Table des matières / Contents

SYNTHÈSE

(Version en français)

Introduction

A. Introduction générale

Malgré l'ouvrage fondamental de Darwin (1871) et la description du "**processus d'emballement**" par Fisher (Fisher 1915, 1930), la **sélection sexuelle** n'a réellement fait l'objet d'études comportementales qu'à partir des années 1960. Elles se sont généralisées après l'article de Trivers (1972) et la démonstration de la réalité du processus fishérien par Lande (1981). A cause de l'**anisogamie** (i.e. la fertilisation d'un gamète femelle de grande taille par un gamète mâle de taille plus réduite), les femelles de la majorité des espèces investissent davantage de ressources dans leur descendance que les mâles. Cette différence d'allocation d'énergie entre les sexes est souvent aggravée par les différences de **soins parentaux** (Trivers 1972). Dans la plupart des espèces, comme par exemple la majeure partie des Mammifères, les mères donnent l'essentiel des soins aux jeunes, le seul investissement énergétique des mâles résidant dans la production des spermatozoïdes lors de la fertilisation. Dans de telles circonstances, le succès de reproduction des mâles est limité par le nombre de partenaires de reproduction, tandis que celui des femelles l'est plutôt par le nombre d'œufs qu'elle peut produire. Il y a ainsi toujours plus de spermatozoïdes que d'ovules à fertiliser. De ce fait, les femelles représentent une ressource limitée pour les mâles, qui vont devoir entrer en compétition pour y accéder. Les mâles vont donc investir leur énergie dans des compétitions entre mâles et dans la production de **caractères sexuels secondaires coûteux,** destinés à "séduire" les femelles, lesquelles vont utiliser ces caractères pour **choisir leur partenaire.** Selon l'**hypothèse du "handicap"**, seuls les caractères particulièrement coûteux peuvent être considérés comme des indicateurs fiables de la qualité du mâle, car seuls les mâles de très haute qualité pourront se permettre de les produire. L'association entre la préférence des femelles pour de tels caractères exagérés et la production de ces caractères coûteux par les mâles de haute qualité serait à l'origine du **dimorphisme sexuel** et du processus d'emballement fishérien : si les femelles préfèrent les mâles aux caractères les plus exagérés, alors les mâles capables de produire ces traits seront favorisés même si leur survie en est diminuée. Dans les cas les plus extrêmes, une sélection sexuelle trop intense pourrait même entraîner l'**extinction** de certaines populations (Doherty, et al. 2003).

Comme la valeur sélective d'un mâle n'est limitée que par le nombre de femelles qu'il peut conquérir, la **polygynie** semble – du moins pour les mâles – le système d'appariement optimal. Par ailleurs, du point de vue des femelles, divers types de bénéfices ont également pu

Encart 1 : Causes possibles de la monogamie sociale. La monogamie est étudiée depuis longtemps, sans doute car elle est souvent considérée comme un système d'appariement « normal » chez les être humains. Diverses hypothèses ont été proposées afin d'expliquer l'apparition et l'évolution d'un tel système (adaptées ici d'après Danchin, et al. 2005):

1) Contraintes écologiques sur les mâles. Si le nombre de partenaires qu'un mâle peut obtenir dépend de la quantité de ressources qu'il contrôle, alors il ne pourra avoir plus d'un partenaire de reproduction tant qu'il ne contrôle pas suffisamment de ressources. Les preuves pour valider un tel scénario chez les oiseaux manquent: ainsi Veiga (1992) n'a trouvé aucune corrélation entre le succès de reproduction des moineaux domestiques mâles et le nombre de nids qu'ils contrôlent.
2) Importance des soins parentaux. Si le succès de reproduction nécessite une collaboration étroite entre les deux parents, alors les mâles ne devraient pas s'engager dans des accouplements hors-couples, car ils ne pourront pas s'impliquer suffisamment dans l'élevage des jeunes pour garantir leur survie. Si l'importance des soins parentaux est assez évidente pour de nombreuses espèces (et notamment la plupart des oiseaux), elle ne peut expliquer tous les cas de monogamie : par exemple, l'antilope dik-dik (*Madoqua kirki*, Komers 1996) est monogame malgré l'inexistence de soins paternels.
3) Territorialité. Pour les espèces territoriales, la monogamie pourrait être une stratégie de coopération afin de défendre un territoire commun (Mattews 2002).
4) Synchronisation de la reproduction. Chez certaines espèces (e.g. les espèces opportunistes), les femelles sont toutes fertiles en même temps, ce qui limite de fait le nombre de femelles qu'un mâle peut fertiliser.
5) Vulnérabilité des femelles à la prédation et aux infanticides. Chez certaines espèces monogames, les mâles ne donnent aucun soin parental, mais sont plus vigilants et défendent les femelles contre les prédateurs et le harcèlement d'autres mâles à la recherche d'un partenaire (Clutton-Brock 1989).
6) Contrôle des femelles. Chez certaines espèces, les femelles appariées sont plus agressives envers les autres femelles qui tentent de pénétrer sur le territoire du couple (Cézilly, et al. 2000b), ce qui pourrait empêcher les mâles d'accéder à d'éventuels partenaires hors couple.

être associés à la **polyandrie**. Ainsi les femelles peuvent acquérir des **bénéfices directs** de leurs partenaires hors couple (e.g. une meilleure fertilité, Baker, et al. 2001), ou des **bénéfices indirects**, sous la forme d'une meilleure valeur sélective de leurs descendants hors couple. Des mâles plus éloignés d'elles génétiquement que leur(s) partenaire(s) habituel(s) pourraient par exemple permettre aux femelles d'augmenter l'hétérozygotie et la valeur sélective de leur progéniture (Foerster, et al. 2003, Freeman-Gallant, et al. 2003). Ainsi, dans une même nichée, les poussins illégitimes des gorges-bleues à miroir (*Luscinia svecica*) ont une meilleure réponse immunitaire que leurs demi-frères (Johnsen, et al. 2000). Cependant, chez de nombreuses espèces, de tels bénéfices indirects de la paternité hors couple n'ont pu encore être identifiés (e.g. le bruant des roseaux, Kleven & Lifjeld 2004).

Jusqu'au début des années 1980, on a cru que la monogamie était la règle chez les oiseaux. En effet, chez la plupart des espèces aviaires, les individus restent en couple jusqu'à la fin de la période de reproduction, et les soins parentaux sont partagés plus ou moins équitablement entre les deux parents. Étant donnés les bénéfices potentiels de la **promiscuité**, diverses hypothèses avaient été proposées pour expliquer cet état de fait plutôt inattendu (cf. Encart 1). Le développement de la génétique en écologie comportementale dans les années 1980 a cependant largement modifié ce point de vue. En effet, les oiseaux **génétiquement monogames** (i.e. les espèces ne présentant pas de poussins illégitimes) sont rares (Griffith, et al. 2002), et environ 90% des espèces socialement monogames présentent des taux non nuls de paternité hors couple ou de parasitisme de nids (la femelle déposant ses œufs dans le nid de son partenaire hors couple, ce qui pourrait être vu comme une forme de maternité hors couple). En fait, le **système d'appariement** génétique de nombreuses espèces socialement monogames est plus proche de la promiscuité que de la monogamie. Le petit pingouin (*Alca torda*) fournit l'un des exemples les plus emblématiques. Bien que cette espèce soit décrite comme typiquement monogame socialement et présentant un haut degré de fidélité au partenaire d'une année sur l'autre, Wagner (1997) y a décrit un système d'appariement génétique proche du lek, femelles et mâles recherchant activement des partenaires hors couple (Richard H. Wagner a ainsi proposé l'hypothèse du « **lek caché** », la promiscuité génétique semblant être dissimulée par la monogamie sociale, Wagner 1997).

Certaines espèces sont pourtant monogames à la fois socialement et génétiquement. C'est le cas de l'espèce étudiée dans cette thèse : la **Mouette tridactyle** (*Rissa tridactyla*). Malgré de nombreuses possibilités de copulation hors couple – les mouettes se reproduisent au sein de colonies à haute densité, ce qui pourrait favoriser les fertilisations hors couple (Morton, et al. 1990) – aucun indice de comportement ou de fertilisation hors couple n'a pu être mis en évidence (Helfenstein, et al. 2004c). Une telle monogamie stricte pourrait avoir de profondes implications sur certains comportements, ce que je vais étudier ici (cf. Figure 0-1).

Tout d'abord, la monogamie sociale et le taux important de fidélité inter-annuelle (Coulson 1966, Coulson & Thomas 1980, Fairweather & Coulson 1995) posent la question de la **reconnaissance individuelle**. Les individus doivent être capables de reconnaître leur partenaire durant plusieurs saisons de reproduction et, lorsqu'ils divorcent, doivent également être capables de reconnaître les autres individus afin de sélectionner le plus rapidement possible un nouveau partenaire en début de saison. Je vais donc dans une première partie étudier la reconnaissance individuelle chez les mouettes, tant entre partenaires, et entre voisins, qu'entre parents et jeunes.

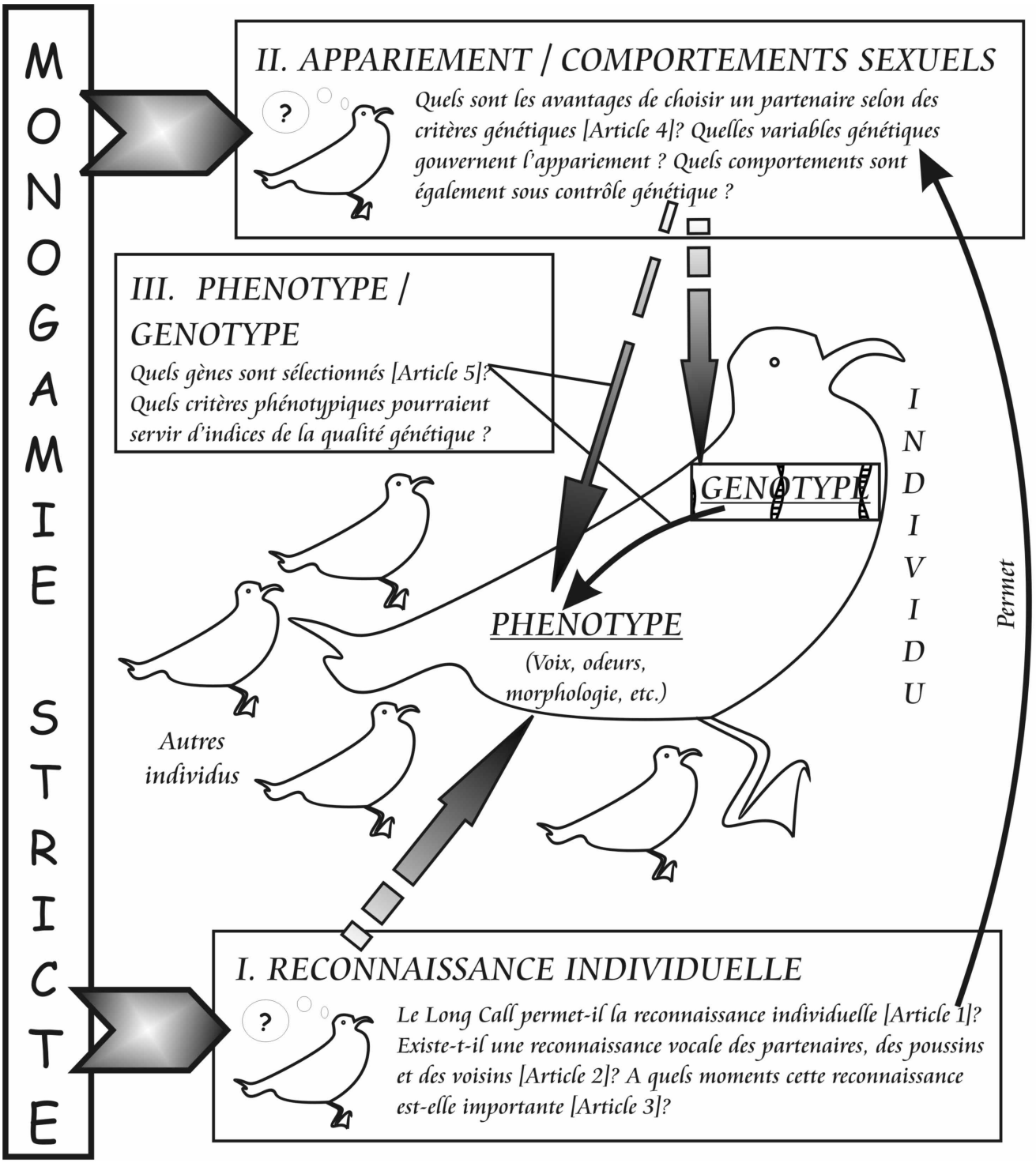


Figure 0-1: Organisation de la thèse.
Le choix du partenaire de reproduction et la fidélité inter-annuelle nécessitent une reconnaissance individuelle. Aussi, chez les espèces strictement monogames, la reconnaissance individuelle (première partie) et le choix du partenaire (deuxième partie) devraient être soumis à une forte sélection. Je me concentre dans cette thèse sur le choix du partenaire selon des critères génétiques et, dans la troisième partie, j'essaie de déterminer quels indices phénotypiques pourraient permettre aux individus d'estimer le génotype de leurs congénères.

L'absence de fertilisation hors couple met en exergue l'importance du choix du partenaire, car ce choix va affecter le succès de reproduction, non seulement de l'année en cours, mais également celle des années ultérieures (du fait de la fidélité inter-annuelle). Cependant, l'absence d'important dimorphisme sexuel (qui rend par ailleurs l'identification des sexes parfois difficile pour les observateurs, Jodice, et al. 2000) semble indiquer chez cette espèce l'absence de caractères sexuels secondaires exagérés, qui auraient pu entrer en jeu lors du choix du partenaire. Par ailleurs, les femelles ne semblent pas copuler en échange de nourriture (Kempenaers, et al. 2006), ce qui pourrait indiquer que le choix du partenaire est lié à d'autres bénéfices que des avantages matériels aussi directs que la nourriture de cour. Les **bénéfices indirects génétiques** (i.e. les individus s'apparieraient afin d'augmenter la qualité génétique de leur progéniture) pourraient donc expliquer le **système d'appariement** monogame strict chez la mouette. Afin de vérifier ou d'invalider cette hypothèse, j'étudie dans la seconde partie de ce manuscrit les éventuels liens entre le système d'appariement et les caractéristiques génétiques des individus. Je recherche également les corrélations possibles entre l'intensité de certains comportements sexuels (notamment les copulations) et les caractéristiques génétiques des couples. Enfin, j'estime l'influence de l'homozygotie des poussins sur leur valeur sélective.

L'identification d'un système d'appariement selon des critères génétiques n'est toutefois pas suffisante pour montrer l'existence d'un réel choix du partenaire sur de tels critères. En effet, je montre dans la deuxième partie que les couples tentent d'augmenter leur distance génétique, ce qui semble indiquer que les individus exercent un choix actif sur ce paramètre. Il est cependant encore difficile de comprendre comment les individus peuvent **estimer le génotype** de leurs partenaires. Dans la troisième et dernière partie, je tente d'identifier différents caractères phénotypiques qui pourraient être utilisés par les mouettes lors de l'estimation du génotype et du choix de leur partenaire.

B. Espèce et population d'étude

La mouette tridactye est l'un des oiseaux de mer les plus communs de l'océan Arctique et du nord des océans Atlantique et Pacifique. Elle passe l'hiver en mer et se reproduit sur des falaises verticales, formant des **colonies à haute densité** et déposant ses œufs dans des nids construits sur d'étroites aspérités de la roche (Baird 1994). Longévive (Cam & Monnat 2000b, Cam, et al. 2003, Coulson 1966, Coulson 1983, Hatch, et al. 1993), elle forme des couples pouvant durer de nombreuses années (Coulson 1966, Coulson & Thomas 1980). Dans sa

thèse, Fabrice Helfenstein (Helfenstein 2002) a montré que cette espèce était génétiquement monogame (Helfenstein, et al. 2004c) et que la durée d'absence des mâles au nid devenait imprévisible à l'approche de la ponte (Helfenstein, et al. 2004b). Ce comportement pourrait être une stratégie de contrôle des femelles ; la durée d'absence des mâles avant la ponte est en effet souvent plus courte que la durée nécessaire à une copulation, et si la femelle était découverte par son partenaire, les coûts engendrés par une copulation hors couple pourraient expliquer l'absence de telles copulations.

Helfenstein, et al. (2004a) ont montré un appariement partiellement basé sur la longueur du tarse dans la population du Cap Sizun (les femelles à long tarse étant appariées préférentiellement avec des mâles à long tarse), mais une telle corrélation n'a pas été retrouvée dans la population de l'île de Middleton (données personnelles). Par ailleurs, les bases génétiques du choix du partenaire n'ont jamais été étudiées chez les Laridés. Afin d'étudier la reconnaissance et l'appariement, nous avons étudié deux populations :

1. La population du Cap Sizun

La première population est située au Cap Sizun (Bretagne, France) et cette étude fait partie d'un projet de suivi à long terme débuté en 1979 (Cadiou, et al. 1994, Cam & Monnat 2000a, Cam, et al. 2003, Danchin 1987, 1988a and b, Danchin, et al. 1998, Monnat, et al. 1990). De 1979 à 2002, 949 adultes et 12246 poussins ont été marqués individuellement par un code unique à 4 ou 5 bagues colorées. Chaque année, les colonies ont été visitées au moins une fois par semaine de février à mi juin, puis tous les jours jusqu'à fin août, ceci afin de déterminer les dates de ponte, d'éclosion, de premier envol et de fin de la période d'élevage pour la plupart des poussins. De 1983 à 1985, les poussins ont été étroitement suivis au moment de l'envol (par Étienne Danchin) ; leur comportement, ainsi que celui de leurs parents et des autres adultes présents dans la colonie, a été relevé [Article 3]. De 1995 à 2002, les adultes reproducteurs et les poussins d'une falaise particulière (nommée 5Z) ont subi un prélèvement de sang. Ces échantillons ont été conservés dans du Tris-EDTA pour les analyses génétiques ultérieures. De 1999 à 2001, les comportements sexuels (montes, copulations, quémandes et nourrissages de cour, etc.) ont également été enregistrés par Fabrice Helfenstein dans le cadre de sa thèse.

2. La population de l'île de Middleton

La seconde population est située sur l'île de Middleton (dans la partie nord du golfe d'Alaska, 58°25' N, 146°19' W). Cette île possède une importante population de mouettes, en

fort déclin depuis plusieurs décennies (166 000 oiseaux en 1981, environ 39000 couples en 1991, moins de 25 000 couples en 1999, Gill & Hatch 2002, Hatch, et al. 1993). Les mouettes étudiées nichent sur des nids artificiels installés le long des murs d'une tour radar de l'U.S. Air Force abandonnée. Afin d'identifier les individus présents sur les nids et le succès de reproduction, chaque nid était observé deux fois par jours. Tous les poussins ont été marqués à la naissance et bagués individuellement à l'âge de 25 jours. Les adultes ont été bagués avec le code métallique américain et 4 bagues colorées. Les dates de ponte et d'éclosion ont été relevées précisément pour tous les couples, mais les observations annuelles cessaient trop tôt pour estimer la date d'envol. Des échantillons de sang ont été prélevés sur un maximum d'adultes (en se concentrant sur les reproducteurs) et conservés dans du tampon Longmire (Longmire, et al. 1988).

Entre 2003 et 2006, une expérience parallèle à celles que j'ai menées a causé d'importantes perturbations sur la colonie, et semble également être à l'origine de nombreux divorces et ré-appariements. De ce fait, je n'ai étudié le choix du partenaire qu'en 2003 et 2004, puisque les données de choix de partenaire pour les années suivantes étaient biaisées. En 2005, des prélèvements de sang ont également été effectués sur les poussins des couples non manipulés à la naissance, et leur croissance (poids, longueur du tarse et de l'aile) jusqu'à 25 jours a été mesurée, ceci afin d'identifier les coûts éventuels de l'homozygotie pour les poussins.

C. Expériences de son

Les « long calls » (Danchin 1987, Tinbergen 1959) des adultes ont été enregistrés, soit lors de l'atterrissage, soit durant le repos sur le nid. J'ai également enregistré les cris émis par les poussins, après 25 jours, lorsqu'ils agitent leurs ailes et émettent un cri assez comparable au long call des adultes. Un microphone AKG D770 était placé directement sur le nid, et relié à un enregistreur Marantz PMD670. Grâce à la configuration particulière de la tour de Middleton, les cris ont été enregistrés en moyenne à moins de 30 cm des individus émetteurs. Les cris ont par la suite été rediffusés grâce à un amplificateur Marantz MA6100 et des haut-parleurs Audax AP080M4. Les sons ont été modifiés grâce au logiciel CTWave32 (Creative Technology Ltd) et analysés avec SAS Lab Pro (Avisoft).

D. Analyses génétiques

Les méthodes d'analyse génétiques sont décrites de façon exhaustive dans l'article 4. En résumé, j'ai utilisé 10 loci microsatellites (K6, K16, K31, K32, K67, K71 Tirard, et al. 2002; et RBG20, RBG27, RBG29 and RBG39, Given, et al. 2002) afin d'estimer à la fois l'hétérozygotie individuelle et la similarité génétique entre les individus du couple. J'ai utilisé 3 indices différents d'hétérozygotie, l'hétérozygotie directe *H*, l'hétérozygotie standardisée *SH* et l'apparentement interne *IR* (proposé par Amos, et al. 2001). Les indices de similarité génétique employés sont l'indice *r* de Queller et Goodnight (1989), et l'indice *Phm* (pour "probabilité de produire des individus homozygotes", un indice que je propose ici pour la première fois et qui pourrait avoir des propriétés intéressantes pour les études ultérieures, cf. [Article 4]). Pour analyser les systèmes d'appariement, j'ai simulé des ré-appariements des individus observés, afin d'estimer la similarité génétique moyenne attendue sous l'hypothèse d'un appariement au hasard. Cette estimation a ensuite été comparée à la similarité génétique des couples observés.

Les allèles de classe II du Complexe Majeur d'Histocompatibilité (CMH) ont été amplifiés grâce aux amorces Pen1/Pen4 (Tsuda, et al. 2001) et LP1/LP2 (Kikkawa, et al. 2005). Ces amorces m'ont permis d'amplifier la section comprenant l'exon 2, l'intron2 et l'exon 3 du locus homologue de DRB1. J'ai utilisé le logiciel MEGA version 4 (Tamura, et al. 2007) pour construire les arbres phylogénétiques et analyser le polymorphisme génétique des séquences observées.

E. Statistiques

Les analyses statistiques ont été effectuées grâce au logiciel SAS® (©SAS Institute Inc.1999, Cary, NC, USA). Sauf indication contraire, tous les tests effectués étaient bilatéraux. Les détails des tests et des procédures utilisés sont explicités dans les articles.

I. Reconnaissance vocale chez les mouettes [Articles 1,2,3]

Les mouettes tridactyles sont strictement monogames (Helfenstein, et al. 2004c) et se reproduisent généralement avec le même individu durant plusieurs années consécutives (Coulson 1966). Une telle fidélité nécessite une reconnaissance individuelle, non seulement au début mais également tout au long de chaque saison de reproduction. Cependant, comme les couples se reproduisent généralement sur le même nid tous les ans, la reconnaissance individuelle pourrait ne pas être indispensable si les individus reconnaissent leur nid. La fidélité au nid et la fidélité au partenaire peuvent cependant évoluer indépendamment l'une de l'autre (Cézilly, et al. 2000). Fairweather & Coulson (1995) ont montré que les mouettes tridactyles étaient généralement plus fidèles à leur partenaire qu'à leur nid (les membres du couple restent appariés même si leur nid est détruit, et vont se reproduire ensemble sur un nouveau site). Il existerait donc un système de reconnaissance individuelle. Nos observations renforcent cette hypothèse : les prospecteurs (ou "squatteurs" Danchin, et al. 1991, Monnat, et al. 1990), i.e. les individus qui n'ont pas encore un site de reproduction définitif (Cadiou, et al. 1994), se déplacent également en couple d'un site à un autre.

En théorie, les individus peuvent utiliser une large panoplie de signaux pour reconnaître l'identité de leurs congénères, tels que la voix, les odeurs, les comportements (par exemple, les squatteurs ont tendance à atterrir silencieusement sur les nids alors que les résidents crient à l'atterrissage, Danchin 1983, 1987), ou encore les indices visuels (taches noires à l'extrémité des ailes, taille et morphologie, couleurs du bec et des commissures, etc.). Dans ce chapitre, je vais essentiellement m'attacher aux signaux vocaux, car ils représentent sans doute l'un des systèmes de reconnaissance les plus utilisés et les plus connus chez les oiseaux. En ce qui concerne les seuls Laridés, il a été démontré que les poussins des mouettes atricilles (*Larus atricilla*, Beer 1969), des mouettes rieuses (*L. ridibundus*, Charrier, et al. 2001) et des mouettes de Buller (*L. bulleri*, Evans 1970) reconnaissent la voix de leurs parents. Les mouettes rieuses et les goélands railleurs (*L. genei*, Mathevon, et al. 2003) pourraient également reconnaître leurs congénères grâce à des signaux vocaux.

Chez les mouettes tridactyles, les preuves de l'existence d'une reconnaissance vocale sont plus rares. Wooller (1978) a démontré que les adultes reconnaissaient leur partenaire de reproduction, mais Aubin et ses collaborateurs n'ont pas réussi à confirmer une telle reconnaissance dans la population de l'île de Hornøya (Norvège, manuscrit non publié). De plus, les résultats des études de reconnaissance entre parents et jeunes sont assez ambigus.

Ainsi Cullen (1956) a mis en évidence que les parents ne semblaient pas reconnaître leurs poussins avant l'âge de 25 jours, et Storey, et al. (1992) a montré que la signature individuelle au sein du cri des poussins était beaucoup moins marquée que dans les cris des poussins d'espèces phylogénétiquement très proches, comme le goéland argenté (*L. argentatus*). La présence d'une reconnaissance de la voix des parents par les poussins n'a à ma connaissance encore jamais été testée chez les mouettes tridactyles. Dans ce chapitre, je vais donc présenter différents résultats mettant en évidence la présence chez la mouette tridactyle d'une reconnaissance vocale entre partenaires de reproduction d'une part, et entre parents et jeunes d'autre part, basés à la fois sur des techniques expérimentales (expériences de rediffusion) et sur des observations comportementales sur le terrain.

