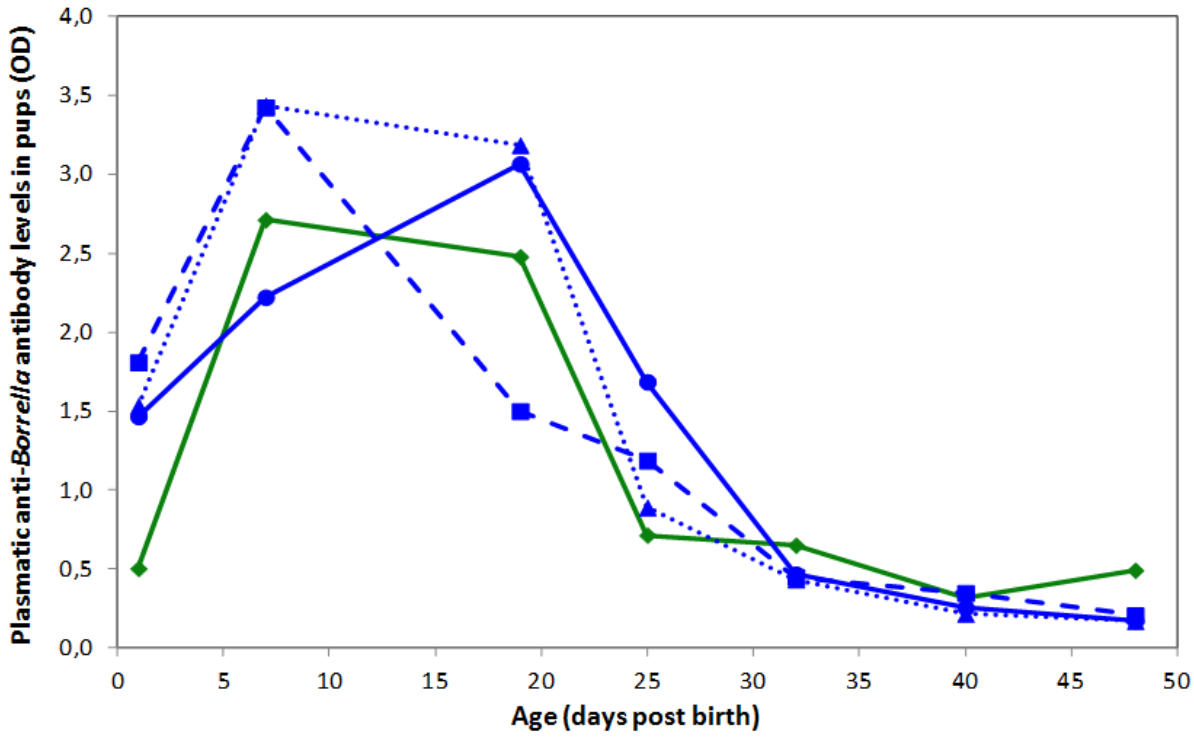
Plasmatic anti-*Borrelia* antibody levels in pups (OD)
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
5
10
15
20
25
30
35
40
45
50
Age (days post birth)

Figure 2

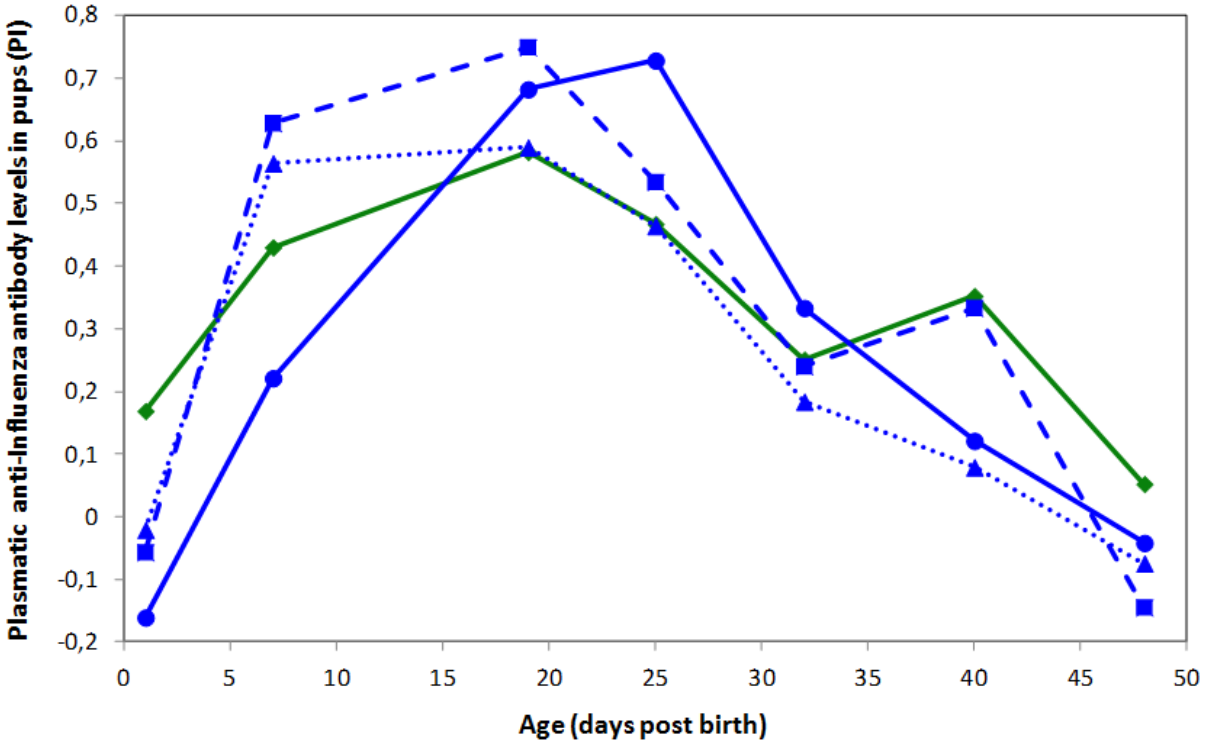
Plasmatic anti-Influenza antibody levels in pups (PI)
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
-0,1
-0,2
0
5
10
15
20
25
30
35
40
45
50
Age (days post birth)

Figure 3

ANNEXE 5

Manuscrit 5:

Length of intervals between epidemics: potential importance of the maternal transfer of immunity

Etat du manuscrit : en préparation pour resoumission

Length of intervals between epidemics: potential importance of the maternal transfer of immunity

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Abstract

Determining what factors affect the length of intervals between epidemics is critical in epidemiology. Recently, particular interest has been given to the role of the dynamics of immunity in determining the intervals between epidemics in wild animal populations. One potentially powerful, but often neglected, process in this context is the maternal transfer of immunity. Here we explore theoretically how the transfer of maternal antibodies can delay the recurrence of epidemics in systems in which epidemic outbreaks followed by pathogen eradication occur. We show that the occurrence of temporary protected newborns can dramatically decrease the proportion of susceptible individuals in the population thus strengthening herd immunity. This effect is likely to be especially strong in seasonally and colonially breeding species exposed to parasites transmitted on the breeding grounds, because the passive protection of newborns can prevent the dilution effect of the newborns on herd immunity. Overall, our results underline how the dynamics of population level immunity can lead to important ecological consequences.

Introduction

The ecology and evolution of both host species and parasites have been widely studied as key factors governing the emergence and re-emergence of pathogens (Schrag & Wiener 1995). Variations in time and space of the distribution of hosts (Altizer *et al.* 2011) and of the immune status of the population (Graham *et al.* 2010) have been shown to influence the epidemiology of a disease. Immunity is indeed a critical mechanism governing the dynamics of diseases in wildlife (Grenfell & Dobson 1995; Tompkins *et al.* 2002). In vertebrates, immune response to parasites relies on both an immediate non specific innate response and a delayed specific acquired response (Frank 2002). The acquired immune response partly results in the induction of specific molecules, the immunoglobulins or antibodies, that directly reduce the deleterious effects of parasites (Frost 1999). These immunoactive compounds can be transmitted to the offspring (Brambell 1970) through the colostrum and the milk in mammals or via the egg yolk in oviparous species. Because this transfer can result in a direct protection against the parasite (see for example Wallach *et al.* 1992; Gustafsson *et al.* 1994), epidemiological implications of this mechanism might be important (Gasparini *et al.* 2001; Grindstaff *et al.* 2003) and yet remain largely overlooked (Boulinier & Staszewski 2008).

The importance of the transfer of maternal antibodies has however received some attention in the context of endemic diseases. In a vole population, the protection of newborns by maternally transferred antibodies has been shown to postpone infection by hantavirus (Kallio *et al.* 2006) and to partly account for the seasonal dynamics of the infection by the hantavirus (Kallio *et al.* 2010). High levels of exposure of mothers prior to reproduction could result in the production of a high proportion of temporarily protected newborns resulting in decreased overall exposure in the following autumn. The temporary protection of newborns could modify the severity and the spread of a disease (Fouchet *et al.* 2006; Fouchet *et al.* 2007), notably because maternal antibodies could favour the occurrence of a “natural

vaccination" against intensively circulating parasites (Zinkernagel 2003). The duration of acquired immune protection and the length of the reproductive season are also of importance in the case of endemic parasites (Fouchet *et al.* 2008).

Such factors may be of importance in systems characterized by epidemics followed by eradication of the parasite, especially if reproduction is synchronized. The gradual loss of immunity by aging individuals has for instance been suggested to partly explain the occurrence of a Canine Distemper Virus epidemic after a long disease free time period in the lion population of the Serengeti (Guiserix *et al.* 2007). Long term effects of immune protection at the population level have been described as the "herd immunity" effect (Anderson & May 1985) that specifies that the protection of part of a population (depending on the characteristics of the parasite) is sufficient to prevent the occurrence of an epidemic. In species with synchronous reproduction, the pulse of naive newborns (Roberts & Kao 1998) has been shown to cause a rapid drop of herd immunity (Keeling & Rohani 2008) which favours the circulation of parasites on breeding grounds. The transfer of maternal antibodies is likely to modify this effect as a lower fraction of the population will effectively be available for the parasite, even after newborns enter the population (Boulinier & Staszewski 2008). A simple analysis of an epidemiological model modified to account for maternal protection (described in appendix 1) can indeed show that maternal antibodies will slow down the build up of the susceptible fraction of the population (figure 1), a critical mechanism for the global epidemiological pattern of a disease (Stone *et al.* 2007). This mechanism has thus the potential to contribute long intervals between epidemics in natural systems.

To explore this potential, we will focus on the European harbour seal (*Phoca vitulina*) populations that have endured two massive mortality events, in 1988 and again in 2002, related to the circulation of a Morbillivirus, the Phocine Distemper Virus (PDV; Osterhaus & Vedder 1988; Jensen *et al.* 2002). In both occasions, the epidemics started on a the same

colony (Härkönen *et al.* 2006) which leads to suppose that the parasite is rarely introduced on the colony (Grenfell *et al.* 1992; Harding *et al.* 2005). The PDV could however well be introduced on the colony more often through for instance contacts with another seal species such as the Grey seal (*Halichoerus grypus*)(Hall *et al.* 2006; Härkönen *et al.* 2006). In that case, a strong herd immunity following an epidemic could prevent short recurrence of epidemics. The transfer of maternal immunity could reinforce this effect by providing a temporary strengthening of herd immunity during the critical period of reproduction.

Here we build a model using realistic parameter values to describe the demography of a European harbour seal population and the epidemiology of the PDV in this population and focus on the predicted intervals between. We focus on how the transfer of maternal antibodies can modify the recurrence of epidemics by comparing models in which acquired immunity can or cannot be passively transferred to offspring. We investigate how the intervals between PDV epidemics are influenced by the basic reproductive number of the parasite and by the synchronicity of the reproduction of the host, an ecological condition that has been shown to influence host-parasite dynamics (Cattadori *et al.* 2005; Adler *et al.* 2008). We also investigate the importance of important the synchronicity of the reproduction of the host and of the transmissibility of the disease. Finally, we investigate the effects of the maternal transfer of immunity in models where introduction of the parasite is subject to stochascticity.

Material and methods: the harbour seal / PDV model

Demography

The demography of the isolated harbour seal colony considered here can be described by an age-structured Leslie model (Caswell 2001) parameterized as described by Härkönen *et al.* (2002). The population is limited through density-dependent fecundities. The carrying capacity of the colony is fixed to 1000 females.

Reproduction happens once a year and can theoretically last up to 180 days to allow for the investigation of an effect of synchronicity of births described by the parameter σ. When $\sigma = 1$, all births happen on day 90. On the contrary, when $\sigma < 1$, births are spread over a number of days equal to $180 - 179*\sigma$ and the reproductive season is centred on day 90. This reproductive season is considered short enough to neglect deaths from natural causes during the season.

At the beginning of each simulation, population structure equals disease-free stable age structure.

Epidemiology

From an epidemiological point of view, individuals can be either protected (by their acquired immune response or by maternally transferred antibodies) or sensitive. Maternal protection, even years after exposure to the virus, has been documented for PDV (Jensen *et al.* 2002) and maternally protected individuals become sensitive 30 days after their birth, so that every newborn is sensitive the year after his birth.

It has been demonstrated that the PDV is not maintained in colonies between epidemics (Swinton *et al.* 1998). According to the hypothesis of an introduction of the pathogen by grey seals, we will first consider that the virus is introduced every year on day 100 of the reproductive season, which can be seen as a worst case scenario. In a first part, we consider

that the virus is efficiently introduced each, and that immunity is the only driver of the epidemiological dynamic. In a second part, we consider that the virus has an annual probability of being introduced (ranging from 0 to 1), and in that case an epidemic only occurs if the introduction is efficient and if immunity allows an epidemic to take place.

With regard to immunity, an epidemic can occur when the fraction of protected individuals falls below the threshold defined for herd immunity $1-{}^{1}\!/_{R_0}$ (Hethcote 2000). If herd immunity is sufficient, the virus does not spread in the colony and we assume that all susceptible individuals remain susceptible the year after. On the contrary, if herd immunity is below the threshold, an epidemic occurs. As shown by retrospective analysis of both the 1988 and 2002 epidemics, adults and newborns suffer increased mortalities compared to subadults (Heide-Jørgensen & Härkönen 1992; Härkönen *et al.* 2007). This is modelled by an age-specific mortality pattern as described in Harding *et al.* (2005). All individuals surviving an epidemic are considered to have developed an acquired immune response, and are therefore added to the resistant individuals. This simplification is supported by observations revealing an exposure of at least 95% of reproductive females on a colony during 1988 epidemic (Heide-Jørgensen & Härkönen 1992).

We explored the effect of different values of the basic reproductive number R_0, with a particular emphasis being given to published estimates ranging from 2.03 to 2.8 (De Koeijer *et al.* 1998; Swinton *et al.* 1998; Klepac *et al.* 2009).

Results

The transfer of maternal antibodies is responsible of an increase in the predicted intervals between epidemics of PDV in the European harbour seal population (figure 2). When considering a basic reproductive number or 2.8 (Swinton *et al.* 1998), considering only acquired immunity leads to intervals of 3 years between epidemics (blue curve) and allowing for the transgenerational transfer of this immune protection increases intervals up to 6 years (green curve). Qualitatively, this result is not sensitive to the exact value of the basic reproductive ratio (figure 3A). Indeed, the mean interval after 300 years of simulation is always longer when the maternal transfer of antibodies is considered, although this difference tends to reduce for low values of basic reproductive number. Quantitatively, the increase in the basic reproductive ratio not surprisingly results in reduced interval between epidemics. However, it is interesting to note that the variation between 2.03 and 2.8, the boundaries of the estimates from the natural situations (gray background on figure 3A), results in about 2 years difference on the intervals between epidemics. The transfer of maternal antibodies has a rather limited effect on the extent of this decay.

How intervals between are extended by the transfer of maternal antibodies also depends on the synchronicity of the reproduction (figure 3B). Increase in the synchronicity of reproduction at first has very low effect on the mean interval between epidemics until a quick increase at high values (green curve). This directly stems from the effect of the pulse of maternally protected newborns. Indeed as newborns are only protected for a limited period of time, when births are not synchronous, the protection of newborns has time to wane before the parasite is introduced in the population which increases the proportion of sensitive individuals and reduces the herd immunity. More surprisingly, highly synchronous births reduce the mean interval between epidemics when only acquired immune response is considered (blue curve). This is because, when newborns are not protected by maternal antibodies, less synchronous

births reduces the number of offspring already born at the time of the exposure to parasite and dampens the birth pulse of sensitive newborns.

Finally, the difference between a situation with an without maternal transfer of antibodies still holds when considering that the introduction of the parasite does not happen every year (figure 4). Interestingly, considering stochasticity on the introduction of the parasite can lead to 14 years interval and such intervals are included in the standard deviation of the mean up to higher annual probability of introduction of the parasite when the transfer of maternal antibodies is considered (figure 4A) as compared to a situation where only the acquired immunity is considered (figure 4B).

Discussion

Transgenerational transfer of antibodies from a mother to her offspring can have strong epidemiological implications because it can reduce the proportion of sensitive individuals in the population at critical times (Boulinier & Staszewski 2008). Our results indicate that this reduction can be relevant from an epidemiological point of view as it can delay the initiation of a new epidemic. The extent of the delay depends on key features of the parasite such as R0 as well as on ecological factors specific of the host species such as the synchronicity of reproduction events. The modification of the dynamics of the susceptible fraction of the population, a critical epidemiological process (Stone *et al.* 2007), by the transfer of maternal antibodies could then partly explain the existence of long intervals between epidemics in some host-parasite systems.

We show in particular the effects of the transfer of maternal antibodies are amplified when reproduction is synchronous, while at the same time synchronicity reduces the intervals between epidemics when only acquired immunity is considered. In other words, the transfer of maternal immunity could be especially important in colonially breeding species, as births are often constrained within a small time window. Indeed, in colonial species such as seabirds or seals the reproductive season is constrained by environmental conditions such as food or breeding ground availability, and these species can reproduce on a very limited time scale and at high densities. The concentration of colonially reproducing individuals on spatially limited breeding areas leads to an increase in contact rates and exposure to parasites (Loehle 1995), but the transfer of protective immunity from mother to newborns reduces the occurrence of epidemics while also providing the newborn with other immunological benefits (Grindstaff *et al.* 2003; Boulinier & Staszewski 2008; Hasselquist & Nilsson 2009). On the one hand, the transfer of maternal antibodies could thus be as an adaptation to colonial reproduction, and in that case, colonially reproducing species may display higher levels of transfer of antibodies.

On the other hand, the transfer of maternal antibodies occurs in all vertebrates, colonial or not, and could represent one of many factors having favoured the evolution of colonial reproduction (Danchin & Wagner 1997).

In colonial species, the epidemiological effects of the maternal transfer of antibodies could also be amplified by specific features of temporal variations in the composition of the colonies, notably in relation to the age of individuals. It has for instance been shown that subadult harbour seals have a different behaviour and do not interact as closely with other individuals as sexually mature seals and lactating pups (Härkönen & Harding 2001). This leads to a relative segregation of juvenile and adult harbour seals during the calving season that could be related to a differential transmission pattern of PDV (Klepac *et al.* 2009). In seabirds, which are colonial and have delayed age at first reproduction, subadults can spend several years away from colonies before starting to prospect on colonies as part of the recruitment process (Reed *et al.* 1999). This low attendance of subadults on colonies observed in many colonial species could thus lead to an even bigger effect of the transfer of maternal immunity on 'local' herd immunity, hence leading to increased intervals between epidemics.

It is important to notice that, for simplicity, we do not model directly the infection process as a function of either the density or the frequency of infected individuals. In that case, the annual introduction would correspond to introducing a number of infected individuals in the population. In that case, the density of susceptible individuals in the population is the key parameter determining the emergence probability of a parasite (for a mathematical definition, see Keeling & Rohani 2008). Indeed, the occurrence of a full scale epidemic can highly depend on stochastic events: even if the parasite is introduced in a population with enough susceptible individuals to sustain an epidemic, the parasite can stochastically go extinct. For instance, if a small number of infected hosts are responsible for the introduction event, they can fail to transmit the parasite (due to the death of infected

individuals as a result of the infection or by chance). This emergence process of the parasite will also affect the size of the induced epidemic. Indeed, the size of the epidemic clearly depends on the fraction of the population that is sensitive at the time of the epidemic (Harding *et al.* 2002; Harding *et al.* 2003). If an epidemic occurs close to the threshold density of sensitive hosts in the population, then the resulting epidemic will be of minimal size. If the epidemic is delayed, for example due to a stochastic emergence phenomenon, the fraction of sensitive individuals in the population will increase. When an epidemic eventually occurs, more sensitive hosts will be available for the parasite and a greater epidemic will take place. Although our simple model shows that the transfer of maternal antibodies can delay the occurrence of epidemics by modifying the susceptibility landscape of the population even when the parasite is not introduced each year, a more detailed epidemiological model would be required to understand how this mechanism modifies on the long term the emergence probability and the size of subsequent epidemics.