A. Signature individuelle et géographique dans le long call [Article 1]

Parmi la panoplie de cris différents utilisés par les mouettes tridactyles, le long call (Cullen 1956, Danchin 1987, Tinbergen 1953, Wooller 1978, Wooller 1979 ; ce cri est décrit dans la Figure I-1) est sans doute le plus élaboré et le plus utilisé. Il est émis par les oiseaux au moment de l'atterrissage, et déclenche généralement une réponse du partenaire si ce dernier est présent sur le nid. Il constitue ainsi l'une des composantes essentielles de la cérémonie d'accueil chez cette espèce (Danchin 1983, Danchin 1987). Il est également émis par des oiseaux depuis leurs nids, généralement à cause de la présence aux alentours de squatteurs tentant de les défier. Comme ce cri semble déclencher une réponse des autres adultes, il pourrait donc contenir des éléments permettant l'identification de l'émetteur.

Dans [Article 1] sont présentés les résultats correspondant à l'enregistrement de différents adultes de la population de Middleton, dans le but de tester que ce cri peut effectivement contenir une signature individuelle. Tous les paramètres estimés (21 variables définies dans Aubin, et al. 2007) variaient davantage entre deux individus différents que pour des cris émis par le même individu, ce qui indique que ces différents paramètres pourraient effectivement être utilisés comme signature individuelle (cf. [Article 1] Tableau 1). Par ailleurs, les analyses discriminantes effectuées à partir de ces 21 paramètres ont permis de distinguer l'identité des émetteurs de la plupart des cris, confirmant les résultats précédents (Aubin, et al. 2007). Le long call semble également évoluer au cours de la saison et d'une année sur l'autre, mais la signature individuelle est suffisamment stable pour permettre une

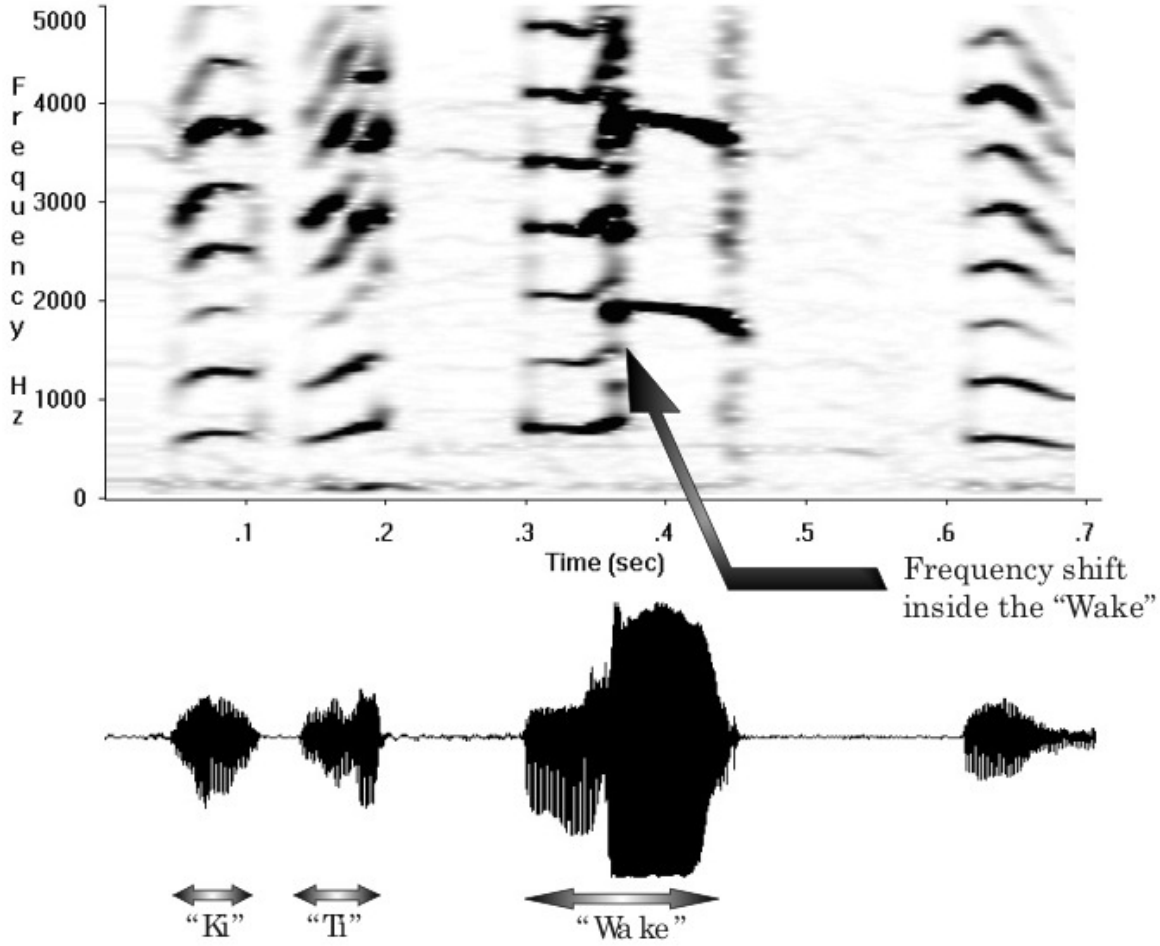


Figure I-1 : Long call d'une mouette tridactyle adulte
Le long call des mouettes tridactyles peut être divisé en trois parties : le "ki", le "ti" et le "wake", l'ensemble de ces trois parties ayant donné le nom commun de cette espèce en anglais (kittiwake). Chez certains adultes, le « wake » contient deux parties divisées par un saut fréquentiel (tel celui présenté ici). Le graphique du haut représente la fréquence en fonction du temps d'un long call d'un adulte enregistré sur l'île de Middleton, et celui du bas l'amplitude en fonction du temps pour le même cri. L'[Article 1] décrit plus précisément l'ensemble de paramètres utilisés pour caractériser ces long calls.

estimation correcte de l'identité de l'oiseau, même dans le cas où des cris émis à différents moments de la saison, voire durant différentes années, sont mélangés dans l'analyse.

Je n'ai trouvé aucune variable qui pourrait permettre une discrimination des oiseaux selon leur sexe, contrairement aux résultats obtenus sur la colonie de l'île de Hornøya (Aubin, et al. 2007). Comme la reconnaissance du sexe est potentiellement importante dans de nombreux comportements (tels le choix du partenaire, la compétition entre mâles ou entre femelles, etc.), les adultes de Middleton devraient posséder d'autres moyens que la voix pour identifier le sexe de leurs congénères. Il est également probable que les comportements

sexuels soient différents entre Middleton et les populations Atlantiques telles Hornøya. Enfin, la plupart des variables utilisées dans cette analyse varient significativement entre les deux populations, ce qui pourrait indiquer que le long call possède également une signature géographique (cf. [Article 1] Tableau 3). Depuis plusieurs décennies, les ornithologistes se demandent si les populations Atlantique et Pacifique de mouettes tridactyles ne devraient pas être considérées comme deux sous-espèces. En effet, les mouettes du Pacifique sont plus grosses, ont un bec plus long et l'extrémité des ailes plus colorée que les mouettes de l'Atlantique (Chardine 2002, Sluys 1982), mais ces différences morphologiques sont souvent insuffisantes pour distinguer complètement ces deux populations (les distributions des variables dans les deux populations se recouvrent partiellement). Plus récemment, McCoy, et al. 2005 ont montré que les fréquences alléliques des loci microsatellites variaient entre les deux populations. Une telle signature géographique n'a cependant pas été retrouvée dans les séquences des gènes codant pour le CMH [Article 5], ce qui pourrait indiquer que les deux populations ne sont pas encore totalement divergentes génétiquement. De plus, comme les populations de l'Atlantique présentent une diversité allélique plus faible que celles du Pacifique, elle pourraient soit (i) être originaires du Pacifique, soit (ii) avoir connu un goulot d'étranglement au cours de leur histoire (ce qui aurait pu diminuer leur variabilité). En effet, les populations de l'Atlantique étaient beaucoup moins nombreuses et beaucoup plus faibles à la fin du XIX^{ème} et au début du XX^{ème} siècle que maintenant, sans doute du fait de leur exploitation par les populations humaines (Coulson 1983, Coulson & Thomas 1985, Lloyd, et al. 1991). Les différences importantes observées sur les paramètres vocaux pourraient donc montrer que la voix évolue plus rapidement que la génétique, même chez une espèce comme la mouette qui ne présente apparemment aucun apprentissage du cri par les jeunes. Ainsi, l'évolution de la voix pourrait être une force importante de spéciation chez les mouettes, ce qui est d'ailleurs pris en compte dans la plupart des modèles de spéciation d'oiseaux en allopatrie (Edwards, et al. 2005).

B. Reconnaissance du partenaire de reproduction d'après le long call [Article 2]

Afin d'étudier la reconnaissance du partenaire chez les mouettes tridactyles, j'ai rediffusé le cri de leur partenaire aux adultes présents sur la colonie. En 2005, une étude préliminaire a montré que le cri devait contenir au moins 6 répétitions du « ki-ti-wake » afin de déclencher une réaction maximale au niveau de l'adulte résident (Figure I-2). Aussi j'ai opté en 2006

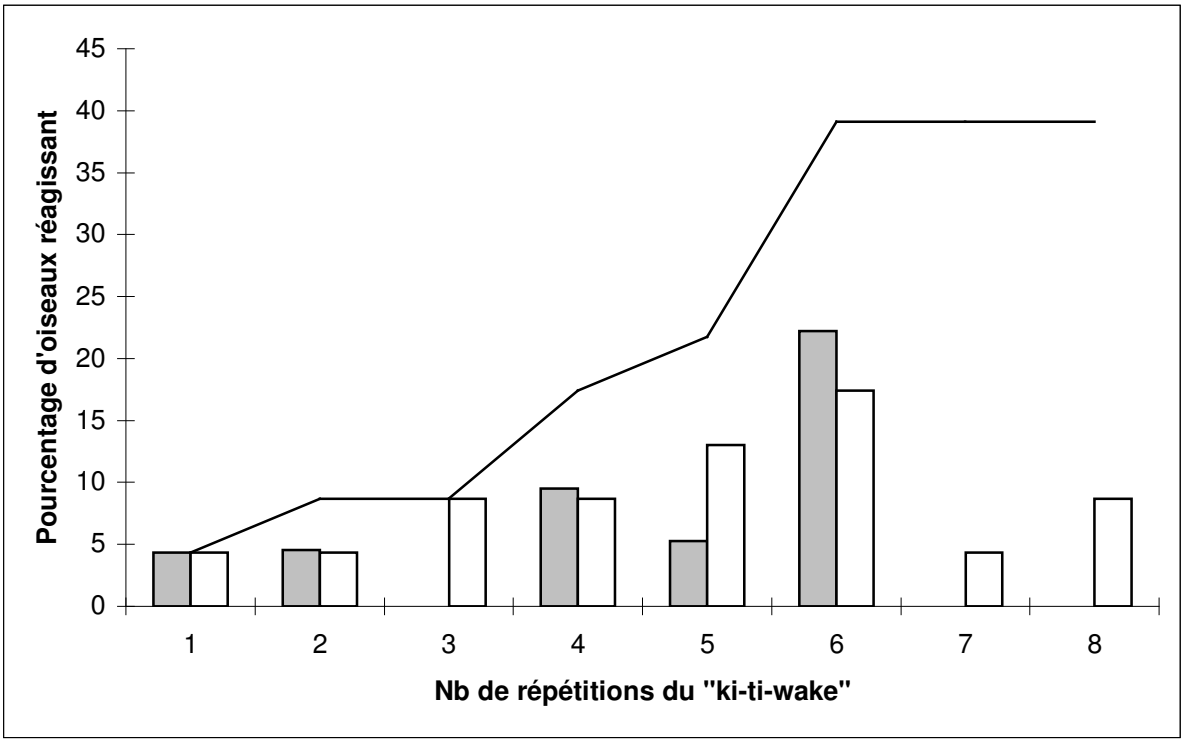


Figure I-2 : Nombre de répétitions du cri nécessaires à délencher une réaction du partenaire.
Seules les réactions les plus évidentes (des mouvements importants de la tête ou des cris en réponse à la repasse) ont été utilisées dans cette figure. La courbe représente le pourcentage total d'oiseaux ayant réagi pour ce nombre de répétitions ou un nombre inférieur. Les barres grises représentent le pourcentage d'oiseaux réagissant pour la première fois à ce nombre de répétitions. Les barres blanches représentent le pourcentage d'adultes réagissant pour ce nombre de répétitions. Dans cette étude préliminaire, le même oiseau était utilisé plusieurs fois, en commençant par la diffusion d'une bande ne contenant qu'un cri puis en augmentant progressivement le nombre de répétitions. Aussi, la chute du niveau de réaction au-delà de 6 répétitions est sans doute dû à un phénomène d'habituation : les oiseaux entendant 7 ou 8 répétitions ayant déjà entendu peu avant tous les autres cas, ils pourraient avoir appris à discriminer les cris rediffusés des cris naturels.

pour une rediffusion de cris contenant exactement 10 répétitions du « ki-ti-wake » (soit non modifiés, soit contenant uniquement la partie « ki-ti » ou la partie « wake ») afin d'optimiser les réactions des adultes observés. Le taux de réaction indiqué dans l'[Article 2] (32%) est très semblable à la proportion totale des oiseaux ayant réagi en 2005 (Figure I-2), indiquant que le niveau de réponse optimal a probablement été atteint.

En 2006, les adultes ont davantage réagi aux cris de leur partenaire qu'aux cris d'autres individus (cris d'inconnus, de voisins, ou leur propre cri rediffusé, cf. [Article 2] Figure 1). Ceci confirme les études précédentes montrant une reconnaissance vocale des partenaires (Wooller 1978). De plus, certains adultes ont réagi au cri de leur partenaire alors que le cri

diffusé ne contenait qu'une partie seulement du long call (soit le "ki-ti", soit le "wake"). La signature individuelle pourrait ainsi être redondante dans le long call. Enfin, comme les oiseaux réagissant au "ki-ti" ne sont pas les mêmes que ceux réagissant au "wake", la signature individuelle pourrait être contenue dans partie différente du cri suivant les individus. Cette interprétation est également confortée par le fait qu'aucune des 21 variables, estimées sur diverses portions du cri et utilisées pour la description des long calls, n'apparaissait comme prépondérante lors de l'analyse discriminante [Article 1].

Je n'ai pas pu montrer une reconnaissance entre voisins. J'ai pourtant essayé divers protocoles expérimentaux, en rediffusant le cri soit depuis un nid, soit depuis l'extérieur de la colonie, et ce alors que l'adulte diffusé était absent ou présent, ou encore lorsque son nid était occupé par un squatteur. Dans aucun de ces cas je n'ai été en mesure de détecter une différence de réaction entre les rediffusions de cris des voisins et d'individus inconnus. Ceci pourrait indiquer que, dans des conditions naturelles, les adultes n'ont pas besoin de réagir aux cris de leurs voisins atterrissant normalement sur leur nid [Article 2]. Par contre, ils devraient réagir aux atterrissages de prospecteurs dans leur voisinage, car de tels adultes peuvent déranger la colonie et entraîner dans certains cas des échecs de reproduction (par exemple, des poussins ou des œufs peuvent être éjectés du nid lors des batailles que ces individus déclenchent souvent). Cependant, les paramètres vocaux ne sont sans doute pas essentiels dans l'identification de tels individus. En effet, les prospecteurs ont un comportement assez original (adoptant la posture « mal à l'aise », Danchin 1983, Danchin 1987) : ils atterrissent silencieusement et gardent leurs ailes le long du corps, se tenant prêts à décoller si nécessaire. En général, les voisins chassent ce type d'oiseaux, ce qui indique qu'ils les ont bien reconnus en tant que non-résidents. Toutefois, cette reconnaissance se base sur le comportement et non sur la voix, le prospecteur étant généralement silencieux. De nombreuses observations sur le terrain suggèrent que les adultes sont effectivement capables de reconnaître, par le comportement ou la voix, la plupart de leurs voisins, mais ne réagissent en général qu'à leur partenaire, une telle réaction étant peut-être liée aux difficultés pour un oiseau à atterrir sur l'étroite bordure du nid à côté de son partenaire. Ce type de réaction pourrait donc réduire les coûts liés à une mauvaise reconnaissance (agressivité), aux risques d'éjection de poussins et d'œufs lors d'atterrissages difficiles, à l'énergie dépensée inutilement dans des vols supplémentaires, etc.

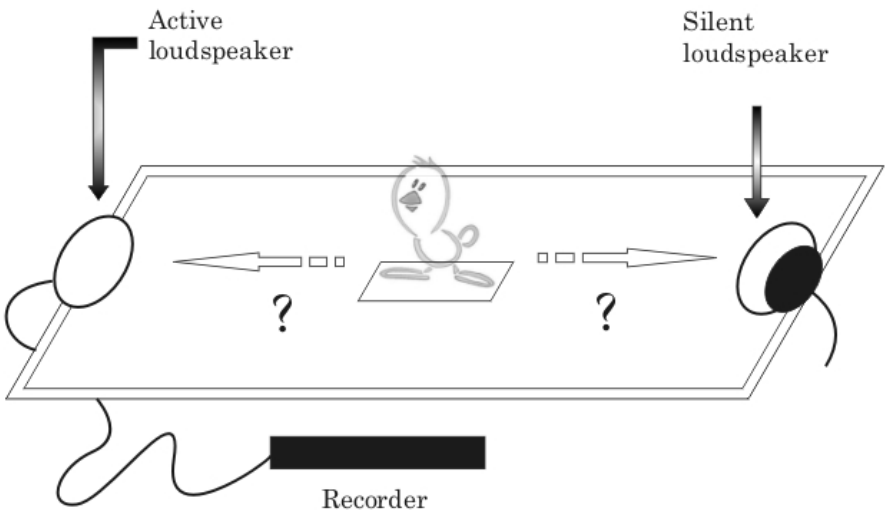


Figure I-3 : Reconnaissance des parents par les poussins : l' « expérience de la table ».
Les poussins sont testés à l'âge de 20, 25 et 30 jours. A chaque extrémité de la table est disposé un haut-parleur, un seul étant relié au magnétophone (« active loudspeaker »). La position du haut-parleur « actif » et l'ordre des séquences diffusées lors des tests sont tirés au hasard. Les mouvements des poussins et leurs cris en réponse ont été relevés, ce qui a permis de montrer qu'ils réagissent davantage aux cris de leurs parents qu'aux cris des autres adultes.

C. Reconnaissance parents-jeunes [Articles 2 & 3]

En 2006, je me suis également intéressé à la capacité des poussins à reconnaître leurs parents, en rediffusant les cris des adultes aux poussins (cf. [Article 2]). J'ai testé les poussins entre 20 et 30 jours. J'ai choisi cette période car (i) les poussins plus jeunes risquent d'être trop stressés par la manipulation (Figure I-3), car ils n'ont pas encore l'habitude d'être séparés de leurs parents (cf. [Article 3] Figure 1 pour la courbe de présence des parents au nid durant l'élevage des poussins), et (ii) les poussins de plus de 30 jours sont suffisamment âgés pour tenter de s'envoler de la table.

Les poussins réagissent plus souvent et de façon plus intense aux cris de leurs parents (ils se déplacent en direction du haut-parleurs diffusant le cri et répondent) qu'aux cris des autres adultes. Dès l'âge de 20 jours, 34% des poussins réagissent davantage à leurs parents qu'aux inconnus, et cette proportion ne change pas significativement jusqu'à 30 jours. Comme pour les adultes, des cris ne contenant que le « ki-ti » ou le « wake » suffisent à déclencher parfois

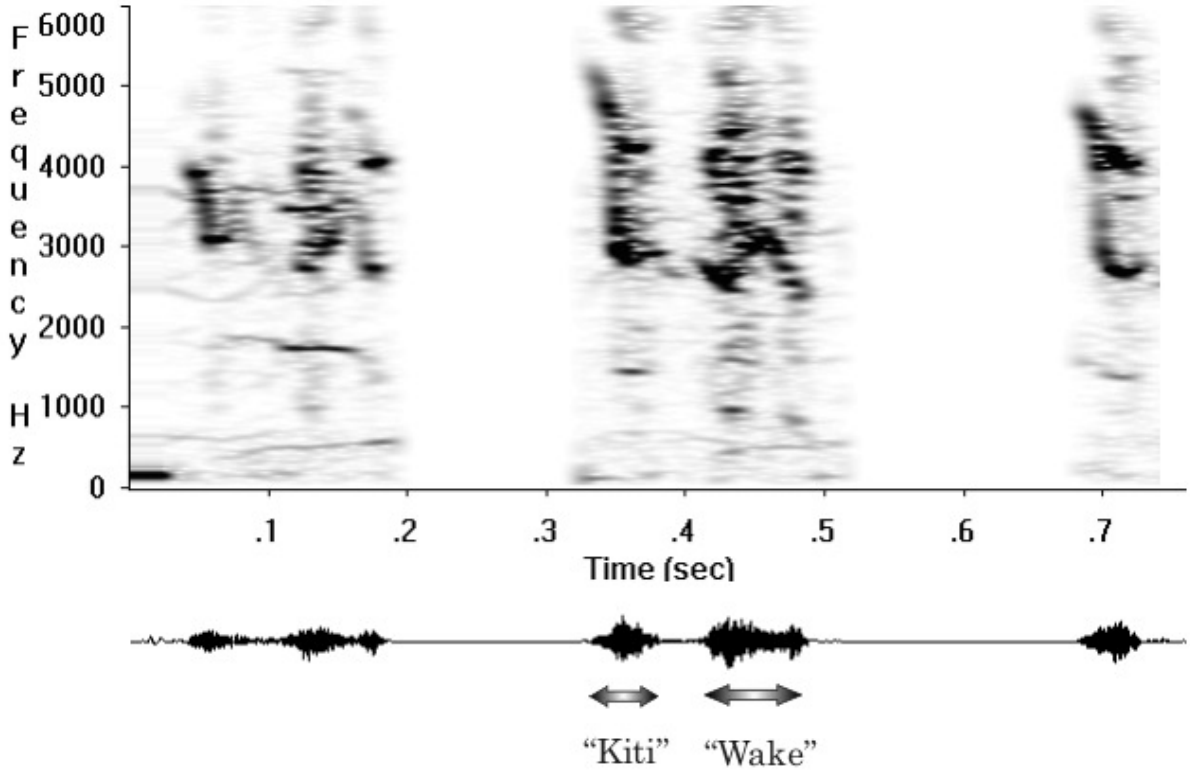


Figure I-4 : Cri d'un poussin (analogue au long call des adultes).
Ce type de cri, probablement dérivé du cri de quémande, est utilisé par les poussins lorsqu'ils battent des ailes sur leur nid ou lorsqu'ils sont effrayés. En utilisant les mêmes distinctions que pour les adultes, ce cri peut être divisé en deux parties, le "kiti", et le "wake". J'ai utilisé quatre paramètres temporels (durées du "kiti" et du "wake", durée des intervalles entre le "kiti", le "wake" et le début du cri suivant) et quatre variables fréquentielles (fréquence au maximum d'amplitude, premier, deuxième et troisième quartiles de distribution d'énergie) pour décrire ce type de cris.

une réaction du poussin, ce qui confirme que ces deux composantes du long call possèdent une signature individuelle. Les poussins sont donc capables de reconnaître la voix de leurs parents dès l'âge de 20 jours, ce qui correspond au moment où les adultes sont de moins en moins présents au nid (cf. [Article 3] Figure 1). L'aptitude des poussins à discriminer les parents des étrangers pourrait donc déjà être importante, car les poussins qui n'adoptent pas un comportement de dominé face aux étrangers atterrissant sur leur nid en l'absence de leurs parents sont parfois agressés et blessés.

Les parents sont également capables de reconnaître leurs jeunes au moment de l'envol. En utilisant des paramètres estimés sur les cris des poussins (Figure I-4), 70% de ces cris étaient correctement assignés au poussin émetteur, indiquant un certain taux d'individualité. Comme ces cris étaient enregistrés avant l'envol (entre le 25ème jour et l'envol), cela pourrait également indiquer que les parents ont la possibilité reconnaître leurs poussins plusieurs jours

avant l'envol. Par ailleurs, les parents de la colonie du Cap Sizun (cf. [Article 3]) réagissent davantage que les autres adultes aux cris de leurs poussins essayant de rentrer au nid. Les parents présents réagissent dans environ 67% des cas aux cris de leurs poussins, et répondent dans 40% des cas, un taux de réaction deux fois supérieur au taux de réaction des poussins aux cris de leurs parents lors de l'expérience de la table. Cette différence est probablement due au fait que les estimations n'ont pas été faites dans les mêmes conditions (observations en conditions naturelles, ou expériences de rediffusion partiellement artificielles).

D. Rôles de la reconnaissance individuelle

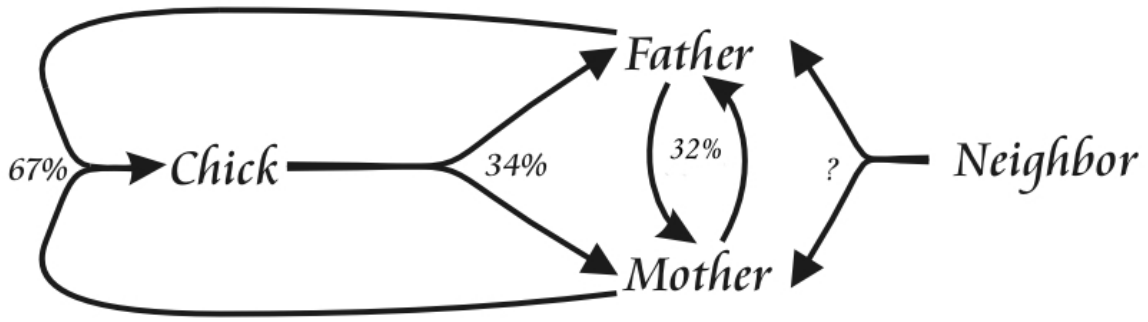


Figure I-5 : Reconnaissance vocale chez la Mouette tridactyle.
Les nombres représentent les pourcentage de réaction aux cris des autres individus; Les flèches indiquent le sens de la reconnaissance (ex : Chick -> Father indique que le poussin réagit à son père dans 34% des cas).

En résumé (Figure I-5), j'ai montré que les partenaires de reproduction pouvaient se reconnaître grâce à la voix, de même que les parents reconnaissent sans doute leurs poussins et vice versa. Cependant, il est important de noter que les niveaux de réaction sont beaucoup plus faibles chez la mouette tridactyle que chez les espèces voisines : ainsi, les poussins des mouettes rieuses réagissent aux cris de leurs parents dans 100% des cas (Charrier, et al. 2001). Ce faible niveau de réaction n'est cependant guère surprenant, étant donné les traits d'histoire de vie de la mouette tridactyle. Comme les poussins restent sur leur nid et sont nourris par leurs parents jusqu'à l'envol, et que les parents se reproduisent d'année en année sur le même site (Coulson 1966), de hauts niveaux de réaction (et peut-être également de reconnaissance) ne sont pas nécessaires dès lors que la propriété du site est établie. Cette interprétation est confirmée par l'étude de Storey, et al. (1992) montrant que la signature individuelle dans le

cri des poussins de mouette tridactyles est moins évidente que celle des poussins – mobiles – des mouettes atricilles. Mathevon, et al. (2003) ont mis en évidence des différences analogues entre les cris des poussins nidicoles de la mouette rieuse et les poussins mobiles (qui forment d'ailleurs des crèches) des goélands railleurs.