Infectious diseases can be a major threat for wildlife conservation (Daszak *et al.* 2000). Our results suggest that the maternal transfer of antibodies, by temporarily protecting newborns, could have important consequences for the timing of epidemics in host-parasite systems and thus the potential to reduce the impact of infectious diseases on population dynamics. It should however be kept in mind that little is known about the actual adaptive value of the maternal transfer of antibodies in natural populations (see (Boulinier & Staszewski 2008) for a review). Some maternal antibodies can have protective effects (Al-Natour *et al.* 2004; Staszewski *et al.* 2007; Nemeth *et al.* 2008), but it is also well known that some antibodies do not protect from the deleterious effect of parasites against which they have been produced (Frank 2002). It is not known though how this relates to the observed patterns of exposure and prevalence to the wide diversity of parasitic agents. Further studies

using natural and experimentally manipulated host-parasite systems are required to address such issues.

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Figure legends

Figure 1: Maternal antibodies prolong the time to reach critical herd immunity, where an epidemic can take hold. The graph illustrates the increase in number of susceptible individuals over time after an epidemic outbreak (ended at time 0) in a general epidemiological model (see supplemental material), including acquired immunity only (gray plain curve) or with both acquired immunity and maternal transfer of antibodies (black plain curve). Herd immunity ($S_{crit,}$ below which $R<1.0$) is indicated by the gray dashed line. Parameters: $N=100$; $\delta=1$; $\omega=0.01$; $\beta=1$; $\alpha=20$; $\gamma=10$.

Figure 2: Population dynamics of an age structured model parameterized as for the Swedish harbour seal population enduring annual introduction of the Phocine Distemper Virus (PDV). Predicted dynamics show shorter intervals between PDV epidemics with acquired immunity only (blue line) than with acquired immunity associated with maternal transfer antibodies (green line). Reproduction happens synchronously, once a year on day 90 of the reproductive season and maternal antibodies are protective for 30 days. Parasite is introduced on day 100, and $R_0 = 2.8$.

Figure 3: Effect of different parameters on the mean interval between PDV epidemics in a model parameterized for the Swedish harbour seal population and after 300 years of simulation when acquired immunity only protects adults (blue curves) or when it can be maternally transmitted to offspring (green curves). (A) Effect of the basic reproductive number (R_0) on the predicted intervals between PDV epidemics. The dots represent the exact values predicted by the model, and the curves correspond to exponential decay curves fitted as linear models ($p<2.2 * 10^{-16}$ in both cases). (B) Effect of the synchronicity of the reproduction

(σ) on the predicted intervals between PDV epidemics. The dots represent the exact values predicted by the model.

Figure 4: Effect of the annual probability of introduction of the PDV on the mean interval between PDV epidemics in a model parameterized for the Swedish harbour seal population when acquired immunity only protects adults (A, blue curve) or when it can be transferred to newborns (B, green curve). Plain curves represent the mean interval after 10000 years of simulation, and dashed lines represent the standard deviation of the mean. The dashed red line represents an interval of 14 years as observed between 1988 and 2002 in the considered population.

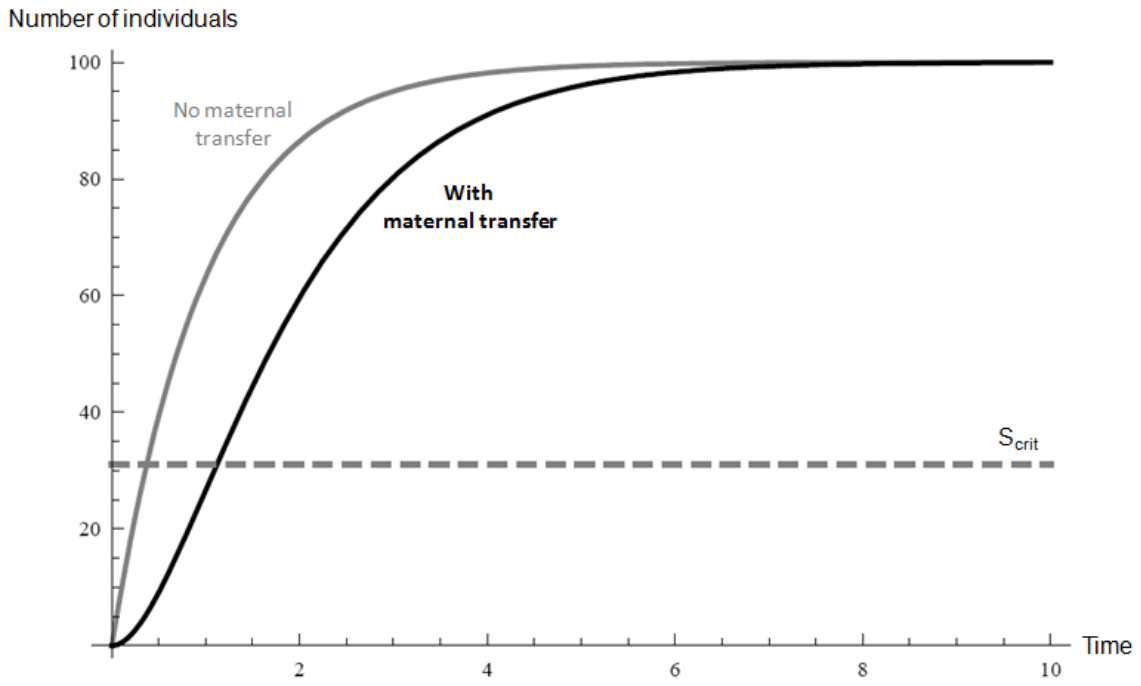
Number of individuals
100
80
60
40
20
No maternal
transfer
With
maternal transfer
S_{crit}
Time
2
4
6
8
10

Figure 1

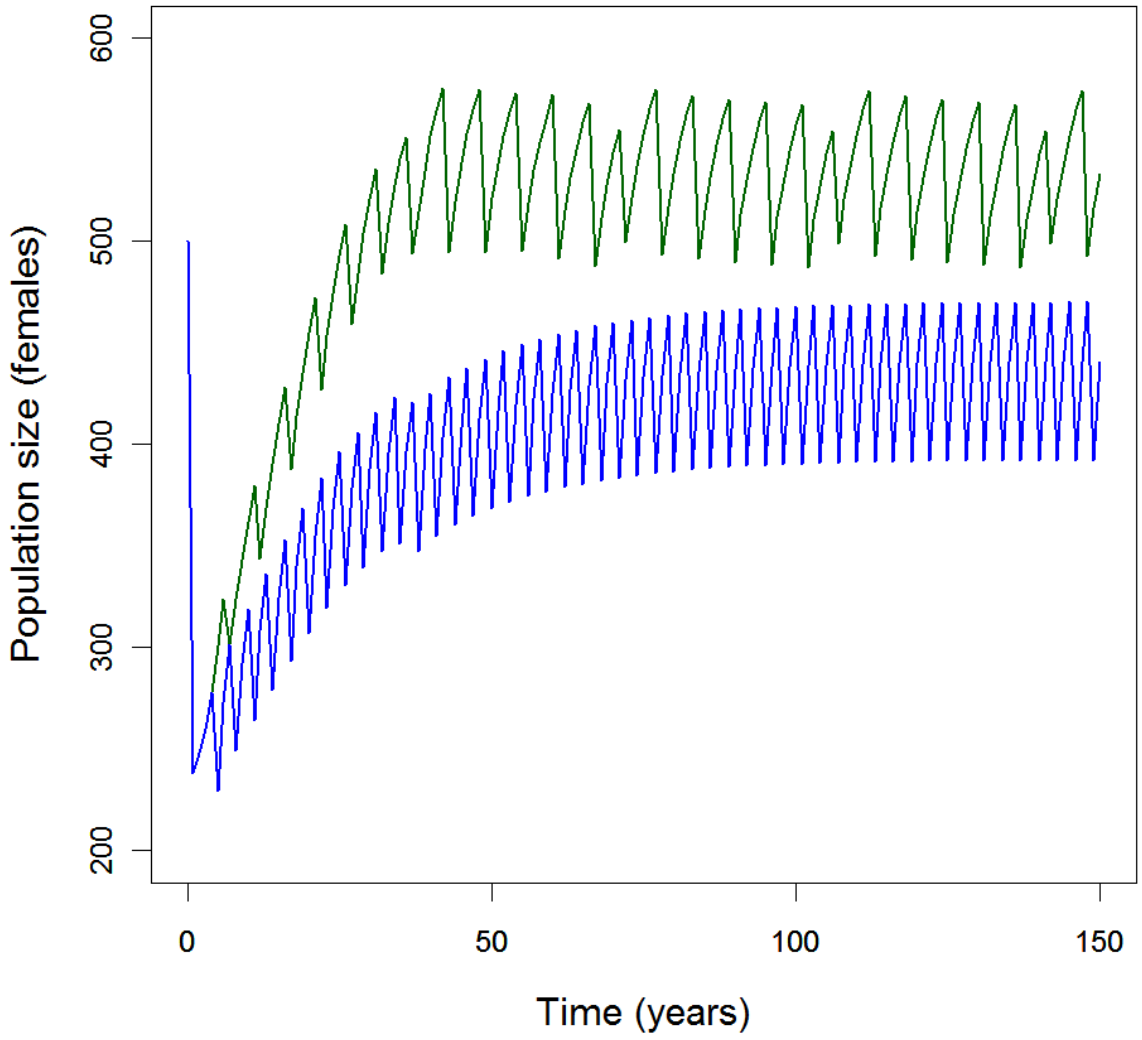
Population size (females)
600
500
400
300
200
0
50
100
150
Time (years)

Figure 2

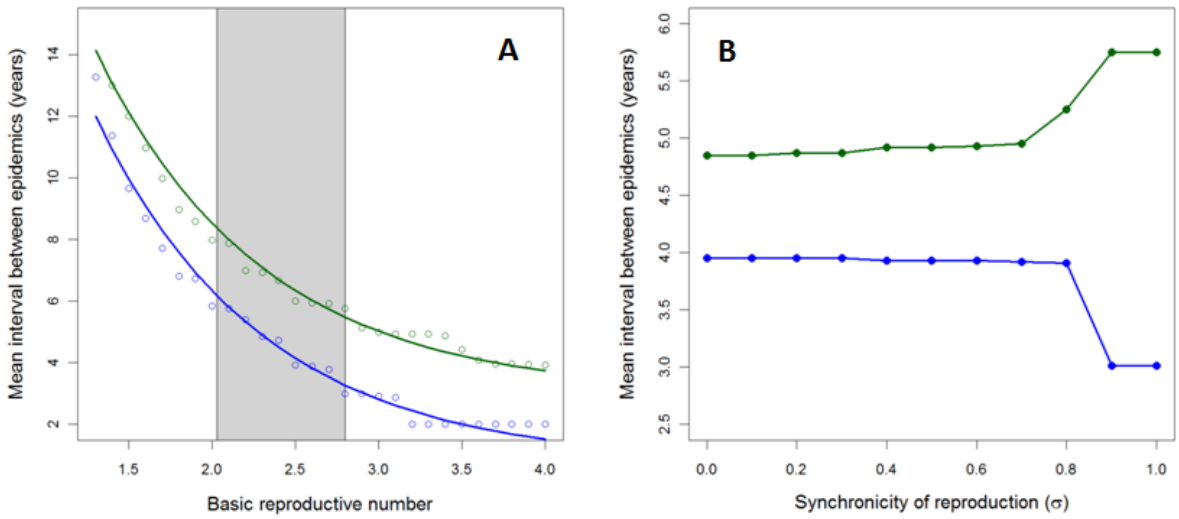
A
Mean interval between epidemics (years)
Basic reproductive number
B
Mean interval between epidemics (years)
Synchronicity of reproduction (σ)

Figure 3

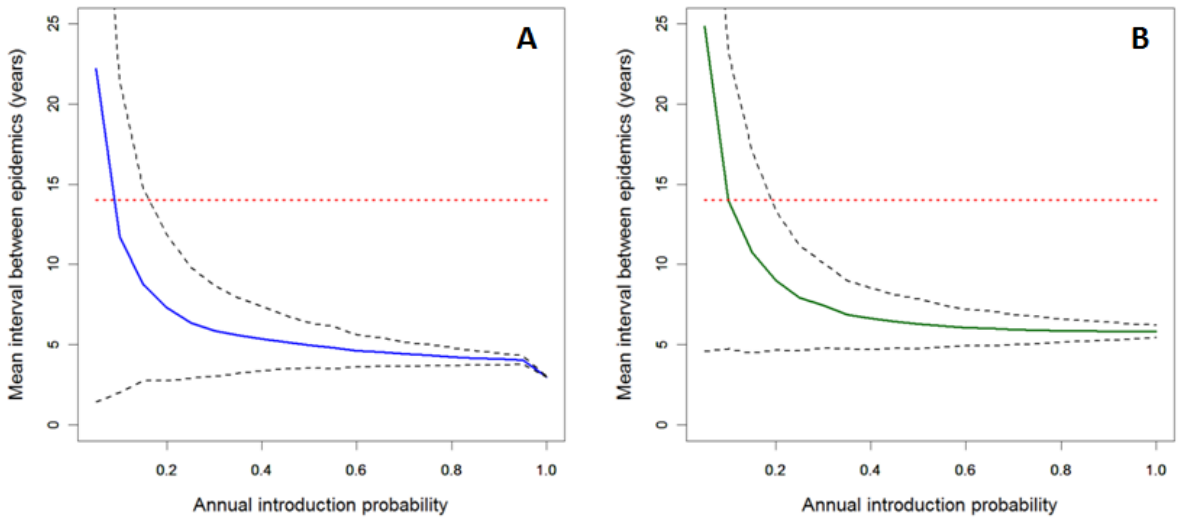
A
B
Mean interval between epidemics (years)
Annual introduction probability
0
5
10
15
20
25
0.2
0.4
0.6
0.8
1.0

Figure 4

Supplementary material

A simple epidemiological model can be written to follow the densities of four different types of hosts (Hethcote 2000): susceptible (S), infected (I), recovered (R) and maternally protected (M) hosts. This model is described by the following system of differential equations:

$$\begin{aligned}
\frac{dM}{dt} &= \lambda\theta\frac{R}{N} - (\omega+\delta)M \\
\frac{dS}{dt} &= \lambda(1-\theta\frac{R}{N}) + \omega M - (\delta+\beta I)S \\
\frac{dI}{dt} &= \beta SI - (\delta+\alpha+\gamma)I \\
\frac{dR}{dt} &= \gamma I - \delta R
\end{aligned} \qquad \text{(S1)}$$

The first line of the system (S1) describes the rate at which animals are born into the maternally protected class (M) from recovered mothers (R). This depends on the fraction θ of recovered mothers that produce maternal antibodies ($\theta = 0$: acquired response only; $\theta = 1$: all resistant females transfer antibodies) and on the density dependent growth function. Susceptible (S) individuals are produced by all classes of individuals, either through density dependent reproduction or through the loss of maternal antibodies (at rate ω). Those susceptible individuals can be infected through a density dependent function. Infected (I) individuals can then either die from the infection (at rate α, the virulence of the parasite) or recover from the infection (at rate γ). Recovered (R) individuals gain a lifelong protection after recovery. All classes can also die due to natural mortality (at rate δ). In order to simplify the analysis of this model, it is hypothesized that the total density of the host population remains constant in time, with dead individuals being immediately replaced by newborns ($\lambda = \delta N + \alpha I$).

An important epidemiological feature of a parasite is its basic reproductive number R_0 which is defined as the expected number of secondary infections produced by a single infected individual introduced in a completely naïve population. For an epidemic to occur in the first place, this basic reproductive number must be over 1, so that an infected individual in the population will result in at least a new infection. In the present model the basic reproductive number is: $R_0 = \frac{\beta N}{\alpha + \delta + \gamma}$

In a partially immune population, there is an analogous criteria for parasite emergence which depends on the "effective" reproductive number $R = R_0(S/N)$. An epidemic will occur if $R > 1$. In other words, there is a threshold density of susceptible individuals $S_{crit} = \frac{\alpha + \delta + \gamma}{\beta}$ above which the effective reproductive number becomes sufficient to allow a new epidemic. Consequently, the effect of maternal transfer of immunity on the recurrence of epidemics boils down to its effect on the time required to build up a large enough population of susceptible hosts to trigger a new epidemic.

If we consider a situation where the host population has suffered a very intense epidemic (i.e. we assume all remaining hosts to have recovered) after which the parasite goes extinct, the system (1) reduces to:

$$\begin{aligned} \frac{dM}{dt} &= \delta\theta R - (\omega + \delta)M \\ \frac{dS}{dt} &= \delta(N - \theta R) + \omega M - \delta S \\ \frac{dR}{dt} &= -\delta R \end{aligned} \qquad \text{(S2)}$$

By integrating over time S2, under the assumption that all individuals are resistant at time step 0 (in particular, S(0) = 0), we express the rate of build up of susceptible individuals in the population with respect to θ and the time since the last epidemic (t):

$$S(t) = \frac{Ne^{-\delta t}\left[\left(e^{-\omega t} - 1\right)\delta\theta + \left(e^{-\delta t} - 1\right)\omega\right]}{\omega}$$

To investigate the effect of the ability of mothers to transfer antibodies on the dynamics of the sensitive fraction of the population, we derive *S(t)* with respect to the parameter θ:

$$\frac{dS}{d\theta} = \frac{\delta N e^{-\delta t}}{\omega}\left(e^{-\omega t} - 1\right)$$

As all the parameters in our model can only have positive values and noting that the expression between parentheses is always negative, the above derivative is always negative. Hence $S(t)$ is a decreasing function of θ, which means that the maternal transfer of antibodies (θ) will always delay the duration between epidemics. This effect is illustrated in figure 1 in the main text.

ANNEXE 6

Manuscrit 6:

Estimating dispersal in the wild: antibodies as a source of information?

Etat du manuscrit : en préparation

Estimating dispersal in the wild: antibodies as a source of information?

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Running title: Estimating dispersal using antibodies

Abstract

Several methods are available to infer dispersal rates in wild animal populations and all have their own drawbacks, in particular when it comes to the time or spatial scale at which they reach their best accuracy. Here, we propose that serological data on the history of exposure of individuals to infectious agents could also be used as a source of information to infer dispersal rates. We first outline the approach by presenting a simple theoretical framework which allows us to explore its potential interest depending on characteristics of the host-infectious agent system considered. We then illustrate its use with a system involving a long-lived colonial seabird, the black legged kittiwake (*Rissa tridactyla*), and two infectious agents, tick-borne Lyme disease bacteria *Borrelia burgdorferi sensu lato* and Avian Influenza viruses. The spatially heterogeneous and temporally stable seroprevalence of anti-*Borrelia* positive individuals indicate that host dispersal is limited at the small spatial scale considered. Conversely, the spatially homogeneous seroprevalence of anti-Avian Influenza virus antibodies provides little information about host dispersal. Given that serological data can be gathered for a broad range of host-parasite systems, we suggest that it represents a neglected but potentially useful source of information about dispersal for vertebrates.

1. Introduction

The movement of individuals is central to animal ecology, affecting both the population biology and evolution of species (Dingle & Holyoak 2001; Nathan *et al.* 2008). Categorizing different types of movements is not an easy task, but some particular movements that share common time or spatial dimensions at the species level appear to be widespread in animal populations. Among these, one of the most widely studied processes is dispersal (Clobert *et al.* 2001). Dispersal can be defined as the movement of an individual from its birth site, or last breeding site, to the place where it reproduces (adapted from Greenwood & Harvey 1982). This definition includes the dispersal of young individuals before first reproduction, i.e. natal dispersal, and the dispersal of adult individuals that shift to new breeding locations between breeding seasons, i.e. breeding dispersal. From this definition, it should be noted that dispersal only occurs when there is a breeding attempt on a new breeding site. Thus, prospecting behaviors (i.e. visits of potential breeding grounds without actual local reproduction; Reed *et al.* 1999), commonly reported in colonial species such as seabirds, are excluded from this definition. Likewise, migratory movements are not specifically associated with dispersal because many species display long range migration, but high fidelity to their breeding location. Given these constraints, dispersal rates can be difficult to infer from field data.