Cependant, la reconnaissance individuelle a sans doute encore un rôle important durant au moins deux moments de la vie des mouettes tridactyles : l'envol, et la formation des couples.

1. Au moment du premier envol des jeunes [Article 3]

La période du premier envol des jeunes est un premier exemple de l'importance de la reconnaissance individuelle (cf. [Article 3]). En effet, comme les jeunes sont encore nourris au nid par leurs parents durant environ deux semaines après l'envol, ils doivent tout d'abord retourner au nid, les parents ne les nourrissant presque jamais en dehors du nid. Au moment de ce premier retour, les jeunes n'ont qu'une vague idée de la localisation de leur nid, et ne peuvent pas utiliser d'informations visuelles puisqu'ils n'ont encore jamais vu leur nid depuis l'extérieur. De ce fait, les jeunes qui tentent leur premier retour atterrissent plus ou moins au hasard sur de nombreux sites aux environs de leur nid d'origine, et risquent donc d'être chassés et blessés par les autres adultes (ou poussins) présents sur ces sites. Un tel comportement peut avoir des coûts importants, et la sélection devrait donc favoriser les parents aidant leurs jeunes lors de leur premier envol. Dans l'article 3, nous montrons que (i) les parents sont davantage présents au moment du premier envol de leur jeune, et (ii) le retour des jeunes est plus rapide lorsque les parents sont présents et réagissent à leurs cris, ce qui permet aux jeunes de localiser le nid et de rentrer.

La reconnaissance parent-jeune pourrait donc être une adaptation au moment du premier envol, et la voix apparaît comme essentielle dans ce processus. Les niveaux de reconnaissance acoustique pourraient donc être plus élevés à ce moment-là qu'à 20 ou 30 jours, mais je n'ai pas pu réaliser de tels tests.

2. Au moment de la formation des couples

Pour l'instant, il n'y a aucune preuve que les individus restent en couple durant l'hiver (Coulson & Thomas 1980, cité dans Baird 1994) et, de ce fait, les partenaires doivent se retrouver à nouveau au début de chaque nouvelle saison de reproduction. Ceci pourrait être la cause du pic d'activité vocale (Wooller 1979) observé en début de saison, qui souligne le rôle que pourrait avoir la reconnaissance vocale à ce moment. De plus, comme le long call évolue

d'une année sur l'autre [Article 1], on peut supposer que les adultes vont crier davantage en début de saison afin de donner à leur partenaire l'opportunité d'enregistrer ces modifications.

En cas de veuvage ou de divorce, les individus doivent également trouver un nouveau partenaire. Ils pourraient pour cela utiliser les informations enregistrées les années précédentes à propos de leurs congénères (ceci est une des prédictions de l'hypothèse du lek caché). Ceci nécessite également une reconnaissance précise des individus.

Étant donnée l'importance que peut prendre la reconnaissance durant ces deux moments de leur vie, les individus sont certainement capables de reconnaître leurs partenaires, leurs apparentés et leurs voisins. Les résultats présentés dans ce chapitre tendent à montrer que l'absence de réaction n'implique pas forcément l'absence de reconnaissance. Plusieurs hypothèses peuvent être évoquées pour expliquer les différences entre les niveaux de réactions observés et les niveaux de reconnaissance attendus du fait de l'histoire de vie de cette espèce :

1. Les expériences étaient sans doute assez artificielles. En effet, les poussins étaient sans doute en situation de stress lors de l'expérience de la table, ce qui peut avoir entraîné une sous-estimation du niveau de réaction. De la même façon, les adultes ont pu rapidement apprendre que les sons émis depuis le site de diffusion (un point d'observation à environ 30m de la tour de Middleton) n'étaient pas associés au retour du partenaire, et auront donc fini par les ignorer.
2. Les individus peuvent utiliser d'autres indices pour la reconnaissance (indices visuels, olfactifs ou comportementaux par exemple). De ce fait, la voix n'est peut-être pas suffisante pour déclencher une réaction dans certaines circonstances. Certaines espèces voisines possèdent une reconnaissance basée sur d'autres indices : les poussins des goélands railleurs peuvent ainsi reconnaître leurs congénères grâce à des indices visuels (Griswold, et al. 1995), bien que ce type de reconnaissance semble secondaire devant la reconnaissance vocale. Les Procellaridés peuvent reconnaître leur nid (Bonadonna, et al. 2003, Bonadonna, et al. 2004) et leur partenaire (Bonadonna & Nevitt 2004) grâce à des indices olfactifs. Des expériences sont en cours pour tester la présence d'une signature individuelle dans les couleurs et les odeurs chez la mouette tridactyle.

3. Signaler son identité peut également être coûteux (Tibbetts & Dale 2007), car il est plus difficile pour un individu facilement identifiable de tricher. Par exemple, un poussin sans caractère particulier est nourri par ses parents mais peut également l'être, en moindre proportion sans doute, par d'autres adultes qui ne lui sont pas apparentés. Chez les mouettes tridactyles, les adoptions après l'envol sont certes rares mais peuvent se produire (Roberts & Hatch 1994, Helfenstein, et al. 2004c), et un poussin à l'identité marquée pourrait avoir moins de chance d'être adopté, et payer ainsi un coût lié à cette signature individuelle. Un tel mécanisme pourrait donc créer des contraintes évolutives diminuant les pressions de sélection sur la reconnaissance des poussins, expliquant ainsi le manque de critères de distinctions dans les cris des poussins de mouettes tridactyles.
4. Enfin, une réaction au cri n'est peut-être pas nécessaire. Comme les adultes reproducteurs et les poussins vivent sur le même nid durant la plus grande partie de la saison, réagir au cri d'individus connus peut n'avoir aucun intérêt, à partir du moment où les adultes (et les poussins) connaissent le chemin pour rentrer au nid. Chez les mouettes, la formation des couples et la propriété du nid est essentiellement établie à partir du comportement d'atterrissage (Danchin 1987), et il serait donc intéressant d'étudier la réaction aux cris plus tôt dans la saison, au moment de la formation des couples, ou, au contraire, au moment de l'envol des jeunes.

II. L'appariement chez les mouettes [Article 4]

Le choix du partenaire a généralement un impact important sur le succès de reproduction, et constitue donc l'un des moments cruciaux de l'histoire de vie des espèces sexuées. Pour ne prendre que l'exemple des femelles, celles-ci peuvent profiter de deux sortes de bénéfices liés au choix du mâle :

1) **Des bénéfices directs** (revus par Andersson 1994). Lorsqu'elle peut se reproduire avec le mâle qu'elle préfère, la femelle peut augmenter sa fertilité, faire profiter ses petits des soins paternels, obtenir davantage de ressources (que ce soit sous la forme de nourriture directement apportée par le mâle ou du territoire qu'il défend et auquel elle a accès), ou encore profiter de la protection du mâle contre la prédation et le harcèlement par d'autres mâles en quête de partenaire.
2) Les femelles peuvent également profiter de **bénéfices indirects**, par le biais de caractères qui ne seront exprimés que dans la génération future. Les jeunes issus d'accouplements avec un mâle spécifique pourraient ainsi avoir une meilleure valeur sélective (meilleure qualité génétique, meilleure résistance contre les parasites, meilleure viabilité et compétitivité, etc.) que des jeunes issus d'accouplements avec des mâles non préférés ou choisis au hasard.

[Chez de nombreuses espèces, le choix du partenaire est bilatéral ; le mâle exerce alors également un choix, et pourrait profiter de bénéfices analogues liés à son choix (Bergstrom & Real 2000, Jones & Hunter 1993).]

Une femelle peut profiter simultanément de ces deux types d'avantages. Par exemple, l'étude de Drickamer, et al. (2000) montre que les souris femelles ayant l'opportunité de s'accoupler avec le mâle qu'elles préfèrent (défini grâce à une expérience de choix) ont des portées plus nombreuses (bénéfice direct), et que leurs petits seront plus viables et auront un statut social plus élevé (bénéfice indirect). Grâce au choix du partenaire, les individus peuvent donc augmenter leur valeur sélective et celle de leurs jeunes.

Aucun bénéfice direct du choix du partenaire n'a pu être identifié pour l'instant chez la mouette tridactyle. L'une des pistes proposées s'est avérée être une impasse : si les mâles donnent bien de la nourriture à leur partenaire juste avant la copulation (comportement de nourrissage de cour, Helfenstein, et al. 2003), des femelles artificiellement nourries *ad libitum* (et qui, de ce fait, n'ont pas besoin de cette nourriture supplémentaire fournie par le mâle) ne copulent pas plus ou moins fréquemment que des femelles non nourries (Kempenaers, et al.

2006), ce qui montre que le nourrissage de cour n'est ni nécessaire ni suffisant pour obtenir une copulation. Si les mouettes choisissent effectivement leur partenaire, les mécanismes de ce choix devraient donc être fondés sur d'autres avantages que des bénéfices nutritionnels, et je vais montrer dans cette partie que ce choix pourrait être lié à des **bénéfices génétiques indirects**.

De nombreuses études ont montré l'existence de coûts importants lies à la consanguinité (au sens large, revue dans Hansson & Westerberg 2002), et les individus devraient donc choisir leur partenaire de façon à limiter ces coûts. Divers oiseaux sont ainsi appariés selon des critères génétiques variés. Ainsi les femelles mésanges bleues (*Cyanistes caeruleus*) ont des poussins hors couple plus hétérozygotes que leurs poussins légitimes (Foerster, et al. 2003). Les moineaux domestiques (*Passer domesticus*) et les bruants des prés (*Passerculus sandwichensis*) sont appariés d'une façon qui augmente la diversité génétique de leurs descendants (Bonneaud, et al. 2006, Freeman-Gallant, et al. 2003). Chez la fauvette des Seychelles (*Acrocephalus sechellensis*, Richardson, et al. 2005), et chez trois espèces d'oiseaux marins (Blomqvist, et al. 2002), les fertilisations hors couple sont plus fréquentes lorsque les membres du couple sont proches génétiquement. Enfin, les femelles bécassines doubles (*Gallinago media*) semblent préférer les mâles possédant des allèles particuliers aux loci du CMH (Ekblom, et al. 2004). Un tel choix du partenaire selon la génétique n'est cependant pas un trait généralisable à l'ensemble des espèces d'oiseaux, différentes études n'ayant pu montrer de telles relations (e.g. Westerdahl 2004).

La plupart des espèces étudiées sont pour l'instant génétiquement polygames, et présentent donc des fréquences variables de fertilisations et de paternité (voire de maternité) hors couple. Chez les espèces monogames génétiquement, de telles fertilisations hors couple sont par définition absentes, et les mécanismes du choix du partenaire pourraient donc subir des pressions de sélection plus importantes que chez les autres espèces n'ayant qu'une monogamie sociale. Ces pressions pourraient encore s'accentuer pour des espèces qui divorcent rarement d'une année sur l'autre. La seule étude publiée à ce jour montrant un appariement selon des critères génétiques chez une espèce génétiquement monogame a décrit un résultat pour le moins inattendu : les frégates du Pacifique (*Fregata minor*) préfèrent les partenaires les plus proches génétiquement (Cohen & Dearborn 2004). Afin de voir si ce résultat paradoxal pouvait être généralisé à d'autres oiseaux strictement monogames, et en l'occurrence chez les mouettes tridactyles, j'ai cherché à savoir :

1) S'il y avait un appariement selon des critères génétiques.

2) Si les couples qui adoptaient un tel mode d'appariement avaient un meilleur succès de reproduction et donnaient naissance à des petits de meilleure qualité que les couples qui ne suivaient pas la règle générale.
3) Si l'intensité de certains comportements sexuels (notamment les copulations) était également corrélée à la proximité génétique des membres du couple.

Cette étude se base essentiellement sur des observations de terrain et des corrélations ; je n'ai pas mis en place d'expériences de choix qui auraient pu prouver la présence d'une préférence active pour des individus ayant certaines caractéristiques particulières.

A. Les individus sont appariés de façon à augmenter la probabilité d'avoir des jeunes hétérozygotes [Article 4]

J'ai utilisé les génotypes obtenus aux locis microsatellites [Article 4] pour tester chez les mouettes les trois hypothèses les plus fréquemment invoquées pour expliquer un choix du partenaire sur des critères génétiques :

(1) l'hypothèse « **bons gènes / hétérozygotie** » (Landry, et al. 2001, Weatherhead, et al. 1999) : les individus préfèreraient s'apparier aux partenaires les plus hétérozygotes, les adultes les plus hétérozygotes étant de meilleures qualité. Si c'est le cas, les individus les plus hétérozygotes devraient s'apparient entre eux, car seuls les mâles les plus hétérozygotes auront accès aux femelles hétérozygotes (et vice versa). De plus, les individus appariés devraient également être plus hétérozygotes que les individus n'ayant pas trouvé de partenaire.

(2) l'hypothèse d' « **évitement de la similarité génétique** » (Bensch, et al. 1994, Blomqvist, et al. 2002, Brown 1996, Hoffman, et al. 2007): un individu donné préfèrerait s'apparier à l'individu ayant un génotype le plus différent possible du sien, ceci afin de produire des jeunes les plus hétérozygotes possibles. Si c'est le cas, les couples formés devraient être constitués d'individus moins semblables génétiquement que si l'appariement était effectué au hasard.

(3) l'hypothèse de « **préférence pour la similarité génétique** » : dans ce cas, les individus préfèreraient s'apparier avec des individus ayant un génotype similaire (Cohen & Dearborn 2004, Kokko & Ots, 2006). En effet, s'apparier avec des individus très différents génétiquement pourrait détruire les associations d'allèles bénéfiques conférant localement une meilleure valeur sélective, et les jeunes issus

de tels accouplements pourraient alors être moins compétitifs que ceux issus d'accouplements d'adultes génétiquement semblables. Les couples formés devraient alors être plus semblables génétiquement que si l'appariement était effectué au hasard.

Pour estimer cette proximité génétique entre les membres du couples, j'ai utilisé deux indices différents, notés *r* et *Phm* (ceci est détaillé dans la partie D de l'introduction). En 2003 et 2004 (Tableau II-1 et [Article 4]), j'ai ainsi trouvé que les couples formés étaient plus différents génétiquement que si l'appariement était fait au hasard, ce qui validerait la seconde hypothèse. Ces résultats étaient significatifs en utilisant l'indice *Phm*, et presque significatifs

	Phm_{xy}	
	2003	**2004**
Nombre de couples *Nombre d'adultes**	74 241	72 289
Tous les loci	Obs = 0.181 Att = 0.196 **P = 0.021**	Obs = 0.184 Att = 0.201 **P = 0.018**
Sans les loci OHW (définis dans [Article 4])	Obs = 0.162 Att = 0.184 **P = 0.005**	Obs = 0.174 Att = 0.191 **P = 0.043**
	$\hat{r}_{xy}$	
Tous les loci	Obs = -0.0297 Att = 0.0013 P = 0.060	Obs = -0.0299 Att = -3.75E-5 P = 0.083
Sans les loci OHW	Obs = -0.037 Att = 0.00076 **P = 0.048**	Obs = -0.016 Att = 0.0013 P = 0.25

Tableau II-1: Différences entre la moyenne observée et attendue des deux indices de similarité génétique : *Phm* (probabilité de produire un jeune homozygote) et *r* (indice de Queller & Goodnight).

Pour chaque année sont reportés le nombre de couples formés et le nombres d'adultes* observés et génotypés dans la zone étudiée. Pour chaque année, les deux indices sont calculés soit en tenant compte de tous les loci microsatellite analysés, soit en retirant les loci OHW (qui présentent un déséquilibre de liaison, ceci est détaillé dans l'article 4). Nous comparons la valeur observée de ces indices (Obs) avec la valeur attendue (Att) obtenue en effectuant 10000 permutations des individus observés en les ré-appariant au hasard. La valeur P est calculée comme étant la proportion de ces permutations ayant une valeur moyenne de l'indice inférieure à la valeur observée (ce qui correspond à des cas où un appariement au hasard conduit à des couples plus différents génétiquement que ce qui est observé).

* Ce nombre contient à la fois les individus appariés, et les prospecteurs, c'est à dire des individus qui ne se sont apparemment pas appariés dans notre zone d'étude (mais qui pourraient l'être ailleurs).

avec l'indice *r*. Ceci signifie que les individus semblent **appariés avec des individus génétiquement différents**, ce qui pourrait leur permettre d'augmenter les chances de produire des jeunes hétérozygotes.

B. Quels sont les bénéfices à s'apparier avec un individu génétiquement différent ? [Article 4]

Puisque les mouettes sont appariées de façon à diminuer la probabilité de produire des jeunes homozygotes, il devrait être coûteux de s'apparier avec des individus génétiquement semblables. J'ai pu identifier plusieurs de ces inconvénients.

Tout d'abord, les couples génétiquement proches ont moins de poussins que les autres (ceci n'a cependant été établi qu'avec l'indice *Phm*, cf. [Article 4] Figure 2). De plus, j'ai trouvé une corrélation positive significative entre l'**hétérozygotie du poussin et sa valeur sélective** en 2005. Cette année-là, j'ai pu génotyper un grand nombre de poussins (n = 82) et suivre leur développement de l'éclosion jusqu'à l'âge de 25 jours. J'ai trouvé que les poussins les plus hétérozygotes grandissaient plus vite (en termes de poids, de longueur du tarse et de l'aile), et avaient une meilleure survie jusqu'à 25 jours que les poussins plus homozygotes (cf. [Article 4]). Ainsi, produire des poussins hétérozygotes est réellement avantageux chez cette espèce, et de tels bénéfices génétiques indirects pourraient expliquer l'appariement observé selon la dissimilitude génétique.

C. Fréquence de copulation et consanguinité du couple

Les mouettes sont ainsi appariées de façon à augmenter leur chance de produire des poussins hétérozygotes, ces poussins ayant une meilleure croissance et une meilleure survie. Un certain nombre de couples sont cependant formés d'individus génétiquement semblables (ou, en tous cas, plus semblables que les autres). Ces appariements sub-optimaux pourraient s'expliquer soit par une mauvaise estimation du génotype du partenaire, soit parce que les individus les plus adéquats n'étaient plus accessibles. Pour de tels couples, la reproduction pourrait représenter un coût très important, puisqu'elle ne serait plus contrebalancée par des avantages tels que la production de poussins de bonne qualité. Ces couples pourraient donc adopter des comportements qui leur permettrait d'éviter la reproduction (c'est une espèce longévive qui peut donc se reproduire plusieurs fois).

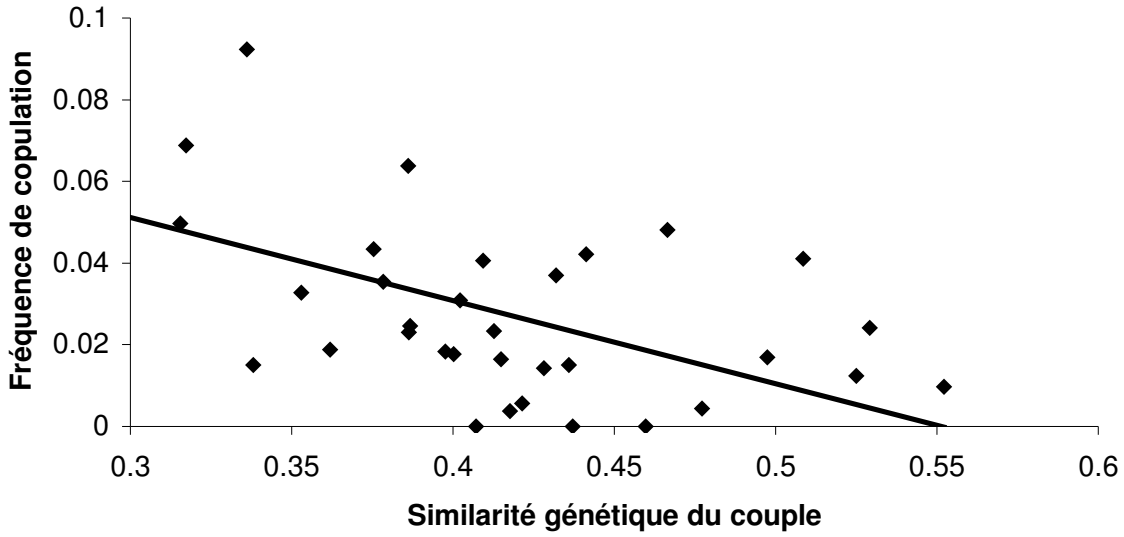


Figure II-1 Corrélation entre la similarité génétique du couple et sa fréquence de copulation.
La fréquence de copulation pour chaque couple est calculée en cumulant les données sur les trois années d'observation. Les analyses sont faites en utilisant une fréquence de copulation par année et par couple (en nombre de copulations par heure d'observations ; modèle mixte, n = 52, F = 7.67, p = 0.013; incluant l'identité du couple comme paramètre aléatoire : z = 1.72, p = 0.04). Le protocole utilisé pour les observations comportementales est décrit dans Helfenstein et al. (2003).

Une étude préliminaire sur les données comportementales recueillies par Fabrice Helfenstein lors de sa thèse (population du Cap Sizun, Helfenstein 2002) montre que la fréquence de copulation est corrélée avec la similarité génétique des couples (calculée ici grâce à l'indice *Phm*, cf. Figure II-1). **Les couples génétiquement similaires copulent donc moins fréquemment** que les couples génétiquement dissemblables. Ce comportement pourrait être interprété comme une stratégie d'évitement de la reproduction.

Les mouettes semblent donc s'apparier de façon à diminuer la probabilité d'avoir des jeunes homozygotes. Ceci s'expliquerait par le faible succès d'éclosion des couples génétiquement semblables, et par la faible qualité des jeunes homozygotes (croissance et survie plus faible jusqu'à 25 jours). Cet appariement pourrait résulter de différents mécanismes. Les individus peuvent effectivement montrer une préférence (active) pour les individus les plus distants génétiquement, utilisant pour cela des indices phénotypiques leur permettant d'estimer le génotype de leurs congénères. On peut également imaginer un **mécanisme passif** : en effet, en supposant que le succès de reproduction est corrélé à la

similarité génétique, et en imaginant que les couples qui échouent leur reproduction divorcent davantage, alors les individus pourraient finalement se retrouver appariés à des individus génétiquement différents (mais ceci pourrait prendre du temps).

Afin de tester le réalisme d'un tel mécanisme passif, j'ai effectué des simulations en utilisant les paramètres estimés d'après la population de Middleton (les paramètres utilisés étaient les fréquences alléliques, le taux de mortalité, le taux de succès, et les taux de divorce après succès ou échec de reproduction ; ces paramètres ont été estimés à partir de l'échantillon d'oiseaux génotypés). Chaque année, les individus sont virtuellement appariés au hasard, et on évalue le nombre d'années nécessaires pour obtenir un état semblable à celui observé en 2003 et 2004. Dans le modèle, chaque couple formé peut échouer ou réussir sa reproduction, et divorcer à la fin de la saison (ceci en accord avec les paramètres estimés). De la même façon, les individus peuvent également mourir, ce qui conduit à la formation de nouveaux couples.

Si le succès de reproduction est aléatoire (les couples génétiquement les plus semblables ayant autant de chance de réussir que les autres), alors le modèle n'atteint jamais l'état observé en 2003 ou 2004. Ceci indique que la corrélation entre succès de reproduction et la similarité génétique doit être particulièrement forte. Par ailleurs, même lorsque seuls les couples génétiquement les plus semblables échouent leur reproduction, il faut un taux de divorce après succès proche de 0% et/ou un taux de divorce après échec bien plus important que celui estimé à Middleton, afin d'atteindre l'état observe dans une durée raisonnable (c'est-à-dire, après un nombre d'années compatible avec le taux de mortalité de la mouette tridactyle). Ces résultats tendent à montrer qu'un mécanisme passif de ce type n'explique pas les observations, puisque la corrélation que nous avons mesurée entre le succès de reproduction et la similarité génétique est assez faible, et que les divorces sont plutôt rares à Middleton.

La sélection naturelle devrait donc favoriser les individus ayant une **préférence marquée** pour les individus distants génétiquement. Ceci semble par ailleurs corroboré par le fait que les couples génétiquement semblables semblent éviter la fertilisation (taux de copulation plus faible), ce qui pourrait indiquer qu'ils sont effectivement capables d'estimer le génotype de leur partenaire et d'adopter un comportement adéquat.

Pour estimer le génotype de leur partenaire, les individus devraient donc utiliser des indicateurs phénotypiques de la qualité génétique. Ceux-ci sont cependant encore largement spéculatifs, car le mécanisme par lequel les mouettes (et les oiseaux de mer en général) choisissent leur partenaire est encore mal connu.

III. Comment les individus estiment-ils le génotype de leur partenaire ? [Article 5]

Helfenstein, et al. (2004a) avaient montré un appariement des individus **selon la longueur du tarse** chez les mouettes du Cap Sizun. Ce paramètre morphologique pourrait donc être utilisé comme point de départ pour étudier le rôle de caractères sexuels secondaires dans le choix du partenaire. Cependant, un tel appariement n'a pu être confirmé pour la population de Middleton, malgré un gros effort de capture. Aucune expérience de choix de partenaire n'a pu être menée jusqu'à présent, ce qui rend le rôle de cette variable dans le formation des couples assez ambigu. Par ailleurs, il est peu probable que ce paramètre puisse être un bon estimateur de la qualité génétique, car nous n'avons trouvé aucune corrélation entre la longueur du tarse et différentes mesures de la qualité génétique (hétérozygotie, etc.). Je vais donc essayer dans cette partie de proposer d'autres indices qui pourraient avoir un rôle dans le choix du partenaire chez les mouettes tridactyles.

A. Choisissent-elles selon des paramètres vocaux ?

Comme les mouettes semblent choisir leur partenaire selon le génotype, et comme elles utilisent le cri pour s'identifier, l'hypothèse la plus parcimonieuse serait que les paramètres acoustiques du cri codent à la fois l'individu et son génotype. Par exemple, on peut imaginer que les cris de deux individus génétiquement distants diffèrent davantage que les cris d'individus plus semblables.

Nous avons besoin d'estimer une « distance vocale » afin de tester une telle hypothèse. Pour cette analyse, j'ai donc (1) estimé les composantes principales de 4 cris, choisis au hasard, pour chaque individu enregistré à Middleton, (2) estimé les composantes principales du « cri moyen », c'est à dire du barycentre de ces 4 cris, et (3) utilisé les composantes du cri moyen de deux individus pour calculer la distance vocale (distance cartésienne) entre ces deux individus. J'ai ainsi pu calculer la distance vocale entre les mâles et toutes les femelles enregistrés dans cette population. Je n'ai cependant trouvé aucune corrélation entre cette distance vocale et la similarité génétique (SAS modèle mixte avec l'identité du mâle comme paramètre aléatoire, les deux variables étaient transformées en rangs normalisés, $F_{1403} = 0.14$, $p = 0.71$). Ce résultat est identique à celui qu'on obtiendrait si la distance vocale était calculée

en donnant à chaque composante principale un poids équivalent, ou si chaque composante était pondérée par sa valeur propre.

Il semblerait donc que les paramètres vocaux ne permettent pas l'estimation du génotype dans cette population de mouettes tridactyles. Les individus pourraient certes utiliser des paramètres vocaux qui ne sont pas pris en compte ici, tels les modulations de fréquence et les attaques, mais ces paramètres sont plus difficiles à estimer. Il est également probable que les individus utilisent des indices visuels ou olfactifs pour estimer leur apparentement avec leurs partenaires potentiels.

A ma connaissance, les seules études montrant une corrélation entre les paramètres acoustiques et la distance génétique ont été réalisées chez le bruant chanteur (*Melanospiza melodia*). Reid, et al. (2005) ont ainsi mis en évidence que la taille du répertoire des mâles est corrélée négativement à leur degré d'apparentement avec les femelles (Reid 2007), ce qui ferait de ce paramètre vocal un indice adéquat de la distance génétique, que les femelles pourraient utiliser lors du choix de leur partenaire (Reid, et al. 2004). La population étudiée est cependant probablement très consanguine (c'est une population insulaire), et les mâles à large répertoire pourraient donc plus simplement provenir d'autres populations, et seraient donc, de fait, différents génétiquement des femelles résidentes. De plus, ces bruants apprennent le chant de leur père, ce qui rend ces résultats plus difficiles à interpréter dans le cadre d'un choix du partenaire selon des critères génétiques.

B. Choisissent-elles suivant les odeurs et le génotype du CMH ? [Article 5]

Les gènes du **Complexe Majeur d'Histocompatibilité** (CMH) sont impliqués dans de nombreux aspects de l'immunité, depuis la reconnaissance du soi et du non-soi à l'activation des voies humorales et cellulaires de la réponse immunitaire. Ces gènes sont les plus polymorphiques que l'on connaisse, et les individus ayant une forte hétérozygotie pour ces loci semblent généralement avoir de meilleures aptitudes immunitaires (Bonneaud, et al. 2004, McClelland, et al. 2003, Penn, et al. 2002, Wedekind, et al. 2004, Westerdahl, et al. 2005). En conséquence, les génotypes CMH semblent également **influencer le choix du partenaire** chez de nombreux vertébrés, les individus cherchant à augmenter la variabilité génétique de leurs descendants (Bonneaud, et al. 2006, Ekblom, et al. 2004, Landry, et al. 2001, Penn & Potts 1998a, Richardson, et al. 2005, Wedekind & Füri 1997).

Dans la plupart des cas, les individus semblent préférer s'apparier à des individus ayant des allèles MHC différents des leurs (Tregenza & Wedell 2000), ou tout au moins des allèles différant le plus possible dans leur composition en acides aminés, notamment au niveau de la région qui se lie aux peptides (e.g. Landry, et al. 2001). En effet, cette région est la plus polymorphique et a pour rôle d'exposer les antigènes aux cellules du système immunitaire afin de déclencher (ou non) la réponse immunitaire. Un tel système d'appariement est assez semblable à celui que j'ai décrit pour les loci microsatellites, et il serait donc intéressant d'étudier si le CMH montre des résultats analogues chez la mouette.

En effet, les allèles du CMH sont par ailleurs **corrélées aux odeurs corporelles** chez certains Mammifères, en particulier chez les souris (Penn & Potts 1998b, Yamazaki, et al. 1990) et les êtres humains (Wedekind, et al. 1995), ce qui fait de ce type d'odeurs de bons estimateurs du génotype. De ce fait, les odeurs corporelles semblent également impliquées dans le choix du partenaire chez les deux espèces (Penn 2002, Wedekind & Füri 1997). Ce lien entre le CMH et les odeurs pourrait peut-être être généralisable à d'autres vertébrés.

Durant ma thèse, j'ai commencé le génotypage des gènes du CMH de Classe II, impliqués dans la réponse immunitaire aux pathogènes extra-cellulaires. Ce génotypage avait pour objectifs de déterminer :

- si les individus sont appariés selon leur genotype CMH
- si ces génotypes sont corrélés à des composants olfactifs

Je ne suis pas encore en mesure de répondre à ces questions, car je n'ai pour l'instant que les séquences des gènes de Classe II chez quelques individus de différentes espèces d'oiseaux de mer (dont la mouette). Ces séquences vont nous permettre de sélectionner les amorces les plus adéquates pour lancer le génotypage à plus grande échelle. Dans l'[Article 5], je publie ces séquences et donnons quelques indices sur l'évolution moléculaire du CMH chez ces oiseaux. Il semblerait que ces gènes ont évolué beaucoup plus rapidement chez les Ciconiiformes que chez les Passeriformes, ce qui pourrait expliquer certains problèmes phylogénétiques auxquels je me suis heurté. Par ailleurs, j'ai trouvé différentes preuves d'une sélection balancée dans ces séquences, ce qui confirme les résultats des études précédentes. Ce type de sélection a tendance à rendre les mutations non silencieuses plus fréquentes que les mutations silencieuses (qui sont plutôt fixées par dérive), ce qui serait la signature d'une pression évolutive en faveur du polymorphisme. Je n'ai pas non plus trouvé d'indices montrant que ces gènes ne seraient pas exprimés, ce qui pourrait donc faire de ces séquences de bons outils pour l'étude des bases génétiques du choix du partenaire chez la mouette tridactyle.

C. Rôles de l'odorat chez les oiseaux

Les paragraphes 1 et 2 font partie d'une proposition de revue (co-signée par moi-même, Sarah Leclaire et Étienne Danchin) que nous souhaitons soumettre bientôt.

1. Les oiseaux ont un sens de l'odorat développé

Bien qu'il était autrefois admis que l'odorat était faiblement développé chez les oiseaux (Prosser 1950, Warden, et al. 1936), les études montrant une **reconnaissance des odeurs chez les oiseaux** sont, en particulier depuis la revue de Roper (1999), de plus en plus nombreuses et touchent de plus en plus de familles. Chez les poulets, les récepteurs olfactifs ont été caractérisés moléculairement (Leibovici, et al. 1996, Nef, et al. 1996) et le bulbe olfactif semble bien développé chez de nombreux oiseaux (Bang & Cobb 1968), ce qui indique que les oiseaux ont un appareil olfactif fonctionnel. Le bulbe olfactif est plus développé chez les espèces nocturnes que chez les espèces diurnes (Healy & Guilford 1990), et des espèces aussi différentes que la caille (Frings & Boyd 1952), certains passeraux (Mäntylä, et al. 2004) ou certains Procellaridés (Cunningham, et al. 2003, Nevitt, et al. 2004) semblent utiliser l'odorat pour trouver leur nourriture. On a démontré expérimentalement que les mésanges utilisaient des plantes aromatiques pour construire leur nid (Petit, et al. 2002), et qu'elles étaient effectivement capables de reconnaître l'odeur de la lavande (Mennerat, et al. 2005). Les pigeons migrateurs utilisent également les variations de concentration atmosphérique en gaz pour s'orienter (Wallraff 2004). Les pétrels reconnaissent leur nid à l'odeur (Bonadonna, et al. 2003), les stariques cristatelles préfèrent l'odeurs de leurs congénères à celles d'autres espèces (Hagelin, et al. 2003) et les prions de la désolation reconnaissent leur partenaire à l'odeur (Bonadonna & Nevitt 2004).

Les oiseaux possèderaient donc un odorat développé, et les odeurs pourraient influencer leurs choix. L'olfaction pourrait donc avoir des conséquences écologiques importantes dans des domaines aussi variés que la reconnaissance des apparentés, le choix du partenaire, la recherche de nourriture, l'orientation géographique ou le choix du site de reproduction. Ces études ne concernent malheureusement qu'un nombre limité d'espèces, et les aptitudes olfactives de la majeure partie des oiseaux sont encore très largement méconnues.

2. L'origine des odeurs corporelles chez les oiseaux

De nombreuses études récentes ont mis en évidence l'importance des odeurs corporelles dans la reconnaissance des congénères et des partenaires de reproduction chez différentes espèces d'oiseaux. Pour comprendre l'usage des odeurs individuelles dans ce contexte, il faudrait aussi étudier l'origine des odeurs corporelles chez les oiseaux. L'une des sources odorifères pourrait être les sécrétions de la glande uropygiales, qui sont étalées par les oiseaux sur leur plumage afin d'en augmenter l'imperméabilité. Ces sécrétions contiennent des composés volatiles dont la concentration varie au cours de la saison (Soini, et al. 2006) et qui pourraient être sous le contrôle des hormones sexuelles (Bohnet, et al. 1991). Ces sécrétions sont par ailleurs utilisées par des mammifères prédateurs d'oiseaux afin de détecter leurs proies (Reneerkens, et al. 2005), ce qui semble confirmer que les sécrétions uropygiales pourraient participer à l'odeur corporelle des oiseaux.

Chez les mammifères, on a suggéré que les odeurs individuelles étaient dues aux interactions entre les molécules du CMH, des peptides volatiles et la faune bactérienne de la peau (Boehm & Zufall 2006, Yamazaki, et al. 1999). De nombreux mammifères (dont l'homme) semblent ainsi s'apparier suivant des odeurs liées au CMH (e.g. Penn & Potts 1998a, Roberts & Gosling 2003, Wedekind & Penn 2000). On a également décrit des appariements selon le CMH chez différents oiseaux comme la fauvette des Seychelles (Richardson, et al. 2005), ou le moineau domestique (Bonneaud, et al. 2006), ce qui suggère que les oiseaux seraient capable d'évaluer le génotype de leurs congénères. Chez les oiseaux comme chez les mammifères, on pourrait donc imaginer que des molécules liées au CMH, déposées sur les plumes lors de l'étalement des sécrétions uropygiales, pourrait être responsables de variations d'odeurs corporelles, ce qui pourrait ainsi permettre d'évaluer le génotype des individus. L'étude des corrélations entre le CMH, les odeurs corporelles et la composition chimique des sécrétions uropygiales pourrait créer un nouveau champ d'investigations dans le contexte de la sélection sexuelle, et pourrait permettre de comprendre l'origine évolutive de l'odeur individuelle chez les oiseaux et les vertébrés en général.

3. Qu'en est-il chez la mouette tridactyle ?

Il n'existe pour l'instant aucune preuve expérimentale montrant que les mouettes, et les Laridés en général, utilisent leur odorat. Les mouettes ont cependant des comportements (épouillage, reniflement des plumes) qui pourraient leur permettre d'enregistrer leur odeur et celles de leurs apparentés, et peut-être d'utiliser ces informations dans la reconnaissance

individuelle. Nous avons commencé pendant la saison 2007 des expériences sur l'usage de l'odorat par les mouettes : nous avons mesuré la réaction des adultes et des poussins à différentes odeurs (vinaigre, musc de mammifère, sécrétions uropygienne de canard, et eau comme contrôle négatif). Nous avons également placé les poussins dans un labyrinthe en Y afin de tester leur capacité à reconnaître l'odeur de leurs parents. Des échantillons de sécrétions uropygiennes ont été prélevés sur différents adultes et poussins tout au long de la saison pour analyser l'évolution de leur composition chimique et leur signature individuelle. Les résultats préliminaires montrent que les adultes réagiraient aux odeurs les plus puissantes (en particulier celle du musc de mammifère) lorsqu'elle est déposée sur leur nid. L'ensemble de ces expériences et leur analyse feront l'objet de la thèse de Sarah Leclaire.

Conclusions et perspectives

J'ai démontré que les mouettes reconnaissaient le cri de leur partenaire, de leurs poussins et que les poussins reconnaissaient également leurs parents grâce au cri. Je n'ai cependant observé aucune réaction au cri des voisins, et le niveau de réaction aux cris des membres de la même famille est globalement plus faible que celui observé dans les espèces phylogénétiquement proches. Ceci pourrait être dû au fait que (i) les adultes comme les poussins restent sur le même nid pendant toute la saison ; à partir du moment où ils sont capables de reconnaître leur partenaire et/ou leurs poussins, ils n'auraient donc plus besoin de réagir à leur cri ; (ii) les mouettes pourraient utiliser d'autres indices pour identifier leurs congénères. La reconnaissance acoustique reste cependant très importante au moment du premier envol, et permet aux poussins de rentrer plus facilement au nid en présence du parent qu'en son absence. Le départ du site d'étude se fait malheureusement trop tôt dans l'année pour pouvoir démontrer expérimentalement que le cri du poussin lors de l'envol du poussin est suffisant pour déclencher une réaction de ses parents.

Il serait également intéressant d'enregistrer précisément ce qui se passe au tout début de la saison, au moment de la formation des couples. Le pic d'activité vocale à cette époque (Wooller 1979) pourrait en effet indiquer que les mouettes utilisent des indices vocaux pour retrouver leur partenaire précédent et/ou sélectionner un nouveau partenaire parmi ceux qu'elles auraient déjà rencontré lors de leurs prospections à la fin de la dernière saison. De plus, comme le long call évolue d'une année sur l'autre, cette intense activité vocale pourrait également permettre aux individus d'enregistrer ces modifications.

Les mouettes sont appariées avec des individus génétiquement différents, ce qui pourrait leur permettre de diminuer les coûts de l'homozygotie des poussins. En effet, les couples formés d'individus génétiquement semblables ont un succès de reproduction plus faible, et les poussins homozygotes grandissent moins vite et ont plus de chance de mourir avant 25 jours. Par ailleurs, les couples génétiquement semblables semblent également s'investir moins dans la reproduction, puisqu'ils copulent moins souvent. Cependant, ces couples copulent et se reproduisent, pondant parfois des œufs et les incubant. Etant donné les effets négatifs de l'appariement avec des individus génétiquement semblables, on pourrait s'attendre à ce que de tels couples évitent complètement de se reproduire, et commencent à chercher un nouveau partenaire le plus tôt possible. Il est tout à fait possible que les couples les plus semblables génétiquement adoptent une telle stratégie : dans ce cas, ils n'apparaîtront jamais dans mes

données, puisqu'ils ne formeront jamais un couple établi. Il pourrait donc exister un seuil de similarité génétique en deçà duquel les couples ne se reproduisent pas du tout (voire ne se forment pas non plus).

Les indices vocaux (définis dans l'[Article 1]) ne sont apparemment pas corrélés à la génétique, et il est donc peu probable qu'ils influencent le choix du partenaire de façon importante. Ce choix pourrait donc se baser sur d'autres indices, olfactifs par exemple. En effet, les souris comme les êtres humains choisissent leur partenaire suivant certaines odeurs définies par le génotype CMH (Penn & Potts 1998b, Wedekind, et al. 1995, Yamazaki, et al. 1990), et les Procellaridés utilisent également l'odeur dans l'identification individuelle (Bonadonna & Nevitt 2004). Or, nous sommes à présent capables de génotyper le CMH de Classe II chez les mouettes tridactyles. Il serait donc particulièrement intéressant de voir si ces gènes présentent les mêmes tendances que les microsatellites, pour ce qui est de l'appariement des individus. Si c'est le cas, l'odeur pourrait potentiellement avoir une importance chez cette espèce, et pourrait être un indicateur du génotype.

J'ai montré des différences importantes, tant comportementales que génétiques, entre les populations de l'Atlantique et celles du Pacifique. Dans l'Atlantique (et notamment au Cap Sizun, France), les mouettes sont appariées selon la longueur du tarse, et il existe un dimorphisme sexuel important des paramètres fréquentiels du long call (population d'Hornøya, Norvège, cf. Aubin, et al. 2007). Ces deux résultats n'ont pas été retrouvés à Middleton, et il pourrait donc exister des différences importantes dans les comportements sexuels entre les mouettes Atlantique et Pacifique. Par ailleurs, les différences génétiques entre les deux populations sont évidentes lorsqu'on regarde les fréquences alléliques des microsatellites (McCoy, et al. 2005), mais nettement moins lorsque l'on regarde les séquences CMH. Ceci pourrait indiquer que les comportements et les cris peuvent évoluer plus rapidement que la génétique, ce qui est consistant avec les modèles courants de spéciation en allopatrie (e.g., Edwards, et al. 2005). Enfin, comme les mouettes sont présentes sur l'ensemble du pourtour de l'océan Arctique, il pourrait exister un gradient autour du pôle Nord dans les différences enregistrées sur les paramètres de long calls (ce qui a par ailleurs été démontré pour certains paramètres morphologiques, comme la répartition des taches noires à l'extrémité des ailes, Chardine 2002). Il pourrait également au contraire y avoir des différences marquées entre certaines populations très proches géographiquement. Ce type d'études pourrait permettre de mieux comprendre l'histoire évolutive des mouettes tridactyles.

Dans cette optique, le fait que les mouettes de l'Atlantique ont une variabilité génétique plus faible à la fois aux microsatellites et au CMH pourrait indiquer que (i) les populations de

l'Atlantique proviendraient du Pacifique, ou (ii) que les populations de l'Atlantique ont subi une goulot d'étranglement, c'est à dire un épisode de diminution drastique de leur taille ce qui aurait pu affecter leur diversité génétique. Cette dernière hypothèse est apparemment confirmée par les études historiques : ces populations étaient effectivement beaucoup plus réduites à la fin du XIXème et au début du XXème siècle qu'aujourd'hui, Coulson 1983, Coulson & Thomas 1985, Lloyd, et al. 1991). Ces différences de variabilité génétique pourraient également affecter les mécanismes du choix du partenaire et les taux de paternité hors couple chez ces populations (Cohen & Dearborn 2004, Kokko & Ots 2006, Krokene & Lifjeld 2000). En effet, on pourrait imaginer que, dans les populations à faible variabilité génétique, les individus préfèrent les individus génétiquement proches, car (i) les coûts liés à la destruction d'associations d'allèles adaptés localement seraient plus importants, et (ii) il est beaucoup plus facile de trouver des individus génétiquement semblables que des individus très différents dans une population peu variable, et donc choisir un tel partenaire permettrait de limiter les coûts liés à la recherche d'un partenaire adéquat.

La monogamie génétique semble donc avoir des implications profondes sur le choix du partenaire et la reconnaissance individuelle, mais ces deux facteurs ne sont sans doute pas les seuls affectés. Par exemple, on peut supposer que les mécanismes de dispersion sont également modifiés : en effet, comme les mouettes sont longévives et utilisent souvent le même nid pendant de nombreuses années, il est sans doute difficile pour les jeunes oiseaux de trouver un nid où s'installer, notamment dans une colonie aussi saturée que la tour de Middleton, où la survie adulte est particulièrement élevée (Hatch, et al. 1993) et le nombre de nids potentiels limités.

SYNTHESIS

(English version)

Introduction

A. General introduction

Despite Darwin's (1871) masterpiece and the description of the "**runaway process**" by Fisher (Fisher 1915, Fisher 1930), **sexual selection** has only started to be the focus of behavioral studies in the 1960's, and especially after Trivers' (1972) paper and the demonstration by Lande (1981) of the reality of the Fisherian process. Due to **anisogamy** (i.e., the fertilization of a large female gamete by a small male gamete), females in most taxa invest more in a single offspring than males. This difference in energy allocation between the sexes is often increased by differences in parental investment (Trivers 1972), and particularly in **parental care**. In many species, as for instance in most mammals, offspring are raised by the female parent alone, the male parent investing only gametes in his progeny. In such circumstances, male reproductive success is mainly limited by the number of sexual partners, while that of female is limited by the number of offspring she can produce. This implies that at any time, there are usually much more sperm than ovules to be fertilized. Females are therefore a limited resource for males, for which males should compete. Thus, males are expected to invest in male-male competition and in the production of **costly secondary sexual traits** in order to "attract" and get access to females, while females are expected to use such traits to **choose their mate**. According to the **"handicap" hypothesis,** only traits that are costly can be reliable clues of male quality, since only high quality males can produce them. The association between the preference of females for such exaggerated traits and the production of these costly traits by high quality males may explain **sexual dimorphism** and the "runaway process": if females show a preference for males with the most exaggerated traits, then males that produce such traits are favored, even if their survival is diminished by the extravagance of the preferred male trait. In extreme cases, intense sexual selection may even lead to the **extinction** of local populations (Doherty, et al. 2003).

As male's fitness is only limited by the number of females he gets, **polygyny** seems, at least for males, the best mating system. From the female point of view, various benefits of **polyandry** have also been found. Indeed, females may get **direct benefits** from their **extra-pair mates**, such as increased fertility (Baker, et al. 2001). They may also acquire **indirect benefits**, in the form of an increased fitness of their extra-pair offspring. For example, by mating with an extra-pair individual genetically more dissimilar than their social mate, females may increase the heterozygosity and the fitness of their offspring (Foerster, et al.

Box 1 : Potential causes of social monogamy.
Monogamy has always been intensively studied, perhaps because social monogamy is often considered as the "natural" mating system in humans. Different hypotheses have been proposed to explain the evolution of this system (adapted here from Danchin, et al. 2005):

1) Ecological constraints on males. If the number of partners for a male depends on the quantity of resources he controls, then below a certain level of controlled resources, males cannot have more than one partner. However, this hypothesis was not very well supported in birds: for example, Veiga (1992) did not find any correlation between the mating success of male house sparrows (*Passer domesticus*) and the number of nests they defend.
2) Importance of paternal care. If the reproductive success depends on a tight collaboration between parents, then fathers should not engage in extra-pair copulations, as they will not be able to interact enough with this other female to raise extra-offspring. If the importance of paternal care may be obvious in some taxa (e.g., in most birds), this explanation is not universal: for example, the antelope dik-dik (*Madoqua kirki*, Komers 1996) is monogamous despite the fact that males do not provide any paternal care.
3) Territoriality. For territorial species, monogamy may be a cooperative strategy enabling the defence of a common territory (Mattews 2002).
4) Reproductive synchrony. In some species, all females are fertile within a limited period of time thus limiting the number of females males can fertilize.
5) Female vulnerability to predation and infanticide. Males of monogamous species may not provide direct parental care, but may be vigilant and defend females from predators and from harassment by other males (Clutton-Brock 1989).
6) Female control. In some species, paired females are more aggressive to other females entering in the pair territory (Cézilly, et al. 2000b). This mechanism may prevent males to get extra-pair partners.

2003, Freeman-Gallant, et al. 2003). This is supported by evidence such as the fact that extra-pair bluethroats offspring (*Luscinia svecica*) have a higher immune response than their within pair half siblings (Johnsen, et al. 2000). However, such an indirect benefit has not been identified in a large number of species (e.g reed bunting *Emberiza shoeniclus*, Kleven & Lifjeld 2004).

Until the early 1980s, **monogamy** was seen as the general rule in birds. Indeed, in most bird species, individuals remain as a pair until the end of the breeding season, and parental care is more or less equally shared by parents. Given the potential benefits of **promiscuity**, various hypotheses have been proposed to explain this unexpected pattern (see Box 1), but the development of genetics in behavioral ecology in the 1980's has substantively modified this view. Indeed, **genetically monogamous** birds (i.e. species with no extra pair offspring) are scarce (Griffith, et al. 2002) and around 90% of the socially monogamous bird species show various levels of extra-pair paternity as well as egg dumping (a form of nest parasitism that

could be seen as "extra-pair maternity"). In effect, the **mating systems** of many socially monogamous species are more akin to a promiscuous mating system than to monogamy. A case study is razorbills (*Alca torda*), which was described as typically monogamous with high inter-annual fidelity, but which shows a genetic mating system close to a lek, with females and males actively seeking extra-pair partners (Richard H. Wagner coined this the "**hidden lek**" hypothesis because it is as if a promiscuous genetic system was hidden behind social monogamy, Wagner 1997).

However, some species remain genetically monogamous. It is the case of **Black-legged kittiwakes** (*Rissa tridactyla*), the study species of this thesis. Despite the numerous possibilities of extra-pair copulations - kittiwakes breed in high density colonies, a fact that may increase extra-pair fertilizations (Morton, et al. 1990) – no evidence of extra-pair fertilizations has been found (Helfenstein, et al. 2004c). Such strict monogamy may have important implications on kittiwake behaviors (see Figure 0-1), some of them being the focus of this thesis.

First, social monogamy and high inter-annual mate fidelity (Coulson 1966, Coulson & Thomas 1980, Fairweather & Coulson 1995) raise the question of **individual recognition**. Individuals need to be able to recognize their mate over several reproductive seasons and when divorcing, they should also be able to recognize other individuals in order to reduce the period of selection of another partner. The extent of individual recognition in kittiwakes is therefore the main topic of the first part, where I study mate-mate, parent-offspring and neighbor recognition.

The absence of extra-pair fertilizations also stresses the importance of mate choice, because this choice affects the success of several subsequent reproductive events. However, this species does not show any strong sexual dimorphism, making sexual assessment sometimes difficult to humans (Jodice, et al. 2000) so that exaggerated sexual secondary traits do not exist in that species. Furthermore, females do not seem to trade copulations for food (Kempenaers, et al. 2006), so mate choice may rely on other benefits than only the direct material benefit of courtship food. **Indirect genetic benefits** (i.e., individuals mate in order to increase the genetic quality of their offspring) may thus explain the **mating patterns** in this species. In the second part of this thesis, I study the existent of such genetically based mating patterns. I also study the correlations between copulation rates genetic quality of pairs, and I identify some fitness costs of homozygosity for chicks.