Dispersal has important implications for the demography and the genetic structure of animal populations (Hanski & Gaggiotti 2004), as well as for the epidemiology and evolution of interactions between hosts and parasites (Gandon & Michalakis 2002). The ecological and evolutionary relevance of dispersal has led to the development of numerous techniques to estimate dispersal rates in natural populations (Clobert *et al.* 2001). Capture-Mark-Recapture (CMR) techniques, based on resighting marked individuals, has allowed biologists to gather crucial information about dispersal. However, proper estimation of dispersal rates using marked animals requires data and models that enable one to account for the imperfect

detection of individuals and for their potential loss via mortality, the latter being especially problematic in the case of dispersal movements (Bennetts *et al.* 2001; Lebreton *et al.* 2003). Multi-site capture-remark-recapture models can disentangle mortality and dispersal, although the large and detailed datasets required for an accurate estimate of dispersal are still scarce (Spendelow *et al.* 1995; Cam *et al.* 2004a; Cam *et al.* 2004b). Measures of genetic structure among populations are also used to infer dispersal. One common method, Isolation by distance, uses the slope of the relationship between the genetic and geographic distances among populations to infer the average parent-offspring dispersal distance (Rousset 1997). This technique can also be applied at the individual level to determine local deme structure (Rousset 2000) Other methods include the specific identification of immigrant individuals using assignment tests based on multilocus genotype data (Rannala & Mountain 1997), where assignments are based on the probability that an individual carries a multilocus genotype from a given population. When genetic differentiation is sufficiently high between populations and under some hypotheses, gene flow can be estimated with some accuracy and thus provide information on dispersal (e.g. Pritchard *et al.* 2000; Wilson & Rannala 2003). However, only a small number of dispersers at each generation is sufficient to blur the genetic signal (Rousset 2001) thus limiting the potential of these techniques at ecologically relevant scales. The measure of stable isotopes has also the potential to signal the origin of an animal(Gómez-Díaz & González-Solis 2007; Ramos *et al.* 2009). However, the temporal scale of the measure is strongly influenced by the ability to sample "memory tissue" that integrates a long enough period to include a dispersal event, and the technique is constrained by the availability of isotopic spatial signatures (Forero & Hobson 2003). Finally, direct measures of dispersal are becoming increasingly available via technical improvements in animal tracking methods (Cagnacci *et al.* 2010). However, the technical devices required remain expensive and there are still considerable logistical constraints that limit their use on certain organisms. For

example, the limited sample sizes involved allow biologists to make qualitative observations on dispersal patterns, but not quantitative estimations of dispersal rates. The currently available tools to estimate vertebrate dispersal rates are therefore limited by the hypotheses they rely on, the amount of field effort necessary and the availability of reliable markers appropriate to the scale considered. Identifying potential complementary sources of information on dispersal would thus be useful.

An alternative for estimating dispersal rates for a vertebrate species, is to gather data on their parasites (Boulinier et al. 2001). For example, when parasites depend directly on their host for dispersal, the genetic structure of parasite populations can provide information on host movements, especially when the genetic structure of the host population is more apparent in the parasite than in the host itself (McCoy *et al.* 2005; Criscione *et al.* 2006; Nieberding & Olivieri 2007). However, when it comes to estimating dispersal at ecological time scales, such indirect markers do not enable one to distinguish between simple movements between breeding areas (in the case of prospecting for instance) and true dispersal (McCoy et al. 2005). Much more information could be obtained if one considers multiple parasites. Indeed, the composition of parasite communities often differ among locations and thus should leave distinct signatures in the host individuals from different sites (Poulin 1998). Here, we propose that immunological evidence of former exposure to local infectious agents could be used to complement the pallet of available sources of information to estimate dispersal in vertebrate species.Indeed, when facing a challenge by an infectious agent, vertebrate species produce immune compounds (antibodies or immunoglobulins) in their serum, specifically directed against the infectious agent (Frank 2002). As circulating antibody levels are often long-lasting (Gatto *et al.* 2006; Staszewski *et al.* 2007), the antibody profile of an individual obtained from a single blood sample will keep track of past encounters with infectious agents and represents an accurate source of data on the infection history of an individual. As exposure to infectious

agents often displays spatial variability, notably in colonial or social species (Holt & Boulinier 2005), and serological data can be readily gathered, we propose that this information could be useful to infer dispersal rates. In particular, individuals living in the same colony or social group should experience closer infectious environments and thus show similar patterns of exposure to infectious agents (Delahay *et al.* 2000; Gasparini *et al.* 2001) resulting in spatial differences in the proportion of individuals displaying antibodies to a given agent (spatial variability in seroprevalence). As seroprevalence will be influenced by the dispersal of individuals that have been exposed to agents at their former breeding location and carry antibodies, this data carries information that could be used to infer dispersal rates between different populations.

Here, we first develop a modeling approach that illustrates how dispersal could be estimated using seroprevalence data, whether or not information on local rates of new infections are available. In a second part, we illustrate the potential application of such an approach using immunological assays against specific infectious agents that circulate in a spatially structured population of a long-lived colonial seabird, the black-legged kittiwake (*Rissa tridactyla*). A major advantage of the method we propose here is that it does not rely on parasite collection, which can prove difficult in wild populations, but rather on host serology, which can be obtained from a single capture and blood sample. We do not claim that the use of this neglected source of information will provide a miracle solution to the long-standing problem of estimating dispersal, but rather that it can complement the pallet of available tools, especially given the increasing interest in wildlife disease ecology and availability of antibody prevalence data.

2. Modeling approach

We here build a simple model to show that under a set assumptions, a range of plausible values for a local dispersal rate can be estimated using data on the seroprevalence of antibodies against prevalent infectious agents.

Let us consider two populations living on two patches, A and B, in which an infectious agent is circulating. We hypothesize that this agent has reached its endemic equilibrium, and thus that its local prevalence on A and B does not vary with time. Two categories of individuals can be distinguished for the purpose of our study: naïve individuals that have not been infected by the infectious agent (A^- and B^-, respectively on patch A and patch B), and seropositive individuals that have mounted an immune response and carry antibodies (A^+ and $B^{+,}$, respectively on patch A and patch B) following infection. We assume that all individuals develop an immune response upon encounter with the parasite, although we are aware that variability in immune function exists in nature. Seroprevalence is measured exclusively on reproducing adults and thus the demography in populations A and B can be described only by the natural mortality rate of adult individuals (μ), the recruitment as reproducing adults of individuals born locally (λ) and the dispersal of individuals between patches (respectively d_A and d_B on patch A and B) (figure 1). The natural mortality rate (μ) and local recruitment rate (λ) are assumed identical on both patches and constant over time. All individuals are supposed to be seronegative when recruited and eventually become infected at a local constant rate I_A (and respectively I_B) describing the incidence of the infectious agent (i.e. the rate of infection of naïve individuals per unit of time). Subsequent blood antibody levels persist during the life of the individual. Finally, dispersal (the proportion of individuals that will change breeding patches between successive breeding seasons) is thought to be the same for infected and non-infected individuals and to happen only between patches A and B. These transitions are summarized in figure 1.

Our goal is to estimate dispersal rates from data that can easily be obtained in the field from simple blood sampling surveys. Using appropriate immunological tests to quantify antibodies in the serum or plasma of sampled individuals, it is possible to measure the number of positive (A^+ and B^+) and negative (A^- and B^-) individuals for a given infectious agent and thus to estimate seroprevalence on each patch. Because we assume that the infectious agent is endemically circulating and has reached equilibrium, a single measure of seroprevalence is representative of the situation on a given patch. Although we do not tackle this issue here, it might be necessary to ensure that this is true in the wild by confirming that antibody levels are stable and seroprevalence repeatable over a relevant time scale in relation to the ecology of the host species considered. This assumption is more likely to hold for long-lived species because their population dynamics are less likely to result in variation in seroprevalence. Using the equations corresponding to the dynamics in serological status in the populations described above, and some simple algebra (see Appendix 1 for calculation details), expressions for dispersal from patch A and B can be obtained:

$$\mathrm{d_A} = \frac{(B^- + B^+)(I_B\, B^- - \lambda\, B^+)}{A^-\, B^+ - A^+\, B^-} \qquad (1)$$

$$\mathrm{d_B} = \frac{(A^- + A^+)(I_A\, A^- - \lambda\, A^+)}{A^+\, B^- - A^-\, B^+}$$

It is interesting to note that obtaining values for dispersal from a patch (i.e., emigration) mainly requires data from the other patch. This is because there is no effect of the infectious agent on dispersal. Dispersal probability is the same for seropositive and seronegative individuals, and thus has no effect on seroprevalence. Local recruitment, local infection of seronegative individuals and dispersal from the other patch are the only mechanisms affecting the dynamics of seroprevalence. This is why dispersal from B is estimated using mostly the dynamics on A and conversely.

Another important remark related to equations 1 is that dispersal can only be directly estimated when there is a minimum contrast between patches (otherwise, equations 1 are intractable). Indeed, the absence of a sufficient difference in seroprevalence can be explained by a wide range of mutual dispersal values between patches. However, even in this situation, information on the dispersal rate can be obtained by estimating the ratio between dispersal from A (d_A) and dispersal from B (d_B):

$$\frac{d_A}{d_B} = \frac{(B^- + B^+)(I_B\, B^- - \lambda\, B^+)}{(A^- + A^+)(I_B\, A^- - \lambda\, A^+)} \qquad (2)$$

This ratio corresponds to a measure of the contrast between the two dispersal rates. Whenever $\frac{d_A}{d_B}$ is over 1, it means that dispersal from patch A is more important than from patch B and the value of the ratio measures the extent of the difference.

However, obtaining an estimation of dispersal from equations 1 or 2 requires knowledge on the local incidence of the infectious agent. In this way, the accuracy of the dispersal estimate depends linearly on the accuracy of the incidence estimate, with the range of the estimated dispersal rates corresponding to the confidence interval of incidence values (see an example with a simple linear error on incidence in figure 2). It is possible to estimate incidence in the field by collecting blood samples over time and analyzing seroconversion events of marked negative individuals, in particular using CMR tools. Such data are rarely collected in field studies and, in turn, estimates of local incidence rates are usually not available and display low accuracy. However, even when incidence is unknown, it is possible to estimate a range of possible dispersal values from equation 1 given that values of I_A are constrained between 0 and 1 and provided we make an additional assumption of identical dispersal (*d)* in both directions (see details in Appendix 1):

$$d = \frac{(A^- + A^+)(I_A\, A^- - \lambda\, A^+)}{A^+\, B^- - A^-\, B^+} \qquad (3)$$

The contrast between the patches in terms of seroprevalence will influence the accuracy of the measure, with more similar patches leading to wider possible intervals of dispersal rates (figure 3).

To summarize, we have shown using this simple framework that dispersal, when considered limited at the scale of two patches, can be estimated whenever local epidemiological dynamics can be precisely described. In this case, the confidence interval of the dispersal rate would notably depend on how accurately incidence can be estimated. When local epidemiological conditions are unknown, our modeling approach show that only a range of dispersal rates can be estimated between two sub-populations from data describing the differential past exposure to a common infectious agent. Finally, we did not tackle here the issue of the estimation of the local recruitment rate λ and assumed that it could be obtained on all patches. In populations with long-term ongoing monitoring through individual marking of newborns, this information could be obtained with a sufficient accuracy. Otherwise, its effect on the estimation of dispersal would be very similar to incidence.

3. Examples of application

Following the modeling results presented above, we provide here an illustration of the approach in a highly spatially structured population of a long-lived colonial seabird, the black-legged kittiwake (*Rissa tridactyla*) and two of its circulating infectious agents (*Borrelia burgdorferi* sensu lato and Avian Influenza virus). The seroprevalence of breeding adults against each of the infectious agents are estimated from a series of sub-populations in order to discuss the potential usefulness of these measures to infer dispersal rates in the seabird host. This natural system, which globally fulfills the assumptions of the model described above, corresponds in its general structure and ecology to many systems for which it may be

interesting to estimate dispersal. To keep the example as illustrative as possible, we assume that no data is available on incidence rates and thus use a symmetric dispersal rate among patches to infer a range of plausible dispersal rates.

3.1 Study area and biological models

Sampling was conducted on Hornøya, an island located in northern Norway (70°22' N, 31°10' E) where more than 10 000 pairs of a small cliff-nesting gull, the black-legged kittiwake, breed each year. This species displays interesting features for our study. At an island-limited spatial scale, several cliffs are used by kittiwakes for breeding and individuals display strong interannual fidelity to their breeding cliff (Boulinier *et al.* 2008). Moreover, as many other seabirds, the kittiwake is long-lived, and thus potentially exposed to numerous infectious agents over its lifespan. Indeed, it has been shown that kittiwakes are exposed to ectoparasites such as the common seabird tick *Ixodes uriae* (McCoy et al. 2005), tick-borne microparasites such as Lyme disease agent *Borrelia burgdorferi sensu lato* (Gasparini *et al.* 2001; Gomez-Diaz *et al.* online early) and to viruses such as Avian Influenza Viruses (Toennessen et al. 2011).

In the Hornoeya population, between 50 and 100 new individuals are caught and individually banded each year as part of a long-term study. A blood sample has been taken from each captured individual since 2003 (Staszewski *et al.* 2007). In this study, we use serum samples obtained from 127 individuals captured in 2009. These samples can be used to estimate the seroprevalence of antibodies against different microparasites using appropriate immunological assays, such as ELISA tests (Enzyme-Linked ImmunoSorbent Assays). Below, we describe results involving two microparasites displaying different patterns of spatial variability in seroprevalence, the bacteria *Borrelia burgdorferi sensu lato* and Avian Influenza Viruses (AIV).

3.2 Borrelia: a textbook example

Circulating levels of antibodies directed against *Borrelia* were determined using a modified commercial ELISA kit (IBL international GMBH, Hamburg, Germany). As this kit has been designed for humans, we replaced the original anti-human IgG by antibodies directed against chicken IgY (A9046, Sigma-Aldrich, St. Louis, MO, USA) to assess antibody levels in kittiwakes (see Staszewski *et al.* 2007).

Among the 127 individuals sampled in eight different breeding cliffs in 2009, 26 individuals displayed detectable antibody levels for *Borrelia* (Table 1), resulting in an overall prevalence of 20%. As expected from a previous study on the local abundance of the tick vector of *Borrelia* (Gasparini et al. 2001), seroprevalence was significantly different among cliffs (χ^2 = 23.79, df = 7, p = 0.001), indicating strong spatial variability in exposure to the parasite (Table 1a). Prevalence ranged from 0% to 56% among cliffs. Although, our analysis only focused on data from 2009, it is known that individual antibody levels are highly persistent between years in this system (Staszewski *et al.* 2007). Overall, we thus have here a highly spatially structured distribution of seropositive individuals that is repeatable over time suggesting that *Borrelia* represents an interesting example of the type of infectious agent that could be used to infer dispersal using the framework described above, at least on a small spatial scale (e.g. between breeding cliffs). For instance, neglecting the occurrence of dispersal between more than two cliffs, the range of possible dispersal rates that could be estimated by our model between a kittiwake breeding cliff with a local seroprevalence of 46% (e.g. cliff 8 in table 1) and another colony in which *Borrelia* antibodies would be detected in 9 % (e.g. cliff 6 in table 1) of the breeding individuals sampled would indeed be between 0 and 0.024 each year.

3.3 A counter example: AIV in kittiwakes

Circulating levels of antibodies directed against AIV were determined using a commercial ELISA kit designed to detect antibodies directed against the A nucleoprotein of these viruses (ID Screen Influenza A Antibody Competition ELISA kit, ID Vet, Montpellier, France). Although there are a wide variety of possible viral strains for influenza viruses, the ELISA kit is directed against a highly conserved protein and the infection by any strain of influenza virus should result in the production of detectable levels of antibodies.

Among the 127 tested individuals for 2009, 77 individuals displayed detectable levels of antibodies resulting in an overall seroprevalence of 61% (Table 2), which appears in accordance with a previous report from an independent study on the same island (71.3%; Toennessen *et al.* 2011). Some of the individuals sampled in 2009 were sampled again in 2010 to assess the temporal stability of antibody levels (measured as the percentage of inhibition). We found that individual antibody levels were correlated between 2009 and 2010 both early in the reproductive season (before Julian day 140; $N = 22$, $r^2 = 0.360$, $p = 0.001$) and late in the reproductive season (after Julian day 140; $N = 19$, $r^2 = 0.592$, $p < 0.001$), indicating that overall anti-AIV antibody levels may be stable between years. The spatial variability in seroprevalence among cliffs was weak for this infectious agent, ranging from 44% to 71% among cliffs (Table 1b). Not surprisingly, the proportions of seropositive and seronegative individuals was not significantly different among cliffs ($\chi^2 = 4.71$, $df = 7$, $p = 0.70$).

These serological analyses outline that a high proportion of kittiwake individuals from different breeding cliffs of Hornøya have been exposed to AIV. However, as stressed by the modeling approach, a crucial requirement for an accurate estimation of dispersal rate is the existence of differences between the seroprevalence of the different sampled locations. Here, the absence of spatial structure in the seroprevalence limits the possibility to use this microparasite as a marker of dispersal at a small spatial scale in the considered host. As an

illustration, if seroprevalences of 44 % (e.g. cliff 6 in table 1) and 57 % (e.g. cliff 3 in table 1) are recorded for two sub-colonies, our model predicts possible values for the dispersal rate between the two colonies to range from 0 to 0.22.

4. Discussion

Here, we propose a novel use of serological data to infer dispersal rates and describe a theoretical approach that allowed us to explore the usefulness of such an approach depending on characteristics of the host-infectious agent system considered. Although the results outline that the determination of an accurate dispersal rate should only be possible when the incidence of the considered microparasite is known, we show that, even when it is not known or even not estimated at all, a range of plausible values (thus likely including the "true" dispersal rate) can be obtained when the seroprevalence of the infectious agent between patches is sufficiently dissimilar.

This model relies on important assumptions associated with both the population dynamics of the host and the epidemiology of the infectious agent. Some of these assumptions would need to be relaxed to make the model more readily useable with field data. A first important step would be to account for more breeding patches (sub-populations), and if possible to generalize the result obtained here to any number of patches. As dispersal may be driven by local factors, this extension may allow the inclusion of environmental factors that could be relevant in the field, such as habitat selection. Another step towards a more biologically realistic model would be to account directly for the lack of precision in the prevalence estimate, as this measure will obviously depend on how sampling is performed in natural populations (for instance, in the kittiwake system, we sampled only 127 individuals in 2009 from a total breeding population of more than 20 000 individuals). The same precision

problem may apply to the estimation of incidence in wild systems. In this case, considering several infectious agents with heterogeneous spatial distributions to estimate dispersal may allow a better comparison of patches. Indeed, given that the error in estimated dispersal solely depends on the confidence interval of incidence, the combination of several independent calculations (i.e., several infectious agents) may narrow down the range of possible dispersal rates. Even when incidence is not known, obtaining data for several infectious agents could provide interesting information. Some parasites may display greater contrasts in prevalence between patches (especially if more than two patches are considered) and thus contribute again to narrow down the range of potential dispersal rates. However, not all infectious agents or host populations are suitable for our purpose. In particular, infectious agents dying out in populations after massive epidemics or host populations with a very high turnover rate of individuals at the temporal scale of the study would prevent strong inferences about dispersal rates.

Despite these limits, we have shown that infectious agents relevant for use in our framework can be found in natural populations. As a general result, we show that evidence of spatial heterogeneity in the distribution of seropositive individuals (and its temporal stability) at a spatial scale relevant for dispersal is an important prerequisite for using specific serological data to infer dispersal. On a limited spatial scale, we can expect vector-borne (and especially tick-borne) parasites to be of more potential interest than directly transmitted parasites to infer dispersal. This is because the spatial structure of vectors will constrain the spatial exposure of hosts to microparasites, and thus will provide information the past history of habitat use by a host at small spatial scales. For instance, tick-borne parasites may display strong spatial structure as a result of limited movements of their hosts, in particular between breeding locations. Individuals captured on tick-free areas and found seropositive to a tick-borne agent (such as *Borrelia*) could thus be used to infer dispersal between parasitized and non-

parasitized locations. On broader spatial scales, directly transmitted parasites, like AIV for which we found no spatial structure on different cliffs of the same island, might be more interesting to consider at the strain level and may reflect broader epidemiological patterns.