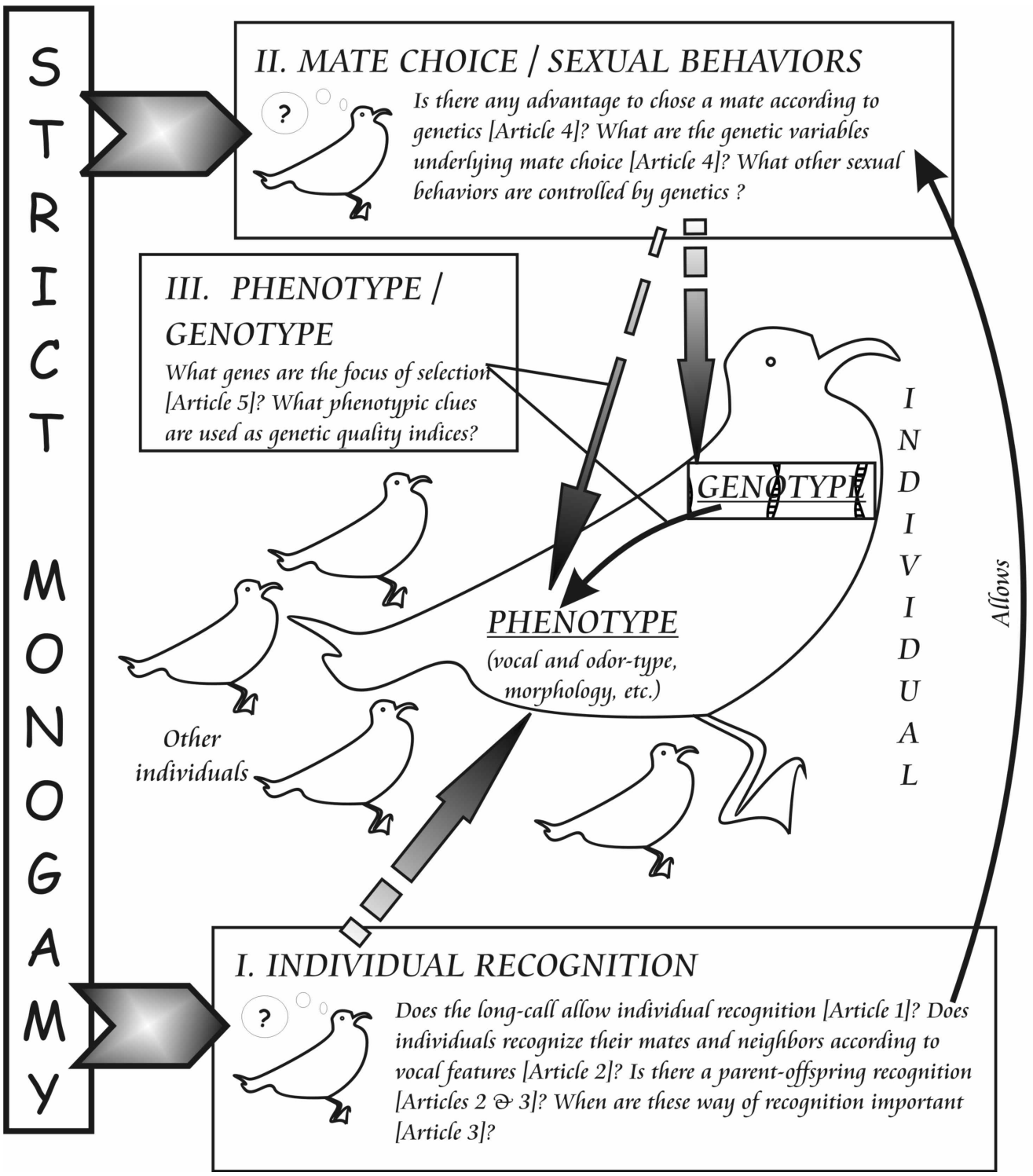


Figure 0-1: Organization of this thesis.
Mate choice implies individual recognition. Under strict monogamy, individual recognition (part I) and mate choice (part II) are expected to be strongly selected (big arrows). I focus on mate choice according to genetics, and I try (part III) to determine by which phenotypic clues individuals may assess their counterparts' genotype.

Identifying a mating pattern is however not sufficient to show the existence of mate choice. Indeed, I show in the second part that genetic similarity seems to be avoided in kittiwakes, but the question of how individuals are able to **assess their mate's genotype** remains unanswered In the third and last part, I try to identify different phenotypic cues that may be used by individuals to assess the genotype of their potential mates.

B. Study species and populations

The black-legged kittiwake is one of the most widely distributed palearctic pelagic seabirds. It winters on the ocean and breeds in **high density colonies** on vertical cliffs, lays eggs in nests built on narrow cliff edges (Baird 1994). It is a long-lived (Cam & Monnat 2000b, Cam, et al. 2003, Coulson 1966, Coulson 1983, Hatch, et al. 1993) birds forming pairs that last for years (Coulson 1966, Coulson & Thomas 1980). In his thesis, Fabrice Helfenstein (Helfenstein 2002) showed that they are genetically monogamous (Helfenstein, et al. 2004c), and that males become **unpredictable** in the timing of their departures and returnsn to the nest at the time of egg-laying (Helfenstein, et al. 2004b). This behavior may be a mate guarding strategy; indeed, male absence duration before egg laying is often shorter than the time needed to achieve a copulation, and the costs of being detected by their mates may explain why females do not engage in extra-pair copulations.

Helfenstein, et al. (2004a) have shown assortative mating by tarsus length in the Cape Sizun population, though no such relationship was found in the Middleton Island population (pers. data). Furthermore, the genetic basis of mate choice has never been studied in Larids. To test for mate choice and recognition, I studied two different populations:

1. Cape Sizun population

The first study population was in Cap Sizun (Brittany, France) and was part of a long term monitoring project that started in 1979 (Cadiou, et al. 1994, Danchin 1987, 1988a and b, Danchin, et al. 1998, Cam & Monnat 2000a, Cam, et al. 2003, Monnat, et al. 1990). From 1979 to 2002, a total of 949 adults and 12,246 chicks were individually marked with a unique code of 4 or 5 color rings. Every year, colonies were visited at least once a week from February to mid June, and then daily until the end of August. This allowed determining dates of egg laying, hatching, first flight and return, and the end of the rearing period. From 1983 to 1985, chicks were closely followed at fledging (by Étienne Danchin); their behaviors, as well as the ones of their parents and other adults in the colony were recorded [Article 3]. From

1995 to 2002, breeders and chicks from a given cliff (named 5Z) were blood sampled. These samples were kept in Tris-EDTA until genetic analyses. From 1999 to 2001, Fabrice Helfenstein followed this same cliff for his thesis and recorded sexual behaviors (mounts, copulations, begging and courtship feedings, see also [5]).

2. Middleton Island population

The second population is located on Middleton Island (north-central Gulf of Alaska, 58°25' N, 146°19' W). This island supports a large but declining colony of Black-legged kittiwakes (from 166,000 birds in 1981, ca 39,000 pairs in 1991, to fewer than 25,000 in 1999, Gill & Hatch 2002, Hatch, et al. 1993). We studied kittiwakes nesting on artificial nests on an abandoned US Air Force radar tower. Nest sites were visited twice a day to assess individual attendance and reproductive success. Every chick was marked at hatching and individually banded when reaching 25 days old. Adults were banded using US metal code and 4 different colored rings. Laying and hatching dates were estimated for all pairs, but annual observations ceased too early to assess fledging dates. Blood samples were taken from as many adults as possible, focusing on mated individuals, and kept in Longmire Buffer (Longmire, et al. 1988).

From 2003 to 2006, another manipulation caused perturbations that might have triggered divorces and re-matings in that colony. Thus, I only studied mating patterns in 2003 and 2004, as later data for mate choice were unreliable. In 2005, I also blood sampled the chicks of un-manipulated pairs at hatching, and followed their growth until 25 days old in order to see if homozygosity is costly for chicks.

C. Sound experiments

Long-calls (Danchin 1987, Tinbergen 1959) were recorded from individuals either during landing or while resting on the nest. When flapping their wings, 25 days old chicks utter a call comparable to the long-call of adults, that I also recorded. A AKG D770 microphone, connected to a Marantz PMD670 recorder, was placed directly on the nest. Thanks to the configuration of the tower on Middleton Island colony, calls were recorded from less than 30 cm away. Play-backs were broadcasted with a Marantz MA6100 and Audax AP080M4 loudspeakers. Sounds were modified using the CTWave32 software (Creative Technology Ltd) and analysed with SAS Lab Pro (Avisoft).

D. Genetic analyses

Genetic methods are fully described in [Article 4]. In summary, I used ten microsatellite loci (K6, K16, K31, K32, K67, K71 Tirard, et al. 2002; and RBG20, RBG27, RBG29 and RBG39, Given, et al. 2002) to assess both individual heterozygosity and pair genetic similarity. I used three different indices of heterozygosity, the direct heterozygosity *H*, the standardized heterozygosity *SH* and the internal relatedness *IR* (proposed by Amos, et al. 2001), and two indices of genetic similarity, the Queller and Goodnight index (Queller & Goodnight 1989), and the Phm index (for "probability of producing homozygous offspring", athat I proposed here and that may have interesting properties for further studies, see [Article 4]). To analyze mating patterns, I simulated artificial re-pairing of the observed birds, in order to have an estimation of the mean genetic similarity of pairs under the hypothesis of random mating. This estimation was then compared to the genetic similarity of observed pairs.

MHC-ClassII-B alleles were amplified using Pen1/Pen4 (Tsuda, et al. 2001) and LP1/LP2 (Kikkawa, et al. 2005) primers. These primers allowed us to amplify a section containing the exon 2, the intron 2 and the exon 3 of the MHC-Class II-B DRB1-like locus. I used the MEGA version 4 software (Tamura, et al. 2007) to build phylogenetic trees and analyze the genetic polymorphism of these sequences.

E. Statistics

General statistical analyses were made using SAS® package (©SAS Institute Inc.1999, Cary, NC, USA). When not stated otherwise, all statistical tests were two-tailed. Details about the tests and the procedures used are given in the articles.

I. Vocal recognition in kittiwakes [Articles 1,2,3]

Kittiwakes are strictly monogamous (Helfenstein, et al. 2004c) and usually mate with the same individual for several consecutive years (Coulson 1966). Such fidelity raises the question of how individuals recognize each other at the beginning of each new reproductive season and during the season. As kittiwake pairs usually breed on the same nest in consecutive years, it could be argued that individual recognition is not needed, as long as individuals are able to recognize their nest. However, mate fidelity and nest fidelity may evolve independently (Cézilly, et al. 2000). Furthermore, Fairweather & Coulson (1995) have shown that kittiwakes are more faithful to their mates than to their nests, moving as a pair to a new nesting area if their previous one was destroyed. I also observed prospectors (or 'squatters' Danchin, et al. 1991, Monnat, et al. 1990), that is individuals that visit breeding colonies in which they are not breeders (Cadiou, et al. 1994), moving as a pair from nest to nest. This strongly suggests the existence of an individual recognition system in kittiwakes.

Theoretically, individuals might use a wide range of cues to assess conspecific identity: voice, odors, behavior (for example, squatters usually land silently on nests they visit for the first time, while resident birds call, Danchin 1983, Danchin 1987), or visual cues (wing tip patterns, size and morphology, bill and gape colors, etc.). In the following chapter, I focus on vocal cues, as it is certainly one of the most widespread and well-known system of recognition in birds. Among Larids for example, chicks of laughing gulls (*Larus atricilla*, Beer 1969), black-headed gulls (*L. ridibundus*, Charrier, et al. 2001) and black-billed gulls (*L. bulleri*, Evans 1970) recognize their parents' voices. Black-headed and slender-billed gulls (*L. genei*, Mathevon, et al. 2003) might also recognize other individuals through vocal cues.

In kittiwakes, evidence of vocal recognition is scarce. If Wooller (1978) found recognition between mates in his population, Aubin and collaborators were unable to show any recognition in their population in Hornøya (Unpublished manuscript). Furthermore, studies on parent-offspring recognition are ambiguous. Cullen (1956) showed that adults do not recognize their chicks before 25 days old, and Storey and collaborators (1992) showed that the individual signature inside kittiwake chicks' calls were much weaker than in closely related species such as the herring gull (*L. argentatus*). To my knowledge, parent recognition by chicks has never been studied in kittiwakes. In this part, I will thus discuss some results showing mate-mate and parent-offspring recognition in this species, using experimental methods (play-back experiments) and behavioral observations.

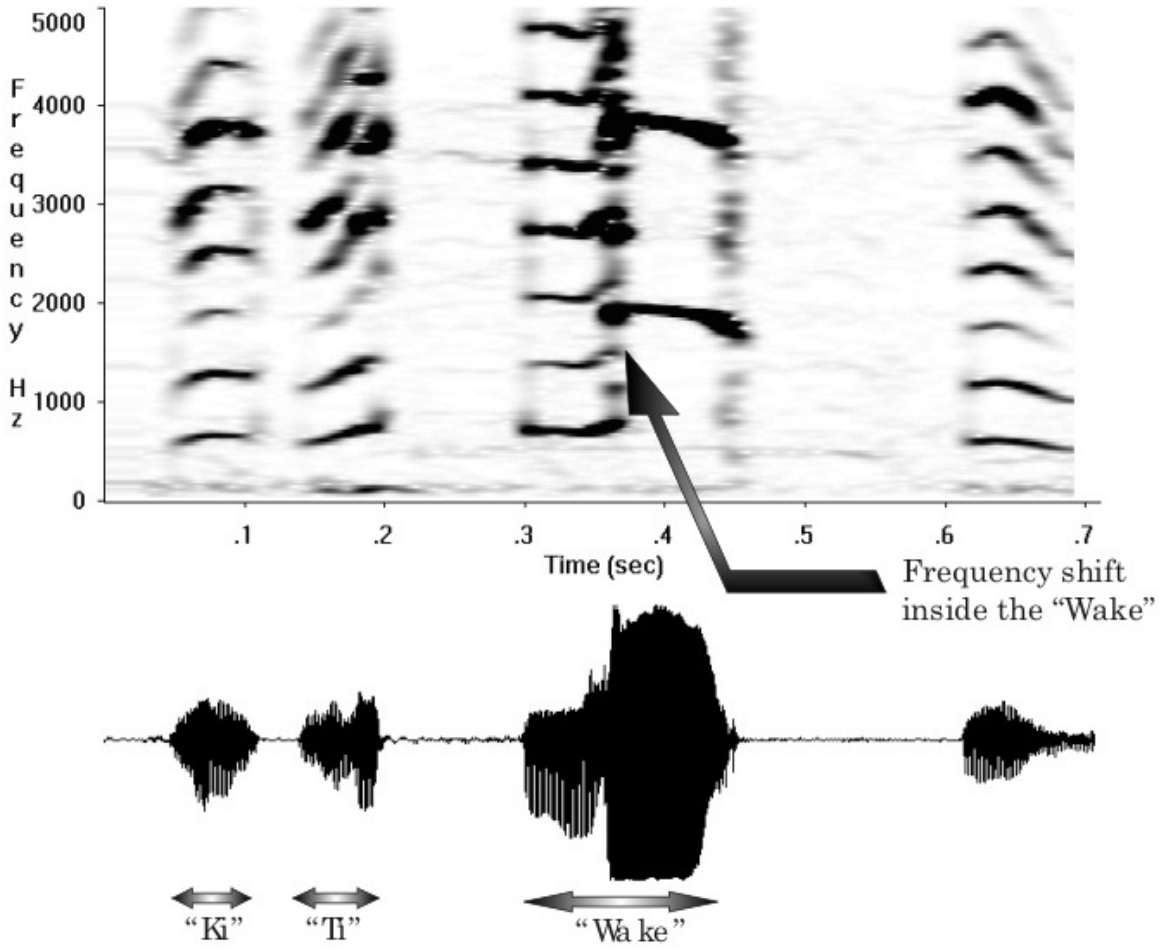


Figure I-1 : Adult kittiwake's long-call
Kittiwake long-calls may be divided in three parts: the "ki", the "ti" and the "wake" parts, the sum of them giving the English vernacular name of the species. For some individuals, the "wake" contains two parts divided by a frequency shift. The upper panel represents the frequency vs. time of an adult's long-call recorded on Middleton Island, the lower panel is the amplitude vs. time of the same long-call. See [Article 1] for the description of the different parameters used to describe long-calls.

A. Individual and geographical signature in the long-call [Article 1]

Among the vocalizations used by kittiwakes, the long-call (Cullen 1956, Danchin 1987, Tinbergen 1953, Wooller 1978, Wooller 1979 see also Figure I-1) is the most frequent and elaborated. Interestingly, it is uttered by incoming birds, and generally the resident bird calls back in response. It is a major component of the greeting ceremony in that species (Danchin 1983, Danchin 1987). It is also often used by birds on the nest, sometimes because

of the presence of squatters harassing them. Since this call is linked to responses from other adults, it may convey an individual signature that may be used by others to assess identity.

In article 1, I tested whether the long-call might convey identity cues in the Middleton population. I found that all the parameters (21 variables defined in Aubin, et al. 2007) used in the analyses vary more between than within individuals, confirming that these variables may be used in individual assignment (see table 1 in [Article 1]). Furthermore, discriminant analyses on these 21 parameters showed that most of the calls were correctly classified across individuals, confirming previous results (Aubin, et al. 2007). The long-call evolves during the season and from year to year, but the individual signature seems sufficiently stable to allow correct discrimination.

Interestingly, I did not find any variable coding for sex, as had been found in Hornøya (Aubin, et al. 2007). As sex assessing is important (for example in mate choice, male-male or female-female rivalry, etc.), Middleton adults are thus expected to have developed other means than vocal cues to assess sex, and sexual behavior between Middleton and Atlantic populations such as Hornøya may differ. Moreover, most vocal variables vary between the two populations, showing that the long-call may convey not only individuality but also geographic origin (see [Article 1] table 3). It has long been discussed whether Pacific and Atlantic kittiwakes should be seen as two subspecies. Indeed, Pacific kittiwakes are larger, have longer culmens than Atlantic kittiwakes and display different wingtip patterns (Chardine 2002, Sluys 1982), but these morphological features are not always sufficient to distinguish them, as there is important overlap. Recently, it has also been found that these two groups differ genetically (McCoy, et al. 2005) according to microsatellite allele frequency. However, I did not find such strong geographical signature in the MHC sequences [Article 5], indicating that the two populations may be not yet completely genetically divergent, and that Atlantic populations, since they show a lower genetic variability, may either: (i) have a Pacific origin, or (ii) have suffered from a bottleneck in their history, that may have diminished their variability. Indeed, Atlantic populations were much smaller at the end of the 19th century and the beginning of the 20th than today, probably because of human exploitation (Coulson 1983, Coulson & Thomas 1985, Lloyd, et al. 1991). The great differences reported in [Article 1] show that calls may evolve faster than genes, even in a species where there is still no evidence of song learning. Evolution of the call might therefore be an important driving force of speciation in this group as it has been suggested in birds in general (Edwards, et al. 2005).

B. Mate recognition according to the long-call [Article 2]

In order to test for mate recognition in kittiwakes, I played-back their mate's calls to resident adults. In 2005, a preliminary analysis showed that the call needs to contain at least 6 repetitions of the "ki-tti-wake" to elicit a full reaction (Figure I-2). Thus play-backed only calls containing 10 repetitions in 2006 (either unmodified or containing only the "ki-ti" or the "wake" part), in order to optimize the focal bird's reactions. Consequently, the reaction percent reported in article 2 (32%) is similar to the total proportion of reacting birds in 2005 (Figure I-2), indicating that I probably reach the optimal response rate.

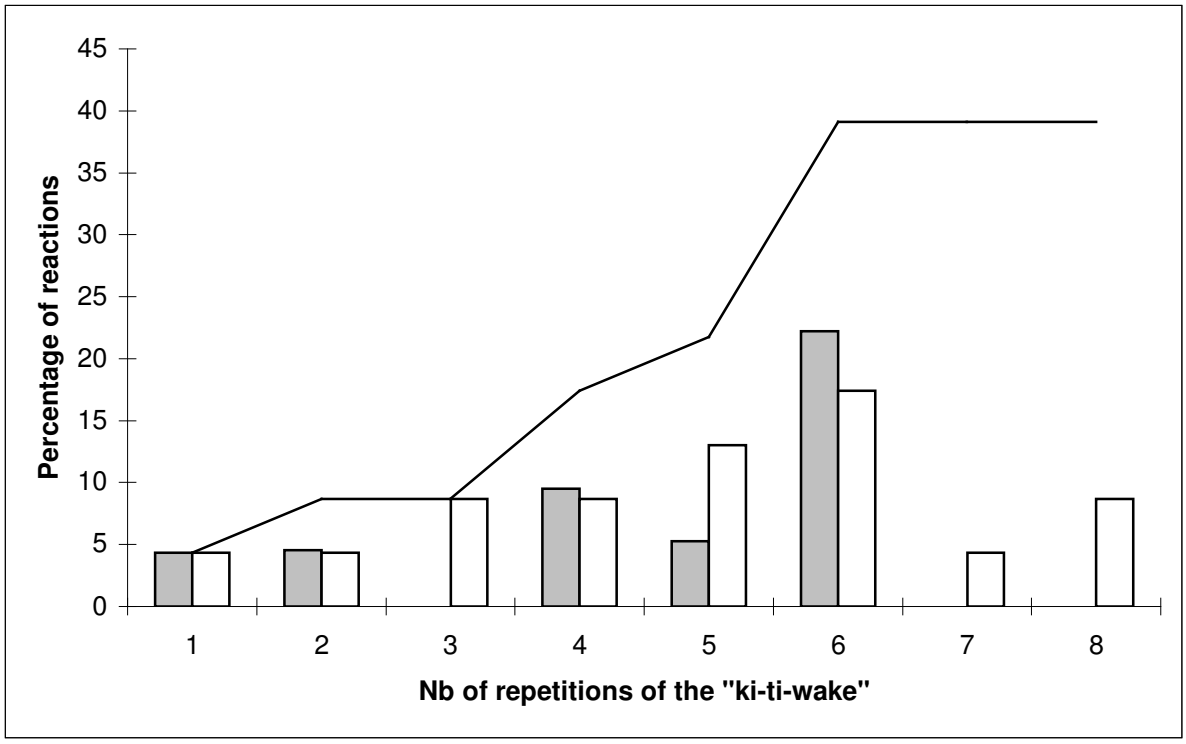


Figure I-2 : Number of repetitions of the call needed to elicit a response from the mate.
Only clear responses (either important head movements or call back) are recorded here. The line represents the cumulative percentage of individuals having reacted. Grey bars are the percentage of individuals that reacted for the first time at this number of repetitions, among the individuals that had not reacted for lower numbers of repetitions yet. White bars are the percentage of individuals reacting to this number of repetitions. In this preliminary study, the same bird was used several time starting with a low number of repetitions and then increasing this number, thus the decrease in reaction after 6 repetitions is probably due to habituation: birds that heard 7 or 8 repetitions of the calls had already heard play-backs with a lower number of repetitions, and may have learned to discriminate played-back calls from natural ones.

Adults reacted significantly more to their mate's calls than to those of any other individuals (unknown, neighbor or own call, see figure 1 in [Article 2]), confirming that adults do recognize their mate's voice (Wooller 1978). Furthermore, some adults reacted to their mate when only a part (either the "ki-ti" or the "wake") of the call was played-back, indicating that the long-call may convey redundant cues, only a part of the call being sufficient to allow recognition. As birds that reacted to the "ki-ti" were not the same as the ones that reacted to the "wake", it may be that the position of the individual signature within the long-call varies among individuals. This interpretation is supported by the fact that, in a discriminant analysis on the 21 variables describing the long-call, no variable ([Article 1]) played a prominent role in the discrimination power of the analysis. In particular, parameters coming from different parts of the call similarly affect individual assignment.

I was unable to detect any recognition among neighbors. I tried various settings, such as play-backing the call of an adult from the focal site or from another nest site while this adult was either present or absent, or when the nest was occupied by another adult. In none of these settings did I detect any significant difference in reaction to the play-backs. As I discuss in article 2, it may be mainly because in a natural situation adults do not need to react to their neighbor's call. This would mean that they recognize them but do not react as there is no information in a neighbor calling from its usual site. To the contrary, they are expected to react to prospectors landing in the neighborhood, because squatters may disturb the colony, sometimes resulting in reproductive failure (for instance when chicks are pushed off the nest during the frantic interactions squatters often trigger). They can use behavioral cues to distinguish prospectors from residents, as prospectors display specific behavior (the awkward posture, Danchin 1983, Danchin 1987), landing silently and keeping their wings ready to fly away. Neighbors are indeed often seen chasing squatters, indicating that they do recognize them as non-local birds. But they would not necessarily need to react every time a resident neighbor is landing on its nest. Recurrent observations suggest that adults are likely to recognize - by behavior if not by voice - most of their neighbors, but react more often to their mate's calls, maybe because of the difficulties to land on a narrow ledge near the mate. This can certainly diminish costs resulting from misrecognition, such as misdirected aggressiveness, risk of pushing chicks or eggs off the nest during hazardous landings, energy spent in unnecessary flights, etc.

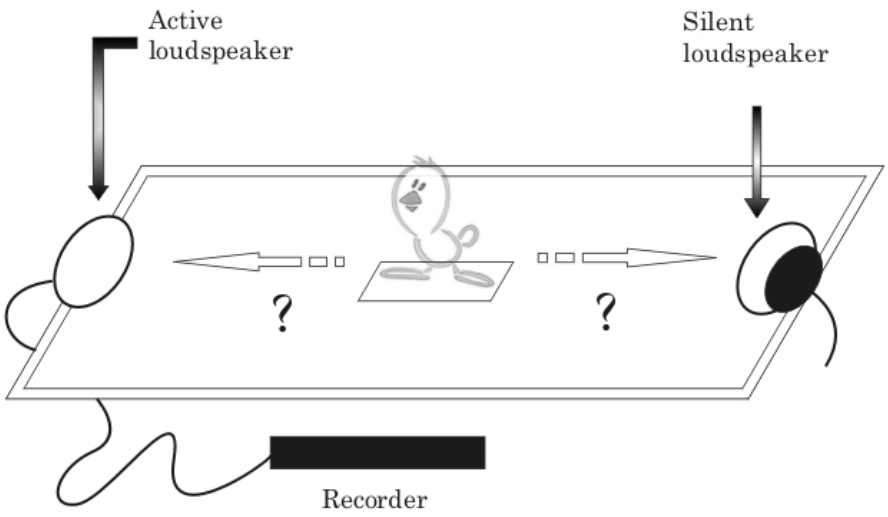


Figure I-3 : Parents' recognition by chicks – The "table experiment".
Chicks were tested every 5 days from 20 to 30 days old. Working loudspeaker and sequence of test calls were random. I recorded chick movements and calls in reaction to the play-backs. They reacted significantly more to their parents' calls than those of other adults.

C. Parent-offspring recognition [2,3]

In 2006, I also tested whether chicks can recognize their parents by playing-back adult calls to chicks (see [Article 2]). I tested only chicks from 20 to 30 days old. I chose that period because, (i) younger chicks might be too stressed by the experiment (Figure I-3) as they are not used to be separated from their parents yet (see [Article 3] figure 1 for a curve of parental attendance during chick rearing), and (ii) chicks older than 30 days try to take off from the experimental table.

Chicks reacted significantly more often and intensely to their parents' call, moving toward the broadcasting loudspeaker and calling back in response, than to the calls of other individuals. At 20 days old, already 34% of the chicks reacted more to their parents' voice than to unknown individuals. This proportion then leveled off until 30 days of age. As for adults, chicks reacted to sounds containing only the "ki-ti" or the "wake" part, confirming that these two long-call components convey identity. Chicks are thus able to recognize their parents at least from the age of 20 days, a time when adults are less and less present on the nest (see [Article 3] figure 1); at that time, the ability of chicks to discriminate between

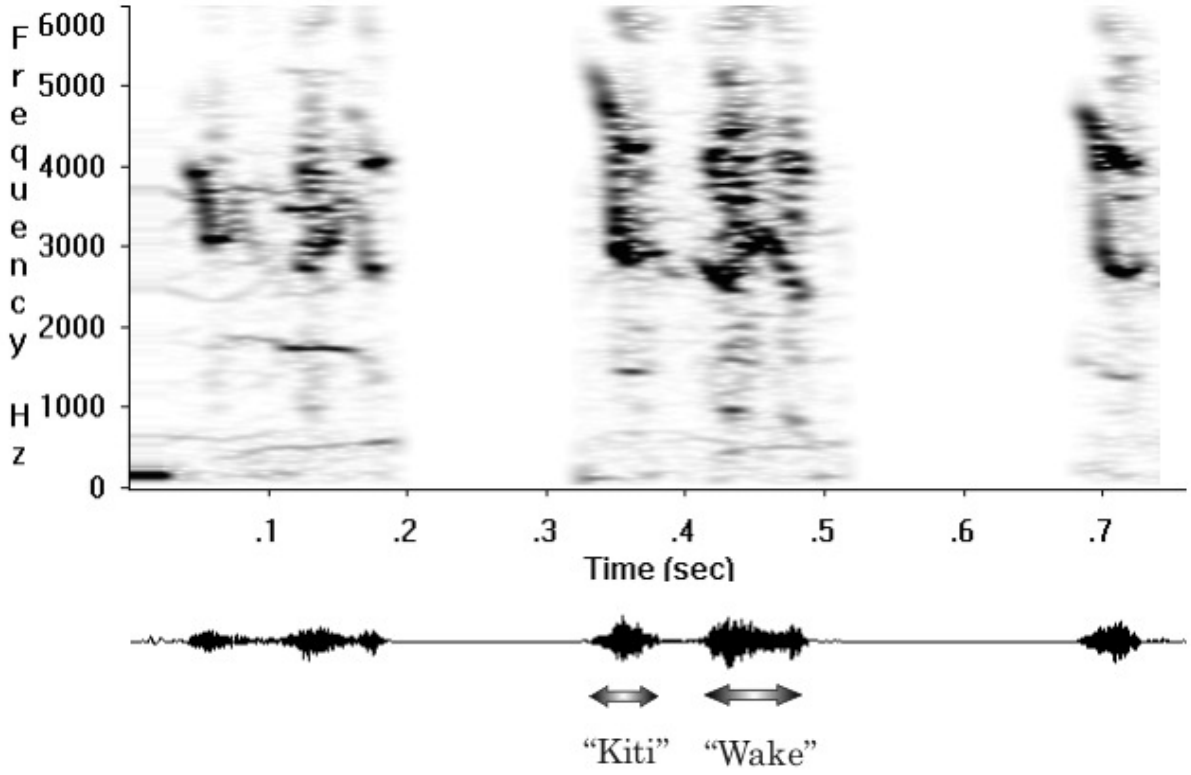


Figure I-4 : "Kittiwake"-like chick's call.
This kind of call, probably derived from the begging sound, is uttered by when chicks wing flap on the nest or when scared. Using the same characters as for adult calls, they can be divided in two parts, one "kiti" and one "wake". Four temporal (duration of the "ki-ti" and the "wake" part, intervals between the "ki-ti" and the "wake" and between the beginning of each call) and four frequency variables (frequency of the maximum of amplitude, first, second and third quartiles of energy distribution) were used to describe such calls.

parents and strangers may already have some importance, as chicks that do not display a dominated posture to landing strangers may be hit and potentially wounded.

Parents are also able to recognize their chicks at fledging. First, using parameters inferred from chick calls (Figure I-4), 70% of the calls can correctly be assigned to a chick, indicating that these calls may convey some information on chick identity. Calls used in this discriminant analyses were recorded before fledging (in fact, between 25 days old and fledging), indicating that parents may be able to recognize their chicks some days before fledging. Second, in the Cape Sizun population (see [Article 3]), parents reacted significantly more than other adults to the call of their fledglings trying to return to their nests. Attending parents reacted in 67% of the cases to their fledglings' call, and called back in 40% of the cases, a rate that is twice higher than the one found for mate and parent recognition by chicks.

This difference is probably due to the fact that the latter data were collected in a natural situation while the others were estimated from artificial experimental settings.

D. Roles of individual recognition

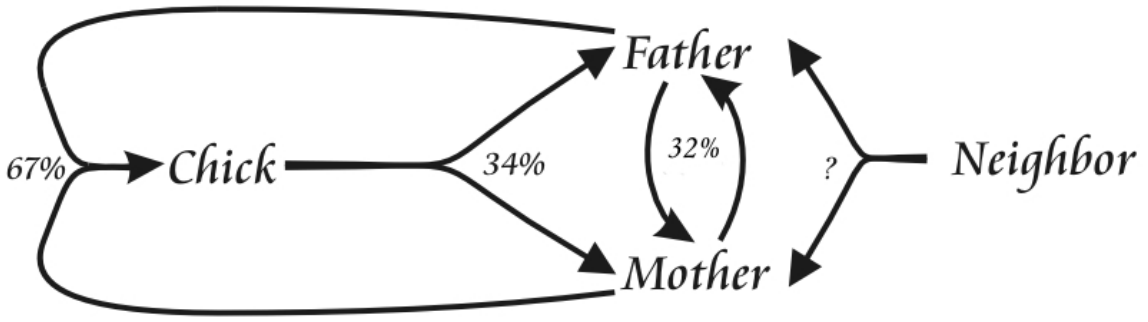


Figure I-5 : Vocal recognition in the Black-legged kittiwakes.
Numbers indicate the percentage of reaction to other individuals' calls. Arrows indicate the direction of the recognition (ex: [34% : Chick -> Father] means chick reacts to its father's calls in 34% of the cases).

In summary (Figure I-5) I have shown that mates are able to recognize each other, as well as parents likely recognize their chicks and vice-versa. However, it is noticeable that all these levels of reaction are lower than those reported in closely related species: for example, Black-headed gull chicks responded in 100% of the cases to parental calls (Charrier, et al. 2001). Such a low level of reaction is not surprising, given the cliff-nesting habits of kittiwakes. Since chicks remains on their nest until fledging with parents feeding them there, and since parents usually breed on the same nesting site for successive years (Coulson 1966), high levels of reaction and recognition are not needed once nest ownership is established. Confirming this view, Storey et al. (1992) showed that the potential of individual coding in the call of kittiwake chicks is much smaller than in the call of the mobile laughing gull chicks, and Mathevon, et al. (2003) showed a similar pattern when comparing the calls of nidicolous black-headed gull chicks to the calls of the mobile crèche-forming slender-billed gull chicks.

However, recognition may still have an important role during at least two events in the life-history of kittiwakes: at fledging and during pair formation.

1. During fledglings first flight [Article 3]

One example of the importance of individual recognition is at the time of the fledgling's first flight (see [Article 3]). After fledging, juvenile kittiwakes are still fed by their parents for two weeks on average. However, in order to be fed, they need to return to their natal nests, since parents most often do not feed their fledglings outside of the nest. While returning, fledglings seem to have difficulty in locating their nest. They cannot rely on visual cues to locate it because they have never seen it from outside.

The consequence is that during their first flight, fledglings try to land more or less randomly on nests of a given area, hence risking being chased and hurt by resident adults or chicks. This may generate severe costs, and selection should favor parents that help their young at this time because this may affect fitness positively. In article 3 I provide evidence that (*i*) parental attendance increases immediately after the fledging of the first chick, and (*ii*) fledglings' first return is faster and easier when parents are present and react to their fledgling's calls by calling back, thus allowing the juvenile to locate the natal nest.

Parent-offspring recognition thus appears adaptive at the time of first flight, vocal cues being essential. I thus expect the level of acoustic recognition to be highest at that time compared to when chicks are 20 to 30 days old. However unfortunately, I was unable to perform such tests.

2. During mate choice

So far, there is no evidence that pairs remain together during the winter (Coulson & Thomas 1980, cited in Baird 1994), and therefore, mates should find each other again at the beginning of the next reproductive season. Accordingly, Wooller (1979) found a peak in the calling activity at the beginning of the reproductive season, suggesting that vocal recognition may play a role at the time of pair formation. Moreover, since long-calls may change from one year to the next [Article 1], I expect adults to call often at the beginning of the season in order to allow mates and neighbors to update their knowledge.

After widowing or divorcing individuals also need to find a new mate. They may then use information gathered about potential mates in previous breeding seasons (this is a prediction of the "hidden lek" hypothesis). These processes necessarily involve subtle mechanisms of individual recognition.

Given the importance that recognition may have at these two stages, I can expect individuals to have acute capacities of recognition in relation to mates and kin. The results developed above suggest that absence of reaction does not necessarily mean absence of recognition. Several hypotheses may explain the gap between the level of reaction I report and the level of recognition I might expect from the life-history of this species:

5. Our experimental setting was certainly "un-natural". Stressed chicks probably showed low reaction to the play-backs implying that I underestimated levels of reaction. The same explanation, though to a lesser extent, may hold for our playing-back of long-calls to adults. Perhaps breeders learned rapidly that sounds coming from a given spot were not associated with the arrival of their mate. They thus may have learned to ignore them.
6. Individuals may use other cues (such as visual, olfactory or behavioral) for recognition. Then voice might not be sufficient to elicit a response in every circumstance. Other Larids show individual recognition based on other cues: for example, laughing gull chicks recognize their conspecifics through visual cues (Griswold, et al. 1995) though this kind of recognition appears to be secondary relative to vocal recognition, and Procellaridae have been repeatedly reported to recognize the odor of their nests (Bonadonna, et al. 2003, Bonadonna, et al. 2004) and their mates (Bonadonna & Nevitt 2004). Experiments are currently being performed to test whether colors and odors may also convey identity in kittiwakes.
7. Signals of identity might be costly (Tibbetts & Dale 2007), because it is more difficult for a recognizable individual to cheat. For example, an indistinctive chick may get food from its parents but also, in a lower proportion, from non-related adults. In kittiwake, cases of adoption after fledging are scarce but may occur (Helfenstein, et al. 2004c, Roberts & Hatch 1994). Distinctive chicks may be less likely to be adopted and therefore pay the cost of individual signature. This may create constraints diminishing selective pressures on chick's recognition, explaining why kittiwake chick calls are not highly individually distinctive.
8. Reaction may not be needed. Breeders and chicks are confined on the same nest for most of the season, hence, as soon as breeders (or chicks) have learned their way back to the nest, ownership is established and there is no more need to react to the voice of known individuals. It is noticeable that in the kittiwake, pair formation and ownership are essentially based on this landing behavior (Danchin 1987). A way to better study these questions would thus be to perform playbacks early in the season, at the time of pair formation, or during juvenile first flight.

II. Mating pattern in kittiwakes [Article 4]

Mate choice is an important aspect of life-histories of sexual species that greatly influences reproductive success. Females for example may gain benefits from mate choice, such as:

1) **Direct benefits** (reviewed in Andersson 1994). By pairing with a preferred male, a female may increase her fecundity, paternal care for its offspring, access to resources (food and territory) or protection against predation or harassment from other males.
2) Females may also **benefit indirectly** through characters that are only expressed in the next generation. For example, offspring coming from matings with specific males may have a higher fitness (e.g., higher genetic quality, better resistance to parasites, higher viability and competitiveness, etc.) than offspring coming from matings with non-preferred or randomly chosen individuals.

[I took here the example of females, but note that males may also choose their mate, and thus mutual mate choice may occur (Bergstrom & Real 2000, Jones & Hunter 1993).]

These two kinds of benefits are not exclusive. For example, Drickamer, et al. (2000) showed that female mice that pair with preferred males have more litters (direct benefit), and offspring of higher viability and social status (indirect benefits), than females mated with non-preferred males. Through mate choice, individuals may thus increase the fitness of their offspring.

In kittiwakes, no direct benefits of mate choice have yet been identified. For example, males give food to their mate before copulation (courtship feeding behavior, Helfenstein, et al. 2003), a behavior that may be an indication of male quality. However, females that are artificially fed *ad libitum* (and thus that do not need the extra-food provided by males) do not copulate less or more often than unfed females (Kempenaers, et al. 2006), indicating that they do not trade copulations for food. Thus if mate choice occurs, it should result from other benefits than nutritional benefits. In this part, I will show that mate choice may be linked to **indirect genetic benefits**.

Numerous studies have shown the fitness costs of **inbreeding** in the broad sense (reviewed in Hansson & Westerberg 2002), and individuals are therefore expected to mate in order to decrease such costs. Recently, birds have indeed been found to choose each other according to various genetic variables. For example, female blue tits (*Cyanistes caeruleus*)

increase the heterozygosity of their offspring through extra-pair matings (Foerster, et al. 2003). House sparrow (*Passer domesticus*) and savannah sparrow (*Passerculus sandwichensis*) yearlings mate in a way that increases genetic diversity (Bonneaud, et al. 2006, Freeman-Gallant, et al. 2003). Extra-pair fertilizations are more common in Seychelles warblers (*Acrocephalus sechellensis*, Richardson, et al. 2005) and in three species of shorebirds (Blomqvist, et al. 2002) when mates are more genetically similar. Female great snipe (*Gallinago media*) seem to choose males with certain MHC-lineage (Ekblom, et al. 2004). However, such genetically driven mate choice does not seem to be a general feature in birds, as various studies failed to show such correlations (e.g., Westerdahl 2004).

Most species studied so far are genetically polygamous, with widely varying frequencies of extra-pair fertilizations and extra-pair paternity. Extra-pair fertilizations are by definition nonexistent in genetically monogamous species, and mate choice in such species is thus likely to be under higher selection pressure than in species that are only socially monogamous. Furthermore, such selective pressures are likely to be even higher in species where pairs rarely divorce between years. Cohen & Dearborn (2004) published the only study so far about mate choice according to genetics in a genetically monogamous bird. Interestingly, they found a quite unexpected result, with birds preferring genetically similar mates. I wanted to see if such a paradoxical result is a general feature among strictly monogamous birds. Thus I tested:

1) If there is a mating pattern according to genetic variables in kittiwakes.
2) If pairs that behave in accordance to this pattern have higher reproductive success and better offspring than pairs that do not follow the general trend.
3) If the frequency of sexual behavior such as copulations is also related to genetic similarity between mates.

I did not conduct any "mate preference" experiment, and all the data presented here come from direct observations in the wild.

A. Individuals are mated in a way that increase the likelihood of producing heterozygous offspring [Article 4]

In article 4, I used the microsatellites' genotypes obtained from the Middleton population to test if kittiwakes are mated according to one of these three different hypotheses of genetically driven mate choice:

(1) "**Good genes as heterozygosity**" hypothesis (Landry, et al. 2001, Weatherhead, et al. 1999): individuals should favor matings with the most heterozygous

individuals. I should then find a positive correlation between female and male heterozygosity, as only heterozygous males will have access to heterozygous females through sexual competition. Furthermore, I might expect unpaired individuals to be less heterozygous than paired individuals.

(2) "**Genetic similarity avoidance**" hypothesis (Bensch, et al. 1994, Blomqvist, et al. 2002, Brown 1996, Hoffman, et al. 2007): individuals should favor matings with individuals carrying different genotypes than their own in order to produce heterozygous offspring. I should then find that formed pairs are less genetically similar than expected by random matings.

	Phm_{xy}	
	2003	**2004**
Number of Pairs *Number of Adults**	74 241	72 289
All loci	Obs = 0.181 Exp = 0.196 **P = 0.021**	Obs = 0.184 Exp = 0.201 **P = 0.018**
Without OHW loci (these loci are defined in [Article 4])	Obs = 0.162 Exp = 0.184 **P = 0.005**	Obs = 0.174 Exp = 0.191 **P = 0.043**
	$\hat{r}_{xy}$	
All loci	Obs = -0.0297 Exp = 0.0013 P = 0.060	Obs = -0.0299 Exp = -3.75E-5 P = 0.083
Without OHW loci	Obs = -0.037 Exp = 0.00076 **P = 0.048**	Obs = -0.016 Exp = 0.0013 P = 0.25

Table II-1: Differences between observed and simulated means obtained with two relatedness indices: the *Phm* index (probability for a pair to produce homozygous offspring), and the *r* index (of Queller & Goodnight).

For each case, I give the number of pairs used, and the number of genotyped adults alive in the considered year; I made 10,000 bootstraps to calculate the simulated mean and the p-value, each bootstrap consisting on randomly making *P* different pairs using the *A* adults available. In each case, first line is the observed mean in the population (Obs), second line is the simulated mean (Exp). Last line is the p-value calculated as the proportion of bootstraps having a mean lower than the observed mean. Significant p-values are in bold. All analyses were done calculating the genetic similarity indices either over all microsatellite loci, or without the OHW loci (for "out of Hardy-Weinberg equilibrium", see [Article 4] for more details.

* This figure incorporates adults that paired as well as unpaired adults and genotyped adults that did not pair within our study plots.

(3) "**Genetic similarity preference**" hypothesis: individuals should favor matings with individuals carrying similar genotypes than their owns (Cohen & Dearborn 2004, Kokko & Ots, 2006). Mating with very dissimilar individuals might destroy associations between different sets of good alleles that may be locally adapted, and offspring issued from such matings might thus be less competitive than offspring issued from genetically similar matings. I should then find that formed pairs are more similar than expected by random matings.

To estimate the genetic similarity of pairs, I used two different indices, the *r* and the *Phm* indices (see Introduction part D.). I found only evidences for the second hypothesis. In 2003 and 2004, pairs were formed in order to give birth to heterozygous offspring. Results were significant using the *Phm* index and very close to significance with the *r* index (Table II-1). This means that **individuals are apparently mated with genetically dissimilar individuals**, a pattern that may maximize their chance of having heterozygous offspring.

B. Benefits of mating with genetically dissimilar individuals [Article 4]

If kittiwakes are mated in a way that decreases the probability of producing homozygous chicks, I should be able to detect benefits of breeding with genetically dissimilar individuals. I found a negative correlation between hatching success genetic similarity (as calculated by the *Phm* index, see [Article 4] figure 2).

I also found a significant positive **correlation between offspring heterozygosity and offspring fitness components** in 2005. In that year, I was able to bleed and genotype a large number of chicks at hatching (n = 82) and to follow their development for 25 days. I found that the most heterozygous chicks grew faster and had higher survival probabilities until 25 days of age than homozygous offspring (see [Article 4]). Thus, producing heterozygous offspring appears advantageous in this species, and this indirect genetic benefits may explain the observed mating pattern in relation to genetic similarity.

C. Copulations and inbreeding avoidance

So far, results show that kittiwakes are mated in a way that reduces the probability of having homozygous offspring, which may be explained by the fact that genetically similar pairs endure a higher hatching failure and a lower chick survival. However, a substantial number of pairs remain genetically similar. This may be because they did not assess correctly their counterpart's genotype, or simply because the most suitable mates were not available. For such pairs, cost of reproduction may not be balanced by the benefits of producing healthy offspring, and they may thus show behaviors linked to reproduction avoidance.

A preliminary analysis on the behavioral data recorded in Fabrice Helfenstein's thesis (Cape Sizun population, Helfenstein 2002) show that copulation rate is correlated with genetic similarity (as calculated with the *Phm* index, see Figure II-1). Thus **genetically similar pairs copulated less frequently** than genetically dissimilar pairs, a behavior that may be interpreted as a strategy to avoid reproduction with genetically similar individuals.

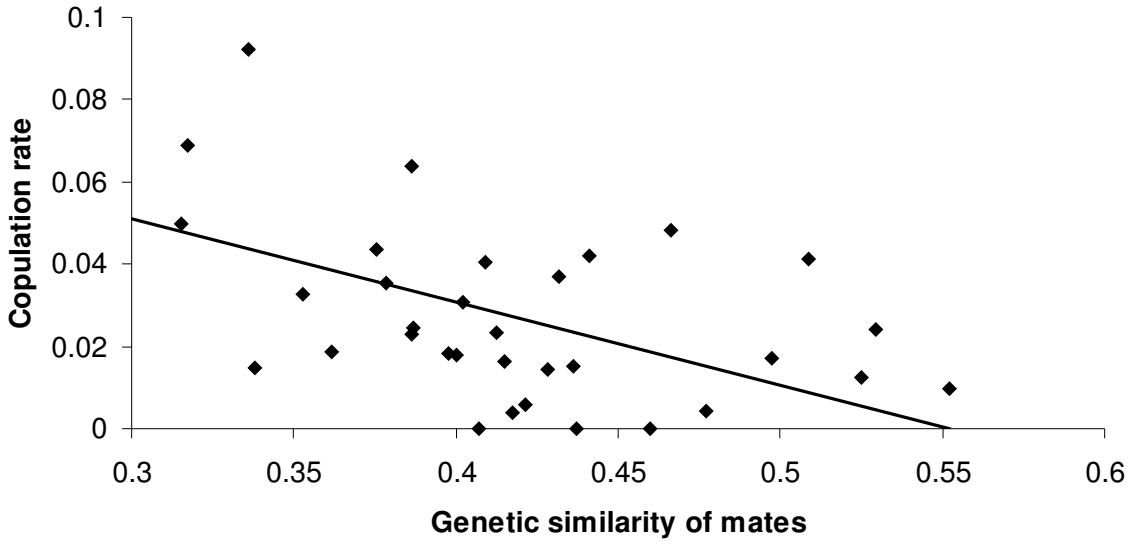


Figure II-1 Relationship between genetic similarity of mates and copulation rates.
For illustration, I show here the copulation rate for each pair pooling data from the many years they were observed. Analyses were made using the rate observed for each year (calculated for a given pair as the number of copulations / number of hours of observation), and including the pair identity as a random parameter (SAS mixed procedure, n = 52, F = 7.67, p = 0.013; for random parameter "pair": z = 1.72, p = 0.04).The protocol used for behavioral observations is described in Helfenstein et al. (2003).

Overall, kittiwakes appear to be mated in a way that diminishes the probability of producing homozygous offspring. This is consistent with the fact that homozygous offspring are of lower quality in that they grow slowly and are more likely to die before reaching the age of 25 days. Such a mating pattern may result from several mechanisms. Mates may select each other on the basis of cues revealing their genetic similarity, or there may be a **passive mechanism.** Assuming that success rate is highly correlated to pair genetic similarity, and failing pairs are more likely to divorce, then, over the long term, individuals may find the most genetically dissimilar mate.

To test the likelihood of such mechanism, I did a simulation model using parameters estimated on Middleton population (such as allelic frequencies, mortality rate, success rate and divorce rates after reproductive success or failure). Each year, unpaired individuals were mated randomly, and I looked at the number of years needed to reach a state with a mating pattern similar to the one observed in 2003 or 2004. In the model, each pair may succeed or fail in their reproduction, and divorce accordingly. Each individual may also die (according to mortality rate), and a new pairs were then formed.

If success is random (that is, genetically similar pairs are as likely to succeed than dissimilar ones), the model never produced a state similar to 2003 or 2004, indicating that a strong correlation between reproductive success and genetic similarity of pairs is needed. Furthermore, even when assuming that only genetically similar pairs fail in their reproduction, the divorce rate after success needs to be close to 0%, or the divorce rate after failure shoud be much higher than observed in order to reach the observed mating pattern after a number of years compatible with kittiwake mortality rates. These results show that such passive mechanism is unlikely to explain the pattern observed in Middleton population, the correlation I observed between genetic similarity and reproductive success being rather weak, and the divorce rates quite low.

Selection should thus favor individuals with **an active preference** for genetically dissimilar mates. Furthermore, the fact that genetically similar pairs behave in a way that may reduce fertilization success (lower copulation rate) could indicate that kittiwakes are indeed able to assess the genotype of their mate and behave accordingly.

I therefore might expect individuals to use phenotypic cues to estimate their relatedness with conspecifics, in order to actively choose the most suitable partner. However, such phenotypic cues remain highly speculative, since little is known about the process of mate choice in kittiwakes, and in seabirds in general.

III. How do individuals assess their mate's genotypes? [Article 5]

Helfenstein, et al. (2004a) previously reported **assortative mating by tarsus length** in the Cape Sizun population. This morphological parameter may thus be a good tool to study the role of sexual secondary trait in mate choice. However, despite intensive capture efforts on Middleton, I did not find any such correlation in that population. Because no mate choice experiment has been undertaken, it is unclear whether this parameter is indeed used in pair formation. Furthermore, I did not find any correlation between tarsus length and individual heterozygosity, indicating that this morphological feature is not likely an estimator of genetic quality in the Middleton population. In this part, I try to tackle the question of the still unknown potential cues of mate choice.

A. Choice according to vocal parameters?

Since kittiwakes are choosing their partners according to genetic components, and since they are also using vocal cues to identify each other, a parsimonious mechanism would be that vocal cues may also signal individual genotype. For example, calls of individuals that are genetically dissimilar individuals may be more different than calls of genetically similar individuals.

To test such hypothesis, I need an estimation of the "vocal distance" between two individuals. I did that by (1) estimating the principal components of 4 randomly chosen calls per individual of the Middleton population, (2) estimating an "average call" for each individual, and (3) calculating the Cartesian distance between the average calls of the two focal individuals. I calculated the vocal distance between all males and females, but I did not find any correlation between this distance and genetic similarity (SAS Mixed procedure with male identity as a random parameter, variables were ranked to reach normality, $F_{1403} = 0.14$, $p = 0.71$). The results were similar if the vocal distance was calculated by giving each principal component a similar weight, or by giving each component a weight similar to its eigen-value.

Thus it appears that vocal parameters may be insufficient to assess genetic similarity in this population of kittiwakes. It is possible that individuals rely on parameters that were not measured here, like the shape of the call in term of frequency modulations, or the attacks, but

such parameters are not easily measured. It is also likely that they use visual or olfactory cues to assess their kinship with potential partners.

The only evidence I know of a correlation between vocal parameters and genetic distance come from studies on song sparrows (*Melanospiza melodia*). Reid, et al. (2005) showed that male repertoire size decreases with its level of inbreeding and with its kinship with female population (Reid 2007) making this vocal parameter a suitable cue for mate choice (Reid, et al. 2004). However, the studied population is likely to be highly inbred (as it is an island population) and therefore males with large repertoire size may just be males coming from other populations that are thus likely to be very genetically dissimilar to females. Furthermore, song sparrows learn their song from their fathers, making the observed pattern much unclear and difficult to apprehend.