Some other ecological characteristics of the species considered might have consequences for estimating dispersal from seroprevalence data. In particular, long-lived species should be more suitable for the use of serological data as they allow a longer historical measure of past exposure to infectious agents. Assuming that antibody levels are long lasting (for Borrelia: Staszewski *et al.* 2007; for AIV: this study, but see Hoye *et al.* 2011), this source of information can provide data at an ecological timescale and has the potential to integrate dispersal events that took place over a series of years. In short-lived species, the data available will, of course, encompass a shorter time period and revealing dispersal behaviors may prove more difficult as it will require the capture of individuals over a shorter time window. Coloniality and sociality should also influence our ability to estimate dispersal. This is because in colonial and social systems, parasite transmission rates are generally increased when the local density of individuals available to a given infectious agent is higher (Loehle 1995). This implies that the similarity in terms of exposure to infectious agent should be higher for individuals inside a given social group or a given colony than between social groups or colonies. This would especially be the case for endemic infectious agents. In the case of social groups, one could even expect some groups to be completely infected (i.e. 100% prevalence) and some others completely naïve. Slight differences in prevalence could in those cases be attributed to dispersal events and antibody prevalence analysis could be a very powerful method.

Dispersal is not the only kind of movement that might have epidemiological implications in animal studies. If the role of migration as a critical period for the spread of parasites is increasingly recognized (Altizer et al. 2011), the importance of seasonal temporary

movements is less well studied. In particular, in colonial species, some individuals are known to visit different colonies during the breeding season to prospect for potential breeding sites (Boulinier *et al.* 1996; Reed *et al.* 1999). As outlined before, when this visit is not followed by a breeding attempt, this does not fall in the definition of dispersal. However, a simple visit of an infected individual might be sufficient to spread a parasite into a population (McCoy et al. 2005). This is why it is important to focus on endemically circulating infectious agents to estimate prevalence because they will have been circulating in the different populations for enough time to prevent their epidemiological dynamics from being strongly affected by such effects.

Obtaining seroprevalence data from the field requires the realization of blood sampling of adults which is not easy for numerous species and in particular in adults. A possible alternative to adult blood sampling may exist to estimate the levels of antibodies in a population by taking advantage of the transfer of maternal antibodies, a common feature of all vertebrate species (Brambell 1970). In vertebrate species, mothers transmit antibodies to their newborns so as to improve their survival during the first days/weeks of life (Grindstaff *et al.* 2003; Boulinier & Staszewski 2008). This mechanism could be diverted to be used for our purpose. Indeed, in birds (and other egg-laying vertebrates), an alternative to adult blood sampling could be to gather eggs or blood samples from young chicks and measure the levels antibodies transmitted by the mother through the egg yolk (Kuiken *et al.* 1998; Pearce-Duvet *et al.* 2009). As levels are highly correlated within clutches, the partial sampling of clutches is sufficient (Hammouda *et al.* in press). Such a sampling approach could notably be conducted on abundant species that are subject to population control measures, such as some large gulls (Pearce-Duvet *et al.* 2009). In mammals, the capture of newborns might be easier than the capture of adults (especially from a handling point of view) and a blood sample of the newborn taken at a carefully chosen moment after birth would also inform on the circulating

levels of antibodies in the mother. Although this way of sampling would obviously induce a sex bias in the estimate of prevalence (only the immunological profile of breeding female can be assessed), the improvement in terms of fieldwork requirements and of number of available samples might still be interesting.

In conclusion, several methods are available to infer dispersal rates in wild animal populations but all have their own drawbacks in particular when it comes to the time or spatial scale at which they reach their best accuracy. Indeed, it is still very complicated to obtain measures of dispersal rates in animals. Here, we have described a new approach that can at least provide estimates of ranges of possible dispersal rates and has a potential high sensitivity over ecological time scales as it relies on data that accurately retrace the histories of living individuals. This approach might also be able to cover different spatial scales by choosing parasites displaying contrasted patterns at the adequate scales. Moreover, even if serological data only provide a range of possible dispersal rates, it might still represent a highly valuable piece of information that could be combined with the results of other sources of information, in particular in a common procedure of inference. In particular, we believe that this data could be part of incremental Bayesian approaches, where information provided by some type of data, along with its associated incertitude, is explicitly used as a prior in the analyses of other types of data. Such a framework could allow more efficient, and certainly more objective, measure of dispersal in natural conditions and help deciphering dispersal mechanisms in wild animal populations.

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Table legends

Table 1: Number (and proportions) of seropositive and seronegative kittiwake individuals for antibodies against *Borrelia burgdorferi sensu lato* (Bbsl) and Avian Influenza Viruses (Influenza) in 2009 in a series of 8 breeding cliffs.

Table 1

a.

Bbsl	Cliff 1	Cliff 2	Cliff 3	Cliff 4	Cliff 5	Cliff 6	Cliff 7	Cliff 8	**Total**
Positive	0 (0%)	1 (17%)	4 (17%)	1 (14%)	0 (0%)	4 (9%)	5 (56%)	11 (46%)	**26 (20%)**
Negative	9 (100%)	5 (83%)	19 (83%)	6 (86%)	6 (100%)	39 (91%)	4 (44%)	13 (54%)	**101 (80%)**

b.

Influenza	Cliff 1	Cliff 2	Cliff 3	Cliff 4	Cliff 5	Cliff 6	Cliff 7	Cliff 8	**Total**
Positive	5 (56%)	3 (50%)	13 (57%)	6 (86%)	26 (60%)	4 (44%)	3 (50%)	17 (71%)	**77 (61%)**
Negative	4 (44%)	3 (50%)	10 (43%)	1 (14%)	17 (40%)	5 (56%)	3 (50%)	7 (29%)	**50 (39%)**

Figure legends

Figure 1: Flux diagram of the transitions within and between patches A and B. Individuals are born at a rate λ and naturally die at a rate μ. They become infected at an incidence rate I_A on patch A and I_B on patch B. Individuals can disperse from patch A to patch B at a dispersal rate d_A and conversely from patch B to patch A at a dispersal rate d_B.

Figure 2: Estimated dispersal rate from patch A to patch B (d_A) corresponding to various levels of error (ε) on the incidence values on patch B (I_B) so that $I_B = I_B \pm \varepsilon I_B$. The solid line represents the estimated value of d_A while the dotted lines represent the corresponding precision of the measure due to errors on the estimation of incidence. Parameter values: $A^+ = 60$; $A^- = 40$; $B^+ = 40$; $B^- = 60$; recruitment rate: $\lambda = 0.1$; $I_B = 0.02$.

Figure 3:.Estimated dispersal rate (d) between patches A and B as a function of the incidence on patch A (I_A) and of the contrast of seroprevalences ($P_A - P_B$) between patches. When prevalences are highly contrasted the restricted set of plausible incidence values leads to a small range of realistic dispersal rates. When the contrast between patches is reduced, a larger set of plausible incidence values is obtained and in turn the accuracy of the estimation of dispersal is weaker. Ultimately, when there is no contrast, incidence cannot be inferred from exposure data and dispersal rate is not estimated. A similar figure could be obtained with regard to the incidence on patch B (I_B). Parameters: $\lambda = 0.1$.

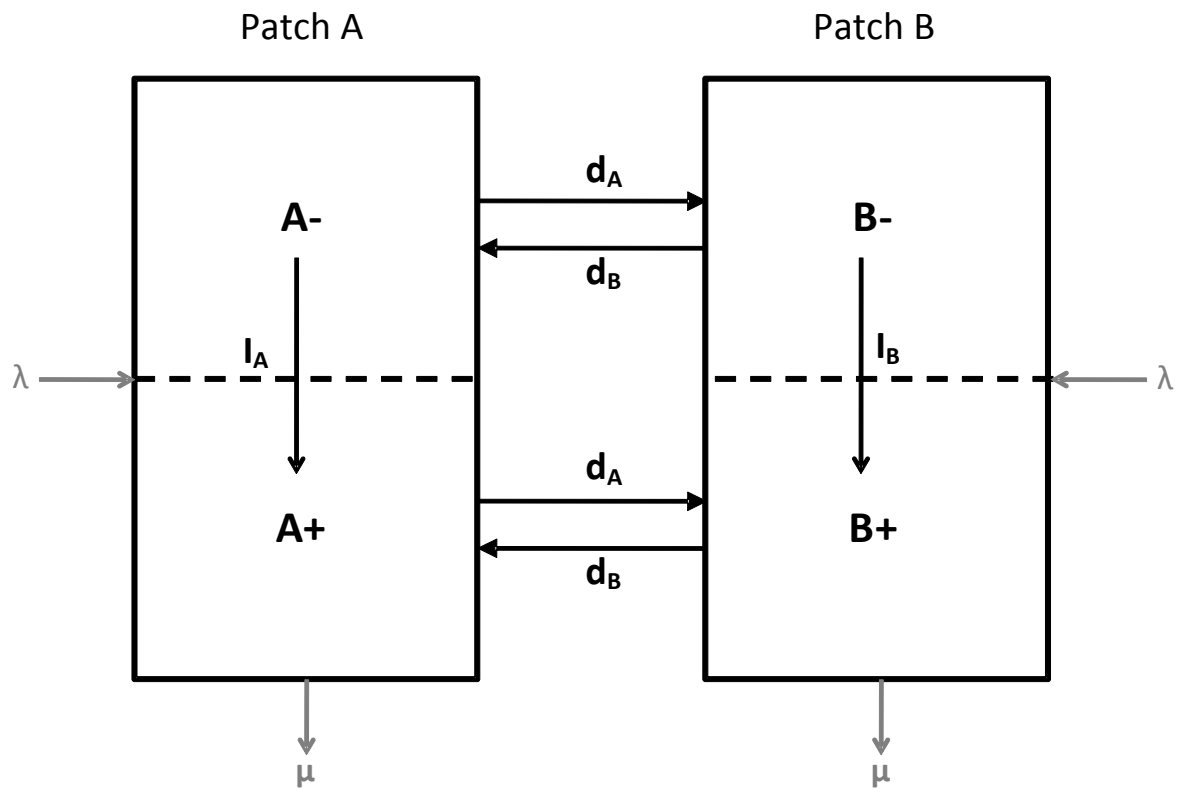

Figure 1


0.25
0.20
0.15
0.10
0.05
0.00
Dispersal from patch A (d_A)
0.0
0.2
0.4
0.6
0.8
1.0
Error (ϵ) associated with the incidence on patch B (I_B)