B. Choice on odors and MHC genotypes? [Article 5]

Genes of the **Major Histocompatibility Complex** (MHC) are involved in many aspects of immunity, from the recognition of self and non-self to the activation of the cellular and humoral pathways of the immune response. They show the highest known level of polymorphism and their heterozygosity has been repeatedly reported to correlate with better immune capacities (Bonneaud, et al. 2004, McClelland, et al. 2003, Penn, et al. 2002, Wedekind, et al. 2004, Westerdahl, et al. 2005). Consequently, MHC genotypes have been also found to **influence mate choice** in various vertebrates, individuals aiming at enhancing the variability of their progeny at these loci (Bonneaud, et al. 2006, Ekblom, et al. 2004, Landry, et al. 2001, Penn & Potts 1998a, Richardson, et al. 2005, Wedekind & Füri 1997).

In most cases the MHC dependent mating preferences seem disassortative: individuals prefer mates with a different set of MHC alleles than their own (Tregenza & Wedell 2000) or alleles that differ as much as possible in amino acid composition, especially on the peptide binding region (e.g. Landry, et al. 2001). Such a pattern is very similar to what I found with microsatellite loci, and thus it would be of particular interest to look more closely at the MHC alleles to see what pattern of mate choice they show and to confirm the putative links between the two processes.

MHC alleles have been found to **correlate with body odors** in mammals, and especially in mice (Penn & Potts 1998b, Yamazaki, et al. 1990) and in humans (Wedekind, et al. 1995). Body odors have thus been found to be used in mate choice in both species (Penn 2002, Wedekind & Füri 1997). This link between MHC and odors may be widespread in

vertebrates, and might explain the repeated reported links between MHC genotypes and mate choice.

During the course of this thesis, I started the genotyping of MHC Class II-B genes, implied in the response against extra-cellular pathogens, in order to answer two questions:

- do individuals choose their partners according to their MHC genotypes?
- are MHC genotypes linked to odor components?

I am still one step behind answering these questions, as for the moment I only managed to amplify the MHC Class II-B sequences in various seabirds (including kittiwakes) in order to find the most suitable primers to start the genotyping of our population on a bigger scale. In article 5, I publish the sequences and give some insights on the phylogeny and the molecular evolution of these seabirds. It appears that MHC Class II has a much faster evolution rate in Ciconiiforms than in Passeriforms, accounting for most of the phylogenetic problems I encountered. Furthermore, the amplified sequences show evidence of balancing selection, confirming what has been reported on this complex. Such selection makes nonsynonymous substitutions more frequent that synonymous ones (the latter being mostly fixed by drift), and is a signature of genes under pressure for polymorphism enhancing. I also did not find any evidence that these sequences are not expressed in the tissues, and they might therefore be of particular interest in the study of the genetic bases of mate choice in kittiwakes.

C. Roles of olfaction in birds

Paragraphs 1 and 2 are part of a review proposal (co-authored by myself, Sarah Leclaire and Étienne Danchin) that we plan to submit soon.

1. Current evidence that birds can smell

Although it was long considered that the sense of smell is poorly developed in birds (Prosser 1950, Warden, et al. 1936), evidence for **bird olfaction** has been accumulating in various families, particularly since Roper's (1999) review. Olfactory receptors have been identified in chickens (Leibovici, et al. 1996, Nef, et al. 1996) and olfactory bulb size has been found to be well developed in many bird species (Bang & Cobb 1968), suggesting that birds may have all the sensory apparatus to smell. In particular, comparative studies on olfactory bulb size show that the use of smell might be more developed in nocturnal than diurnal species (Healy & Guilford 1990). Furthermore, birds of taxa as varied as quails

(Frings & Boyd 1952), warblers (Mäntylä, et al. 2004) and procellariforms (Cunningham, et al. 2003, Nevitt, et al. 2004) have been suggested to use smell to find food. Passerines such as tits have been experimentally shown to choose aromatic plants to build their nests (Petit, et al. 2002), and to recognize the odor of lavender (Mennerat, et al. 2005). Migratory pigeons have been recurrently demonstrated to use trace gas concentration to navigate (Wallraff 2004). Petrels use their smell to find their burrows (Bonadonna, et al. 2003), crested auklets prefer conspecific than interspecific odors (Hagelin, et al. 2003) and Antarctic prions have been experimentally shown to recognize their mates by smell (Bonadonna & Nevitt 2004).

These studies thus suggest that birds do possess and use smell in many fitness affecting decisions. Bird olfaction is thus probably much more widespread than previously thought, and is likely to have important ecological implications for decision-making in contexts as varied as kin recognition, mate choice, foraging, navigation or breeding habitat choice. However, numerous groups of birds have not been tested yet, and the extent of olfactory abilities in birds remains largely unknown.

2. The origin of body odors in birds

Surprisingly enough, several recent studies have emphasized the importance of individual body odors in conspecific and mate recognition in several bird taxa. The use of individual odors in this context needs to be studied in relation to the origin of body odors. One source of body odors may be preen gland secretions, which are spread by the birds all over the plumage to insure feather waterproofing. These secretions contain volatile compounds that vary seasonally (Soini, et al. 2006) and may be under the control of sexual hormones (Bohnet, et al. 1991). Furthermore, these secretions appear to be used by predatory mammals to detect their bird prey (Reneerkens, et al. 2005). This supports the idea that preen glands may participate to body odor in birds.

In mammals, it has been suggested that individual odors are influenced by interactions among MHC molecules, volatile secreted peptides and bacteria on the skin (Yamazaki, et al. 1999, Boehm & Zufall 2006). For instance, in various mammals (including humans) mating preferences have been found to be influenced by MHC linked body odors (e.g. Penn & Potts 1998a, Roberts & Gosling 2003, Wedekind & Penn 2000). Recently however, individuals of some bird species such as Seychelles warblers (Richardson, et al. 2005), or house sparrows (Bonneaud, et al. 2006) have also been found to choose mates in a way that is non-random relative to the MHC genotype. This suggests that birds can somehow detect the MHC genotype of other individuals and raises the question of how birds evaluate the MHC

genotypes of potential mates. We propose that in birds as in mammals, MHC linked molecules at the surface of the body or put on the feathers with preen wax may be responsible for variations in body odors revealing the individuals' MHC genotype. Studying such questions has the potential to lead to the emergence of a new field in bird biology and more specifically within the context of sexual selection. Future studies on the potential link between MHC, body odors and preen gland chemistry should therefore provide interesting insights on the origin and roles of individual odors in birds and higher vertebrates in general.

3. And in kittiwakes?

So far, there is no evidence for the use of smell in kittiwakes or in any Larid. However, kittiwakes show behavior (preening, sniffing…) that may allow them to record the odors of their kin, and potentially use it in recognition. During the 2007 field season, we started experiments to study smell in kittiwakes: adults and chicks were tested for their reactions to different odors (vinegar, mammal musk, duck preen oil, and water as a negative control). Chicks were also tested for parental odor recognition through a Y-maze. Preen samples were taken for several adults and chicks throughout the season to test for seasonal evolution and individuality of preen components. Preliminary results showed that adults might be able to smell strong odors such as mammal musk when they are spread on the nest. These analyses will be the subject of Sarah Leclaire's PhD thesis.

Conclusions and perspectives

I provide evidence suggesting that kittiwakes recognize their mates, their chicks and their parents according to vocal parameters, but do not react to neighbors' calls. The level of reaction to known calls is overall weaker than in closely related species, maybe because: (i) both adults and chicks are highly constrained to the nest, thus once they can recognize their mate or offspring, reaction from the mate (or the parent) is no longer needed, and (ii) kittiwakes use other cues in identification. Vocal recognition remains important at fledging, leading chicks to return more easily to their natal nest in the presence of their parents. However, I was unable to determine whether vocal cues in chicks calls are sufficient to elicit a response from their parents, as I left the colony too early to make play-backs at the time of first flight.

It would be also interesting to follow what happens at the beginning of the season, during the pair formation period. The peak in calling activity at this time (Wooller 1979) may indicate that kittiwakes use vocal cues to find their previous mate and/or select a new mate they may have already chosen when prospecting at the end of the previous season. Furthermore, as the long-call evolves slightly from one season to the next, this activity peak may also allow re-paired individuals to update their knowledge of their mate's voice.

Kittiwakes appear to be paired with genetically dissimilar individuals, a pattern that may diminish the costs of offspring homozygosity because genetically similar pairs hatched fewer chicks and homozygous chicks grew slower and were more likely to die before fledging. Genetically similar pairs appear also to invest less in their reproduction, copulating less often than genetically dissimilar pairs. However, they still copulate and reproduce, laying eggs and incubating them. Given the highly deleterious effects of mating with a genetically similar individual, I would expect such pairs to avoid breeding overall and start prospecting for new mates as soon as possible. Pairs that are the most genetically similar may perhaps indeed forgo reproductive efforts and search for a partner as soon as possible; as such, they will not be recorded in the data as they would not be seen as an established pair. Thus there may be a level of genetic similarity under which pairs do not attempt to breed, and perhaps do not form at all.

Apparently, vocal cues (as defined in [Article 1]) are unrelated to genetics, and are thus not likely to influence mate choice greatly. Mate choice may therefore be influenced by other cues, such as smell. Indeed, mice and humans have already been shown to choose their mates according to odor-types driven by MHC genotypes (Penn & Potts 1998b, Wedekind, et al. 1995, Yamazaki, et al. 1990), and Procellariiforms have been found to use smell in individual identification (Bonadonna & Nevitt 2004). We are now able to genotype the MHC Class II-B in kittiwakes, and it would therefore be interesting to see whether these genes show the same pattern as microsatellites. If this is the case, then it may be really interesting to look more closely at the use of smell in kittiwakes, in order to see if odors convey genotypic information.

The differences I found between Pacific and Atlantic kittiwakes are also intriguing. In the Atlantic, there was assortative mating by tarsus length (Cape Sizun, France) and sexual dimorphism in frequency parameters in the long-call (Hornøya, Norway, see Aubin, et al. 2007). These two findings were not found in the Pacific population, which may imply important differences in sexual behavior. Such strong geographical differentiation was confirmed with microsatellite studies (McCoy, et al. 2005), but did not appear clearly at MHC sequences. This may indicate that behavioral and vocal components may evolve faster than genes, consistently with previous models of speciation in allopatry (e.g., Edwards, et al. 2005). As kittiwakes are present throughout the Arctic Ocean, it would also be interesting to study whether there is a gradient in long-call differences around the North Pole (following in that what has been found for morphological features such as black patterns on wing tips, Chardine 2002), or whether there is an abrupt shift at some point. This would give insights on the evolutionary history of black-legged kittiwakes.

The fact that Atlantic kittiwakes are less variable in both microsatellites and MHC loci may indicate that (i) the Atlantic population originated from the Pacific, and/or (ii) Atlantic populations have suffered from a bottleneck in their history (and they were indeed low at the end of the 19th century and the beginning of the 20th, Coulson 1983, Coulson & Thomas 1985, Lloyd, et al. 1991). This difference of genetic variability may also influence mate choice and extra-pair paternity in these populations (Cohen & Dearborn 2004, Kokko & Ots 2006, Krokene & Lifjeld 2000). Indeed, in populations that have low genetic variability, individuals may prefer genetically similar individuals, since (i) the costs of destroying beneficial local associations of alleles may be higher, and (ii) they are more likely to find such individuals than in large outbred populations, and may thus reduce the costs linked to the time spent in the search for a suitable mate.

Strict monogamy seems to have an implication on both mate choice and individual recognition, but these two factors are likely not the only ones to be affected. For example, dispersal may also be affected: since kittiwakes are long-lived and sometimes use the same nest for successive reproductions, the opportunities for young birds to find a nest to settle are scarce, especially in a crowded colony such as on the tower on Middleton where adult survival is high (Hatch, et al. 1993) and the number of potential nests limited.

Références utilisées dans la Synthèse / Literature cited in the Synthesis

Amos, W., Worthington Wilmer, J., Fullard, K., Burg, T. M., Croxall, J. P., Bloch, D. & Coulson, T. 2001. The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society of London B*, **268,** 2021-2027.

Andersson, M. 1994. *Sexual selection*. Princeton: Princeton University Press.

Aubin, T., Mathevon, N., Staszewski, V. & Boulinier, T. 2007. Acoustic communication in the kittiwake *Rissa tridactyla*: potential cues for sexual and individual signatures in long calls. *Polar Biology*.

Baird, P. H. 1994. Black-legged kittiwake. In: *The Birds of North America*, pp. 1-24: A. Poole and F. Gill, Editors.

Baker, R. H., Ashwell, R. I. S., Richards, T. A., Fowler, K., Chapman, T. & Pomiankowski, A. 2001. Effects of multiple mating and male eye span on female reproductive output in the stalk-eyed fly *Cytodiopsis dalmanni*. *Behavioral Ecology*, **12,** 732-739.

Bang, B. G. & Cobb, S. 1968. The size of the olfactory bulb in 108 species of bird. *Auk*, **85,** 55-61.

Beer, C. G. 1969. Laughing gull chicks: recognition of their parents' voices. *Science*, **166,** 1030-1032.

Bensch, S., Hasselquist, D. & Von Schantz, T. 1994. Genetic similarity between parents predicts hatching failure nonincestuous inbreeding in the Great reed warbler? *Evolution*, **48,** 317-326.

Bergstrom, C. T. & Real, L. A. 2000. Towards a theory of mutual mate choice: Lessons from two-sided matching. *Evolutionary Ecology Research*, **2,** 493-508.

Blomqvist, D., Andersson, M., Küpper, C., Cuthill, I. C., Kis, J., Lanctot, R. B., Sandercock, B. K., Székely, T., Wallander, J. & Kempenaers, B. 2002. Genetic simimarity between mates and extra-pair parentage in three species of shorebirds. *Nature*, **419,** 613-615.

Boehm, T. & Zufall, F. 2006. MHC peptides and the sensory evaluation of genotype. *Trends in Neurosciences*, **29,** 100-107.

Bohnet, S., Rogers, L., Sasaki, G. & Kolattukudy, P. E. 1991. Estradiol induces proliferation of peroxisome-like microbodies and the production of 3-hydroxy fatty acid diesters, the female pheromones, in the uropygial glands of male and female mallards. *Journal of Biological Chemistry*, **266,** 9795-9804.

Bonadonna, F., Cunningham, G. B., Jouventin, P., Hesters, F. & Nevitt, G. A. 2003. Evidence for nest-odour recognition in two species of diving petrel. *Journal of Experimental Biology*, **206,** 3719-3722.

Bonadonna, F. & Nevitt, G. A. 2004. Partner-specific odor recognition in an Antarctic seabird. *Science*, **306,** 835.

Bonadonna, F., Villafane, M., Bajzak, C. & Jouventin, P. 2004. Recognition of burrow's olfactory signature in Blue petrels, *Halobaena caerulea*: an efficient discrimination mechanism in the dark. *Animal Behaviour*, **67,** 893-898.

Bonneaud, C., Chastel, O., Federici, P., Westerdahl, H. & Sorci, G. 2006. Complex MHC-based mate choice in a wild passerine. *Proceedings of the Royal Society of London B*, **273,** 1111-1116.

Bonneaud, C., Mazuc, J., Chastel, O., Westerdahl, H. & Sorci, G. 2004. Terminal investment induced by immune challenge and fitness traits associated with major histocompatibility complex in the House sparrow. *Evolution*, **12,** 2823-2830.

Brown, J. L. 1996. A theory of mate choice based on heterozygosity. *Behavioral Ecology*, **8,** 60-65.

Cadiou, B., Monnat, J.-Y. & Danchin, E. 1994. Prospecting in the kittiwake, *Rissa tridactyla*: different behavioural patterns and the role of squatting in recruitment. *Animal Behaviour*, **47,** 847-856.

Cam, E. & Monnat, J.-Y. 2000a. Apparent inferiority of first-time breeders in the kittiwake: the role of heterogeneity among age classes. *Journal of Animal Ecology*, **69,** 380-394.

Cam, E. & Monnat, J.-Y. 2000b. Stratification based on reproductive state reveals contrasting patterns of age-related variation in demographic parameters in the kittiwake. *OIKOS*, **90,** 560-574.

Cam, E., Monnat, J.-Y. & Hines, J. E. 2003. Long-term fitness consequences of early conditions in the kittiwake. *Journal of Animal Ecology*, **72,** 411-424.

Cézilly, F., Dubois, F. & Pagel, M. 2000. Is mate fidelity related to site fidelity? A comparative analysis in Ciconiiforms. *Animal Behaviour*, **59,** 1143-1152.

Chardine, J. W. 2002. Geographic variation in the wingtip patterns of black-legged kittiwakes. *Condor*, **104,** 687-693.

Charrier, I., Mathevon, N., Jouventin, P. & Aubin, T. 2001. Acoustic communication in a black-headed gull colony: how do chicks identify their parents ? *Ethology*, **107,** 961-974.

Cohen, L. B. & Dearborn, D. C. 2004. Great frigatebirds, *Fregata minor*, choose mates that are genetically similar. *Animal Behaviour*, **68,** 1229-1236.

Coulson, J. C. 1966. The influence of the pair-bond and age on the breeding biology of the kittiwake gull *Rissa tridactyla*. *Journal of Animal Ecology*, **35,** 269-279.

Coulson, J. C. 1983. The changing status of the Kittiwake *Rissa tridactyla* in the British Isles, 1969-1979. *Bird Study*, **30,** 9-16.

Coulson, J. C. & Thomas, C. S. 1980. A study of the factors influencing the pair-bond in the kittiwake gull. *Proceedings of the International Ornithological Congress*, **17,** 823-833.

Coulson, J. C. & Thomas, C. S. 1985. Changes in the biology of the kittiwake Rissa tridactyla: a 31-year study of a breeding colony. *Journal of Animal Ecology*, **54,** 9-26.

Cullen, E. 1956. Adaptations in the kittiwake to cliff-nesting. *Ibis*, **99,** 275-302.

Cunningham, G. B., Van Buskirk, R. W., Bonadonna, F., Weimerskirch, H. & Nevitt, G. A. 2003. A comparison of the olfactory abilities of three species of procellariiform chicks. *Journal of Experimental Biology*, **206,** 1615-1620.

Danchin, E. 1983. Les comportements liés à l'occupation du site de reproduction chez la mouette tridactyle (*Rissa tridactyla*) : les comportements de pré-atterrissage. *CNRS-CRBPO***,** 226-246.

Danchin, E. 1987. The behaviour associated with the occupation of breeding site in the kittiwake gull *Rissa tridactyla*: the social status of landing birds. *Animal Behaviour*, **35,** 81-93.

Danchin, E. (1987). Description and functional signification of the behaviour and the displays of the kittiwake (*Rissa tridactyla*). *(Unpublished).*

Danchin, E. 1988. Social interactions in Kittiwakes colonies: social facilitation and/or favourable social environment. *Animal Behaviour*, **36,** 443-451.

Danchin, E., Boulinier, T. & Massot, M. 1998. Conspecific reproductive success and breeding habitat selection: implications for the study of coloniality. *Ecology*, **79,** 2415-2428.

Danchin, E., Cadiou, B., Monnat, J.-Y. & Estrella, R. R. 1991. Recruitment in long-lived birds: conceptual framework and behavioural mechanisms. In: *Acta XXth Congressus Internationalis Ornithologicus*, pp. 1641-1656. Wellington: Hutcheson, Bowman and Stewart Ltd.

Darwin, C. 1871. *The descent of man and selection in relation to sex*. London: John Murray.

Doherty, P. F., Sorci, G., Royle, J. A., Hines, J. E., Nichols, J. D. & Boulinier, T. 2003. Sexual selection affects local extinction and turnover in bird communities. *Proceedings of the National Academy of Sciences USA*, **100,** 5858-5862.

Drickamer, L. C., Gowaty, P. A. & Holmes, C. M. 2000. Free female mate choice in house mice affects reproductive success and offspring viability and performance. *Animal Behaviour*, **59,** 371-378.

Edwards, S. V., Kingan, S. B., Calkins, J. D., Balakrishnan, C. N., Jennings, W. B., Swanson, W. J. & Sorenson, M. D. 2005. Speciation in birds: genes, geography, and sexual selection. *Proceedings of the National Academy of Sciences USA*, **102,** 6550-6557.

Ekblom, R., Saether, S. A., Grahn, M., Fiske, P., Kålås, J. A. & Höglund, J. 2004. Major histocompatibility complex variation and mate choice in a lekking bird, the Great snipe (*Gallinago media*). *Molecular Ecology*, **13,** 3821-3828.

Evans, R. M. 1970. Parental recognition and the 'mew call' in Black-billed gulls (*Larus bulleri*). *Auk*, **87,** 503.

Fairweather, J. A. & Coulson, J. C. 1995. Mate retention in the kittiwake, *Rissa tridactyla*, and the significance of nest site tenacity. *Animal Behaviour*, **50,** 455-464.

Fisher, R. A. 1915. The evolution of sexual preferences. *Eugenics Review*, **7,** 184-192.

Fisher, R. A. 1930. *The genetical theory of natural selection*. Oxford: Clarendon Press.

Foerster, K., Delhey, K., Johnsen, A., Lifjeld, J. T. & Kempenaers, B. 2003. Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature*, **425,** 714-717.

Freeman-Gallant, C. R., Meguerdichian, M., Wheelwright, N. T. & Sollecito, S. V. 2003. Social pairing and female mating fidelity predicted by restriction fragment length polymorphism similarity at the major histocompatibility complex in a songbird. *Molecular Ecology*, **12,** 3077-3083.

Frings, H. & Boyd, W. A. 1952. Evidence of olfactory discrimination by the Bobwhite quail. *American Midland Naturalist*, **48,** 181-184.

Gill, V. A. & Hatch, S. A. 2002. Components of productivity in black-legged kittiwakes *Rissa tridactyla*: response to supplemental feeding. *Journal of Avian Biology*, **33,** 113-126.

Given, A. D., Mills, A. & Baker, J. 2002. Isolation of polymorphic microsatellite loci from the red-billed gull (*Larus novaehollandiae scopulinus*) and amplification in related species. *Molecular Ecology Notes*, **2,** 416-418.

Griffith, S. C., Owens, I. P. F. & Thuman, K. A. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology*, **11,** 2195-2212.

Griswold, D. A., Harrer, M. F., Sladkin, C., Alessandro, D. A. & Gould, J. L. 1995. Intraspecific recognition by laughing gull chicks. *Animal Behaviour*, **50,** 1341-1348.

Hagelin, J. C., Jones, I. L. & Rasmussen, L. E. L. 2003. A tangerine-scented social odour in a monogamous seabird. *Proceedings of the Royal Society of London B*, **270,** 1323-1329.

Hansson, B. & Westerberg, L. 2002. On the correlation between heterozygosity and fitness in natural populations. *Molecular Ecology*, **11,** 2467-2474.

Hatch, S. A., Roberts, B. D. & Fadely, B. S. 1993. Adult survival of Black-legged kittiwakes *Rissa tridactyla* in a Pacific colony. *Ibis*, **135,** 247-254.

Healy, S. & Guilford, T. 1990. Olfactory-bulb size and nocturnality in birds. *Evolution*, **44,** 339-346.

Helfenstein, F. 2002. Stratégies de reproduction et conflits sexuels. In: *Ecologie*, pp. 137. Paris: Université Pierre et Marie Curie.

Helfenstein, F., Danchin, E. & Wagner, R. H. 2004a. Assortative mating and sexual size dimorphism in black-legged kittiwakes. *Waterbirds*, **27,** 350-354.

Helfenstein, F., Danchin, E. & Wagner, R. H. 2004b. Is male unpredictability a paternity assurance strategy? *Behaviour*, **141,** 675-690.

Helfenstein, F., Tirard, C., Danchin, E. & Wagner, R. H. 2004c. Low frequency of extra-pair paternity and high frequency of adoption in Black-legged kittiwakes. *Condor*, **106,** 149-155.

Helfenstein, F., Wagner, R. H., Danchin, E. & Rossi, J.-M. 2003. Fonctions of courtship feeding in black-legged kittiwakes: natural and sexual selection. *Animal Behaviour*, **65,** 1027-1033.

Hoffman, J. I., Fordaca, J., Trathan, P. N. & Amos, W. 2007. Female fur seals show active choice for males that are heterozygous and unrelated. *Nature*, **445,** 912-914.

Jodice, P. G. R., Lanctot, R. B., Gill, V. A., Roby, D. D. & Hatch, S. 2000. Sexing adult black-legged kittiwakes by DNA, behavior, and morphology. *Waterbirds*, **23,** 405-415.

Johnsen, A., Andersen, V., Sundling, C. & Lifjeld, J. T. 2000. Female bluethroats enhance offspring immunocompetence through extra-pair copulations. *Nature*, **406,** 296-299.

Jones, I. L. & Hunter, F. M. 1993. Mutual sexual selection in a monogamous seabird. *Nature*, **362,** 238-239.

Kempenaers, B., Lanctot, R. B., Gill, V. A. & Hatch, S. A. 2006. Do females trade copulations for food? An experimental study in Black-legged Kittiwakes. *Behavioral Ecology*, **147,** 192-193.

Kikkawa, E. F., Tsuda, T. T., Naruse, T., Sumiyama, D., Fukuda, M., Kurita, M., Murata, K., Wilson, R. P., Le Maho, Y., Tsuda, M., Kulski, J. K. & Inoko, H. 2005. Analysis of the sequence variations in the *MHC DRB1*-like gene of the endangered Humboldt penguin (*Spheniscus humbodlti*). *Immunogenetics*, **57,** 99-107.

Kleven, O. & Lifjeld, J. T. 2004. Extrapair paternity and offspring immunocompetence in the reed bunting, *Emberiza schoeniclus*. *Animal Behaviour*, **68,** 283-289.

Kokko, H. & Ots, I. 2006. When not to avoid inbreeding. *Evolution*, **60,** 467-475.

Krokene, C. & Lifjeld, J. T. 2000. Variation in the frequency of extra-pair paternity in birds; a comparison of an island and a mainland population of Blue tits. *Behaviour*, **137,** 1317-1330.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. *Proceedings of the National Academy of Sciences USA*, **78,** 3721-3725.

Landry, C., Garant, D., Duchesne, P. & Bernatchez, L. 2001. 'Good genes as heterozygosity': the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). *Proceedings of the Royal Society of London B*, **268,** 1279-1285.

Leibovici, M., Lapointe, F., Aletta, P. & Ayer-Le Lièvre, C. 1996. Avian olfactory receptors: differenciation of olfactory neurons under normal an experimental conditions. *Developmental Biology*, **175,** 118-131.