Figure 2

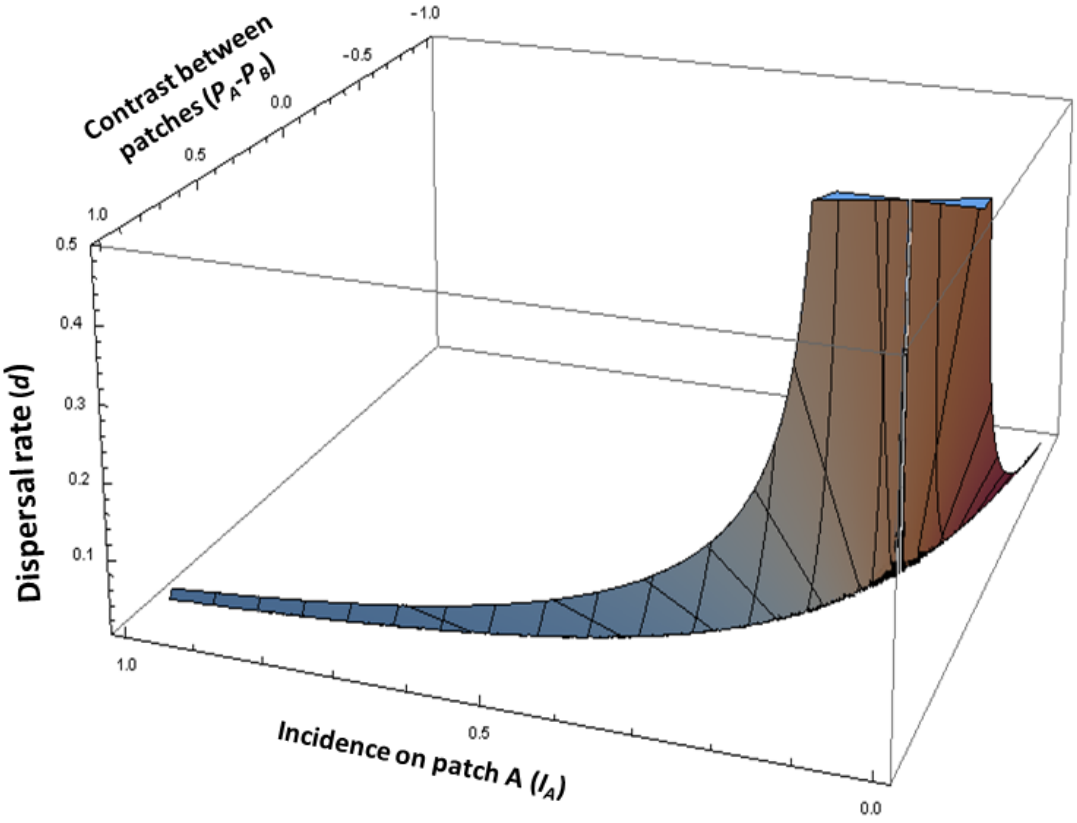
Contrast between patches (P_A-P_B)
-1.0
-0.5
0.0
0.5
1.0
Dispersal rate (d)
0.5
0.4
0.3
0.2
0.1
Incidence on patch A (I_A)
1.0
0.5
0.0

Figure 3

Appendix 1

Using appropriate immunological tests to quantify antibodies in the serum or plasma of sampled individuals, it is possible to obtain the number of positive (A^+ and B^+) and negative (A^- and B^-) individuals for a given infectious agent and thus to estimate the local seroprevalences for patches A and B as:

$$P_A = \frac{A^+}{A^+ + A^-} \quad \text{(A1)}$$

$$P_B = \frac{B^+}{B^+ + B^-} \quad \text{(A2)}$$

Given that we assume that the parasite has reached an endemic equilibrium and that the host population has reached a demographic equilibrium, the local prevalence calculated from a single sampling campaign will be assumed not to change through time. From a mathematical point of view, this can be written (with δ referring to derivatives):

$$\frac{\delta P_A}{\delta t} = \frac{\delta P_B}{\delta t} = 0 \qquad \text{(A3)}$$

Or using simple derivative operations on equations A1 and A2 (primes denote derivatives):

$$\frac{\delta P_A}{\delta t} = \frac{A^{+'}(A^+ + A^-) - A^+(A^{+'} + A^{-'})}{(A^+ + A^-)^2} = 0 \quad \text{(A4)}$$

and

$$\frac{\delta P_B}{\delta t} = \frac{B^{+'}(B^+ + B^-) - B^+(B^{+'} + B^{-'})}{(B^+ + B^-)^2} = 0 \quad \text{(A5)}$$

Moreover, these quantities can be expressed as a system of differential equations describing the transitions summarized in figure 1 (main text), which summarizes the processes affecting the temporal changes in the different types of individuals:

$$A^{-'} = \frac{\delta A^-}{\delta t} = \lambda(A^+ + A^-) - (\mu + I_A)A^- + d_B B^- - d_A A^-$$

$$A^{+'} = \frac{\delta A^{+'}}{\delta t} = I_A\, A^- - \mu\, A^+ + d_B B^+ - d_A A^+$$

$$B^{-'} = \frac{\delta B^-}{\delta t} = \lambda(B^+ + B^-) - (\mu + I_B)B^- + d_A A^- - d_B B^-$$

$$B^{+'} = \frac{\delta B^{+'}}{\delta t} = I_B\, B^- - \mu\, B^+ + d_A A^+ - d_B B^+$$

(A6)

Equations A4 and A5 can thus be solved independently, giving rise to two equations for the dispersal rate from patch A to patch B (d_A) and from patch B to patch A (d_B), respectively depending on the incidence on patch B (I_B) and on the incidence on patch A (I_A):

$$d_A = \frac{(B^- + B^+)(I_B\, B^- - \lambda\, B^+)}{A^-\, B^+ - A^+\, B^-} \quad \text{(A7)}$$

$$d_B = \frac{(A^- + A^+)(I_A\, A^- - \lambda\, A^+)}{A^+\, B^- - A^-\, B^+} \quad \text{(A8)}$$

If the incidence could be estimated on patch A or B, the value of the dispersal rate could thus be calculated from equations A7 and A8. Unfortunately, this measure is almost never available in natural populations (as it would require estimating the proportion of naïve individuals becoming infected per unit of time). Making the further assumption of an identical dispersal rate in both directions ($d_A = d_B = d$), it is possible to rewrite the equations A7 and A8 in order to obtain two equivalent expressions for the dispersal rate (d):

$$d = \frac{(A^- + A^+)(I_A\, A^- - \lambda\, A^+)}{A^+\, B^- - A^-\, B^+}$$
$$d = \frac{(B^- + B^+)(I_B\, B^- - \lambda\, B^+)}{A^-\, B^+ - A^+\, B^-} \quad \text{(A9)}$$

A corollary to the hypothesis of a uniform dispersal is that there should exist a relationship between the two patches in terms of local incidence, as the local infection of naïve hosts is the

only remaining mechanism explaining possible differences in prevalence. From the equations A9, a relationship indeed arises between the incidence on patch A (I_A) and on patch B (I_B):

$$I_B = \frac{-(A^- + A^+)(I_A A^- - \lambda A^+) + \lambda B^- B^+ + \lambda B^{+2}}{B^-(B^- + B^+)} \qquad \text{(A10)}$$

The values of I_B, I_A and d are thus constrained by equations A9 and A10, which enables us to infer ranges of plausible values for those three variables given the values of A^+, A^-, B^+, B^- and the assumptions made. Whenever the two conditions given below are met, it is thus possible to obtain an interval of plausible values for the dispersal rate (see figure 3 in the main text) corresponding to the interval of plausible incidence values arising from equation A10 (figure S1):

- Both expressions of the dispersal rate, d (equations A9), have to give the same result and this result has to be a positive value.
- The value of incidence on patch B calculated using equation A10 has to be a positive value.

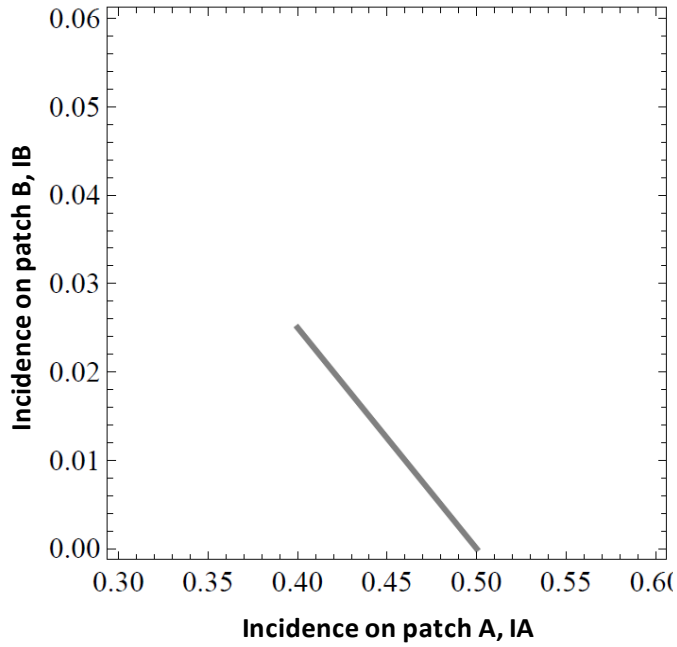

Figure S1: Restricted set of incidence values I_A and I_B, respectively on patch A and B, that can explain the observed patterns of seroprevalence, and that fulfill the assumptions described. Parameter values used in the example are: $A^+ = 80$; $A^- = 20$; $B^+ = 20$; $B^- = 80$; birth rate: $\lambda = 0.1$. In this example, the high seroprevalence of positive individuals on patch A (80 %) and low prevalence on patch B (20 %) implies that plausible values for the dispersal rate vary from 0 to 0.03, while plausible values of the incidence vary between 0.4 and 0.5 on patch A and between 0 and 0.025 on patch B.