Lloyd, C., Tasker, M. L. & Partridge, K. 1991. Kittiwake *Rissa tridactyla*. In: *The status of seabirds in Great Britain and Ireland*, pp. 182-193.

Longmire, J. L., Lewis, A. K., Brown, N. C., Buckingham, J. M., Clark, L. M., Jones, M. D., Meincke, L. J., Meyne, J., Ratliff, R. L., Ray, F. A., Wagner, R. P. & Moyzis, R. K. 1988. Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family Falconidae. *Genomics*, **2,** 14-24.

Mäntylä, E., Klemola, T. & Haukioja, E. 2004. Attraction of willow warblers to sawfty-damaged mountain birches: novel function of inducible plant defences? *Ecology Letters*, **7,** 915-918.

Mathevon, N., Charrier, I. & Jouventin, P. 2003. Potential for individual recognition in acoustic signals: a comparative study of two gulls with different nesting patterns. *Comptes-Rendus Biologies*, **326,** 329-337.

McClelland, E. E., Penn, D. J. & Potts, W. K. 2003. Major histocompatibility complex heterozygote superiority during coinfection. *Infection & Immunity*, **71,** 2079-2086.

McCoy, K. D., Boulinier, T. & Tirard, C. 2005. Comparative host-parasite population structures: disentangling prospecting and dispersal in the Black-legged kittiwake *Rissa tridactyla*. *Molecular Ecology*, **14,** 2825-2838.

Mennerat, A., Bonadonna, F., Perret, P. & Lambrechts, M. M. 2005. Olfactory conditioning experiments in a food-searching passerine bird in semi-natural conditions. *Behavioural Processes*, **70,** 264-270.

Monnat, J.-Y., Danchin, E. & Estrella, R. R. 1990. Assessment of environmental quality within the framework of prospecting and recruitment: the squatterism in the Kittiwake. *Comptes-Rendus de l'Académie des Sciences de Paris*, **311,** 391-396.

Morton, E. S., Forman, L. & Braun, M. 1990. Extrapair fertilizations and the evolution of colonial breeding in purple martins. *Auk*, **107,** 275-283.

Nef, S., Allaman, I., Fiumelli, H., De Castro, E. & Nef, P. 1996. Olfaction in birds: differential embryonic expression of nine putative odorant receptor genes in the avian olfactory system. *Mechanisms of Development*, **55,** 65-77.

Nevitt, G. A., Reid, J. M. & Trathan, P. 2004. Testing olfactory foraging strategies in an Antarctic seabird assemblage. *Journal of Experimental Biology*, **207,** 3537-3544.

Penn, D. J. 2002. The scent of genetic compatibility: sexual selection and the major histocompatibility complex. *Ethology*, **108,** 1-21.

Penn, D. J., Damjanovich, K. & Potts, W. K. 2002. MHC heterozygosity confers a selective advantage against multiple-strain infections. *Proceedings of the National Academy of Sciences USA*, **99,** 11260-11264.

Penn, D. J. & Potts, W. K. 1998a. MHC-disassortative mating preferences reversed by cross-fostering. *Proceedings of the Royal Society of London B*, **265,** 1299-1306.

Penn, D. J. & Potts, W. K. 1998b. Untrained mice discriminate MHC-determined odors. *Physiology & Behavior*, **63,** 235-243.

Petit, C., Hossaert-McKey, M., Perret, P., Blondel, J. & Lambrechts, M. M. 2002. Blue tits use selected plants and olfaction to maintain an aromatic environment for nestlings. *Ecology Letters*, **5,** 585-689.

Prosser, C. L. 1950. *Comparative animal physiology*. Philadelphy: W.S. Saunders Co.

Queller, D. C. & Goodnight, K. F. 1989. Estimating relatedness using genetic markers. *Evolution*, **43,** 258-275.

Reid, J. M. 2007. Secondary sexual ornamentation and non-additive genetic benefits of female mate choice. *Proceedings of the Royal Society B-Biological Sciences*, **274,** 1395-1402.

Reid, J. M., Arcese, P., Cassidy, A., Hiebert, S. M., Smith, J. N. M., Stoddard, P. K., Marr, A. B. & Keller, L. F. 2004. Song repertoire size predicts initial mating success in male song sparrows, Melospiza melodia. *Animal Behaviour*, **68,** 1055-1063.

Reid, J. M., Arcese, P., Cassidy, A. L. E. V., Marr, A. B., Smith, J. N. M. & Keller, L. F. 2005. Hamilton and Zuk meet heterozygosity? Song repertoire size indicates inbreeding and immunity in Song sparrows (*Melospiza melodia*). *Proceedings of the Royal Society of London B*, **272,** 481-487.

Reneerkens, J., Piersma, T. & Sinninghe Damsté, J. S. 2005. Switch to diester preen waxes may reduce avian nest predation by mammalian predators using olfactory cues. *Journal of Experimental Biology*, **208,** 4199-4202.

Richardson, D. S., Komdeur, J., Burke, T. & Von Schantz, T. 2005. MHC-based patterns of social and extra-pair mate choice in the Seychelles warbler. *Proceedings of the Royal Society of London B*, **272,** 759-767.

Roberts, B. D. & Hatch, S. A. 1994. Chick movements and adoption in a colony of Black-legged kittiwakes. *Wilson Bulletin*, **106,** 289-298.

Roberts, S. C. & Gosling, L. M. 2003. Genetic similarity and quality interact in mate choice decisions by female mice. *Nature Genetics*, **35,** 103-106.

Roper, T. J. 1999. Olfaction in birds. *Advances in the Study of Behavior*, **28,** 247-332.

Sluys, R. 1982. Geographical variation of the kittiwake. *Rissa tridactyla*. *Le Gerfaut*, **72,** 221-230.

Soini, H. A., Schrock, S. E., Bruce, K. E., Wiesler, D., Ketterson, E. D. & Novotny, M. V. 2006. Seasonal variation in volatile compound profiles from preen gland secretions of the Dark-eyed junco (*Junco hyemalis*). *Journal of Chemical Ecology*.

Storey, A. E., Anderson, R. E., Porter, J. M. & McCharles, A. M. 1992. Absence of parent-young recognition in kittiwakes: a re-examination. *Behaviour*, **120,** 302-323.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. 2007. *MEGA4*: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution***,** 10.1093/molbev/msm092.

Tibbetts, E. A. & Dale, J. 2007. Individual recognition: it is good to be different. *Trends in Ecology & Evolution***,** doi:10.1016/j.tree.2007.09.001.

Tinbergen, N. 1953. *The herring gull' world*. London: Collins.

Tinbergen, N. 1959. Comparative studies of the behaviour of gulls (*Laridae*): a progress report. *Behaviour*, **15,** 1-70.

Tirard, C., Helfenstein, F. & Danchin, E. 2002. Polymorphic microsatellites in the Black-legged kittiwake *Rissa tridactyla*. *Molecular Ecology Notes*, **2,** 431-433.

Tregenza, T. & Wedell, N. 2000. Genetic compatibility, mate choice and patterns of parentage: Invited review. *Molecular Ecology*, **9,** 1013-1027.

Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual selection and the descent of man, 1871-1971* (Ed. by Campbell, B.), pp. 136-179. Chicago, IL: Aldine Publishing Company.

Tsuda, T. T., Tsuda, M., Naruse, T., Kawata, H., Ando, A., Shiina, T., Fukuda, M., Kurita, M., Le Maho, Y., Kulski, J. K. & Inoko, H. 2001. Phylogenetic analysis of penguin (*Spheniscidae*) species based on sequence variation in MHC Class II genes. *Immunogenetics*, **53,** 712-716.

Wagner, R. H. 1997. Hidden leks: sexual selection and the clumping of avian territories. In: *Extra-pair mating tactics in birds* (Ed. by Parker, P. G. & Burley, N.), pp. 123-145. Washington, D.C.: Ornithological Monographs, American Ornithologists' Union.

Wallraff, H. G. 2004. Avian olfactory navigation: its empirical foundation and conceptual state. *Animal Behaviour*, **67,** 189-204.

Warden, C. J., Jenkins, T. N. & Warner, L. H. 1936. Comparative psychology. In: *Vertebrates*. New York: Ronald Press.

Weatherhead, P. J., Dufour, K. W., Lougheed, S. C. & Eckert, C. G. 1999. A test of the good-genes-as-heterozygosity hypothesis using Red-winged blackbirds. *Behavioral Ecology*, **10,** 619-625.

Wedekind, C. & Füri, S. 1997. Body odour preferences in men and women: do they aim for specific MHC combinations or simply heterozygosity? *Proceedings of the Royal Society of London B*, **264,** 1471-1479.

Wedekind, C. & Penn, D. 2000. MHC genes, body odours, and odour preferences. *Nephrology Dialysis Transplantation*, **15,** 1269-1271.

Wedekind, C., Seebeck, T., Bettens, F. & Paepke, A. J. 1995. MHC-dependent mate preferences in humans. *Proceedings of the Royal Society of London B*, **260,** 245-249.

Wedekind, C., Walker, M., Portmann, J., Cenni, B., Müller, R. & Binz, T. 2004. MHC-linked susceptibility to a bacterial infection, but no MHC-linked cryptic female choice in Whitefish. *Journal of Evolutionary Biology*, **17,** 11-17.

Westerdahl, H. 2004. No evidence of an MHC-based female mating preference in great reed warblers. *Molecular Ecology*, **13,** 2465-2470.

Westerdahl, H., Waldenström, J., Hansson, B., Hasselquist, D., Von Schantz, T. & Bensch, S. 2005. Associations between malaria and MHC genes in a migratory songbird. *Proceedings of the Royal Society of London B*, **272,** 1511-1518.

Wooller, R. D. 1978. Individual vocal recognition in the kittiwake gull, *Rissa tridactyla* (L.). *Zeitschrift für Tierpsychology*, **48,** 68-86.

Wooller, R. D. 1979. Seasonal, diurnal and area differences in calling activity within a colony of kittiwakes *Rissa tridactyla* (L.). *Zeitschrift für Tierpsychology*, **51,** 329-336.

Yamazaki, K., Beauchamp, G. K., Imai, Y., Bard, J., Phelan, S. P., Thomas, L. & Boyse, E. A. 1990. Odortypes determined by the major histocompatibility complex in germfree mice. *Proceedings of the National Academy of Sciences USA*, **87,** 8413-8416.

Yamazaki, K., Singer, A. G. & Beauchamp, G. K. 1999. Origin, functions and chemistry of H-2 regulated odorants. *Genetica*, **104,** 235-240.

ARTICLES

ARTICLE I: "Dialects" in a non-oscine bird: long-call differences between Atlantic and Pacific kittiwakes

Hervé Mulard, Thierry Aubin, Joël F. White, Etienne Danchin

In preparation for *Biological Journal of the Linnean Society*

Abstract

Acoustic features are important in recognition in both songbirds and non-oscine birds. Dialects occur in many songbirds, but geographical divergence in vocal features have been little studied in birds that do not have any song learning process. Here we show that two populations of the black-legged kittiwake, a seabird where the call is apparently not learned from any of the parents, differ greatly in their vocal parameters measured on the long-call. In both populations, the call seems to convey individual information, but frequency and temporal parameters are greatly different. Moreover, no gender signature has been found in the calls of adults from the Pacific population (Middleton, Alaska), while such signature was present in the Atlantic population (Hornøya, Norway), and this may potentially affect sexual behaviors. The long-call seems also to evolve throughout the reproductive season and from one year to the next, but the individual signature remains fairly stable. Overall, our results show that call divergence may be an important driving force for speciation in birds.

Introduction

In songbirds, song is generally learned from other adults, and acoustic characteristics are passed on to succeeding generations by vocal imitative learning. Errors in this learning process may, in the long term, cause major divergences between populations (Chilton and Lein, 1996, Ellers and Slabbekoorn, 2003). Thus, song learning is thought to be at the origin of geographic variations, dialects between different populations appearing as the product of a divergent cultural evolution (reviewed in Kroodsma & Miller 1996). Overproduction of previously memorized song, and counter-selection of those that fail to match the general dialect of the population, could also lead to dialectal differences between populations (Nelson & Marler 1994). According to the habitat-dependent selection hypothesis, acoustic features evolve according to the characteristics of the environment, in order to reach maximal transmission (Slabbekoorn & Smith 2002, Tubaro & Segura 1995, Van Dongen & Mulder

2006). Song divergence has also been shown in ring species (Irwin 2000), and is thought to be one of the main causes of speciation in oscine birds (Grant & Grant 1997, Price 1998, Slabbekoorn & Smith 2002).

Acoustic features are also important in birds with no song learning process. For example, most seabirds form large colonies when breeding, and the level of noise in such high density environments is usually very high (Aubin 2004), emphasizing the importance of vocal communication in such species. The ability to recognize without ambiguity the mate or the young is particularly important in colonies, where individuals are densely packed, making the possibility of confusion great (Slabbekoorn 2004). In these colonial seabird species, the combination of monomorphic adult plumage and vocal greeting displays suggests also that sound is likely to play a major role in mate and parent-offspring recognition (Falls 1982). For example, penguins chicks recognize their parents according to acoustic features (Aubin & Jouventin 2002), and such parent-offspring vocal recognition seems also common in Larids species (Beer 1969, Charrier, et al. 2001, Evans 1970, Hutchinson, et al. 1968, Mathevon, et al. 2003). Nevertheless, in numerous Larids, vocal recognition between mates and between parent and offspring remains to be demonstrated because published evidence is controversial.

In such species, since calls are apparently not learned from any of the parents, acoustic features of the call should depend on genetic control (reviewed in Kroodsma 2004). In this case, two genetically different populations should produce distinct acoustic features, but such hypothesis has been little studied so far.

The present paper aims to compare the potentiality for gender and individual vocal recognition in two distant populations of adult Black-legged kittiwakes, *Rissa tridactyla*. Aubin et al. (2007) have previously shown an individual and a gender signature in the long-call of kittiwakes of the Atlantic area (study realized in Hornøya island, Barents sea, Norway). Atlantic populations have been found to differ genetically from Pacific populations (McCoy, et al. 2005), thus Pacific populations may also differ in their long-call characteristics. The call of this species is also suspected to evolve with the course of the reproductive season (Wooller 1978). However, temporal evolution, as well as geographical differences, have never been studied in this species. In the present study, the three main objectives will thus be: (1) to see whether individual and gender signature is a general feature among kittiwakes, using kittiwakes of the Pacific area (Middleton island, Gulf of Alaska, USA) as a test-population; (2) to test for temporal evolution of the long-call using records made at different time of the year and during two consecutive years; (3) to test for potential geographical divergences of the long-calls by comparing Pacific to Atlantic populations.

Methods

Studied populations

Middleton Island (north-central Gulf of Alaska, 58°25' N, 146°19' W) supports a large population of black-legged kittiwakes (25,000 birds in 1999, Gill & Hatch 2002). We studied kittiwakes nesting on artificial nest sites on the edges of an abandoned US Air Force radar tower which has been modified to enable close observations and easy captures. This study plot is characterized by vertical walls and uniform nest spacing, with birds nesting on wooden ledges built specifically for cliff-nesting seabirds (Gill & Hatch 2002). Adults were banded using US metal code and 4 different colored rings.

Hornøya Island (Barents sea, Norway, 70°21'N, 31°02'E) supports a population of more than 10,000 breeding pairs. Records were focused on ringed sexed adults (for further details, see Aubin, et al. 2007).

Recordings

Long-call (also named "kittiwakes" call) were recorded from individuals either while landing or while on the nest. This call is usually emitted while landing or by pairs of individuals when they are greeting each other on their nest (Danchin 1987). A microphone AKG D770 (linked to a Marantz PMD670 digital recorder, sampling frequency 48kHz) was placed directly on the nest, so the calls were recorded from less than 30cm from the emission point. Adults were recorded in May and early June in 2005 and 2006 (corresponding to the pre-laying period), and again in late July and August in both years (corresponding to the chick rearing period).

For details concerning records in Hornøya population, refer to Aubin, et al. (2007).

Long-call analyses

For call measurements, we used the SASLab software (Avisoft). The parameters used in this study are the same as the ones used by Aubin et al. (2007):

- time parameters:
    - DurKi: duration of the "ki" part
    - DurKiTi: duration between the end of the "ki" and the beginning of the "ti" part

- DurTi: duration of the "ti" part
- DurTiWk: duration between the end of the "ti" and the beginning of the "wake" part
- DurWake: duration of the "wake" part
- DurWakeBeg: duration of the first part of the "wake" part when existing
- DurIntKi: duration between two consecutive "ki". In Middleton Island, we measured for some calls the DurIntKi and the duration between two consecutive wake (used for Hornøya population, Aubin, et al. 2007), and found no significant difference between both measures, thus, since DurIntKi is easier to record in Middleton, we will use that one in this paper.

- frequency parameters:

- F0KiBeg: fundamental frequency of the beginning of the "ki" part
- F0KiMax: maximum fundamental frequency of the "ki" part
- F0KiEnd: fundamental frequency of the end of the "ki" part
- F0TiBeg: fundamental frequency of the beginning of the "ti" part
- F0TiEnd: fundamental frequency of the end of the "ti" part
- F0WakeBeg: fundamental frequency of the beginning of the "wake" part
- F0Wake: general fundamental frequency of the "wake" part, or of the first part of the "wake" when this one is spliced in two
- Q25, Q50 and Q75: first, second and third quartile of the "wake" part, or of the first part of the "wake" when this one is split in two parts
- F0WakeB: fundamental frequency of the second part of the "wake" part, when existing
- Q25B, Q50B, Q75B: first, second and third quartile of the second part of the "wake" part

Statistical analyses

Statistical analyses were done using the SAS® package (SAS Institute 1999). Following Aubin et al. (2007), we defined the PIC (potential for individual coding) as the coefficient of variation between individuals divided by the coefficient of variation intra-individual. This analysis was made using 4 randomly chosen calls per individual to avoid pseudo-replication. Similarly, the PSC (potential for sex coding) and the PPC (potential for population coding)

were the coefficient of variation between sexes (or populations) divided by the coefficient intra-sex (or intra-population), and these analyses were made using one randomly chosen call per individual. Significance of PIC, PSC and PPC were estimated using Kruskal-Wallis tests, and is given as: * for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$.

Results

Calls are individually distinct

We calculated the coefficient of variation and the potential of individual coding (PIC) on

Parameters	CVbetween	Mean CVind	PIC	ANOVA Kruskal-Wallis
DurKi (78)	20.25	14.89	1.36	154.83***
DurKiTi (78)	203.42	122.80	1.66	183.34***
DurTi (78)	27.75	19.16	1.45	199.34***
DurTiWk (78)	46.09	24.32	1.90	235.71***
DurWake (78)	20.93	11.36	1.84	227.18***
DurWakeBeg (60)	28.91	17.91	1.61	169.46***
DurIntKi (78)	11.00	6.18	1.78	211.20***
F0KiBeg (78)	11.90	8.42	1.41	182.06***
F0KiMax (78)	11.36	8.34	1.36	175.32***
F0KiEnd (78)	17.40	10.52	1.65	209.84***
F0TiBeg (78)	19.02	11.43	1.66	204.33***
F0TiEnd (78)	10.64	8.51	1.25	156.38***
F0WakeBeg (78)	10.15	7.94	1.28	154.12***
F0Wake (78)	10.70	7.22	1.48	188.00***
Q25 (78)	18.92	15.76	1.20	133.91***
Q50 (78)	12.95	10.40	1.25	143.62***
Q75 (78)	17.73	14.19	1.25	122.42***
F0WakeB (60)	34.56	24.67	1.40	126.39***
Q25B (60)	23.39	18.61	1.26	121.76***
Q50B (60)	14.78	12.45	1.19	81.18*
Q75B (60)	32.46	25.00	1.30	116.07***

Table 1: Individual signature in the long-call in Middleton population of kittiwakes. For each parameter, the number of observations is given by the number in brackets. PIC (potential of individual coding) is the quotient of the coefficient of variation between individuals (CVbetween) by the coefficient of variation intra-individual (CVind). Its significance was tested by a Kruskal-Wallis test (last column, first number is the χ^2 and significance of the test follows using: * if $p < 0.05$, *** if $p < 0.001$)

312 calls coming from 78 individuals (4 randomly chosen calls per individual to avoid pseudo-replication; variables for the second part of the wake were assessed only for 60 individuals). This calls were chosen without taking into account the recording time (pre-incubation or rearing period, 2005 or 2006). The table 1 shows that all variables are potentially coding for individuality, the Kruskal-Wallis tests made on these variables being highly significant.

On the basis of a discriminating analysis using principal components calculated for all variables, we classify correctly 75% of the calls according to individual (760 calls coming from 78 individuals with a minimum of 4 calls/individual, 16 variables), indicating that the individual signature is stable in the long-call.

For calls with a break of frequency inside the "wake", we were able to assess 5 more variables for the second part of the "wake" part. Then, we reach a correct discrimination of 88% of the calls according to individual (538 calls coming from 60 individuals with a minimum of 4 calls with a break of frequency, 21 variables). With principal components calculated from temporal variables only (7 variables), we have a correct discrimination of 58%, while with frequency variables only (14 variables), we have a correct discrimination of 57% of these calls.

Evolution of the long-call during the season and during years

Analyses presented in this part were made without taking into account variables calculated from the second part of the "wake" part, in order to have a sufficient sample size.

On the basis of a discriminant analysis using principal components calculated for all variables, we were able to classify correctly 81% of the calls according to individuality and to breeding period (pre-incubation or rearing period; analysis made on 144 calls coming from 10 individuals with a minimum of 3 calls/individual/period). Among 24 misclassified calls, 14 were at least well classified according to individuality (leading to a correct discrimination of 93% of the calls according to individuality only, for individuals recorded at two different periods of the year).

For individuals recorded in 2005 and 2006 at the pre-incubation period, we made a discriminant analysis using individuality and recording year as classification variables. We were able to classify correctly 85% of the calls according to both individuals and years (analysis made on 253 calls coming from 21 individuals with a minimum of 3 calls/individual/year). Among 39 misclassified calls, 18 were at least well classified according to individuality (leading to a correct discrimination of 92% of the calls according to individuality only, for individuals recorded in the pre-incubation period in 2005 and 2006).

No sexual dimorphism in Middleton Island long-calls

To avoid pseudo-replications, the data set used in the following analysis was composed of only one randomly chosen call per individual. Kruskal-Wallis tests on the different variables (table 2) show no differences between sexes. Indeed, only F0WakeBeg was significantly lower for males than for females, and F0WakeB was higher for males, but these

Parameters	Females	Males	CVb	CVw	PSC	Kruskal
DurKi (ms) (35-41)	73.1+/-15.4 (68-189)	73.1+/-14.7 (37-106)	20.49	20.73	0.99	0.01 NS
DurKiTi (ms) (35-41)	8.6+/-19.7 (0-69)	10.9+/-20.4 (0-21)	203.60	209.51	0.97	0.43 NS
DurTi (ms) (35-41)	71.0+/-16.8 (35-117)	64.8+/-21.3 (20-114)	28.94	28.49	1.02	1.42 NS
DurTiWk (ms) (35-41)	83.9+/-42.2 (0-187)	93.4+/-45.6 (0-205)	49.59	49.86	0.99	0.90 NS
DurWake (ms) (35-41)	176.9+/-40.6 (108-276)	179.0+/-33.6 (121-238)	20.70	20.99	0.99	0.17 NS
DurWakeBeg (ms) (27-31)	122.2+/-32.3 (68-183)	112.0+/-34.9 (51-183)	29.12	29.08	1.00	1.06 NS
DurIntKi (ms) (35-41)	569.9+/-59.4 (473-723)	570.5+/-60.6 (440-751)	10.49	10.59	0.99	0.14 NS
F0KiBeg (Hz) (35-41)	518+/-65 (403-722)	501+/-52 (394-656)	11.61	11.61	1.00	0.84 NS
F0KiMax (Hz) (35-41)	589+/-67 (488-778)	570+/-56 (450-692)	10.70	10.69	1.00	1.10 NS
F0KiEnd (Hz) (35-41)	479+/-93 (328-778)	475+/-78 (319-656)	17.85	18.07	0.99	0.00 NS
F0TiBeg (Hz) (35-41)	480+/-87 (328-647)	485+/-94 (319-703)	18.78	18.89	0.99	0.00 NS
F0TiEnd (Hz) (35-41)	711+/-72 (600-863)	691+/-68 (570-834)	10.10	10.10	1.00	0.83 NS
F0WakeBeg (Hz) (35-41)	679+/-67 (553-814)	647+/-60 (542-788)	9.81	9.61	1.02	4.82*
F0Wake (Hz) (35-41)	632+/-62 (504-760)	620+/-65 (510-790)	10.22	10.24	1.00	1.01 NS
Q25 (Hz) (35-41)	2532+/-378 (1757-3280)	2352+/-593 (760-3694)	21.02	21.20	1.04	2.04 NS
Q50 (Hz) (35-41)	3404+/-379 (2625-4107)	3422+/-477 (2350-4620)	12.70	12.62	1.01	0.09 NS
Q75 (Hz) (35-41)	4958+/-794 (3474-6837)	4685+/-685 (3520-7230)	15.53	15.41	1.01	3.07 NS
F0WakeB (Hz) (27-31)	1219+/-447 (640-2070)	1432+/-374 (840-1910)	31.65	31.68	1.00	4.37*
Q25B (Hz) (27-31)	2787+/-630 (1590-3980)	2893+/-661 (1610-3840)	23.08	23.34	0.99	0.02 NS
Q50B (Hz) (27-31)	3698+/-430 (3000-5080)	3733+/-543 (3050-5250)	13.24	13.20	1.00	0.03 NS
Q75B (Hz) (27-31)	6236+/-1848 (3140-11130)	6530+/-2371 (3350-11530)	33.46	33.25	1.01	0.05 NS

Table 2: Sexual signature in the long-call in Middleton population of kittiwakes. For each parameter, the number of observations is given by the number in brackets (first number for females, second for males). We give the mean +/- standard deviation (minimum-maximum) for each parameters and each sex. PSC (potential of sex coding) is the quotient of the coefficient of variation between sexes (CVb) by the coefficient of variation intra-sex (CVw). Its significance was tested by a Kruskal-Wallis test (last column, first number is the χ^2 and significance of the test follows using: * if $p < 0.05$, NS = non significant).

distinctions become non-significant after Bonferoni correction for multiple tests. Furthermore, in both cases, the PSC was very close to 1, indicating that these variables are not likely to code for sex.

Regional differentiation

In Hornøya, kittiwakes were recorded only during the pre-incubation period (Aubin, et al. 2007). Thus, we only kept calls coming from this same period in Middleton to compare both populations. To avoid pseudo-replications, the data set used in the following analysis was composed of only one randomly chosen call per individual.

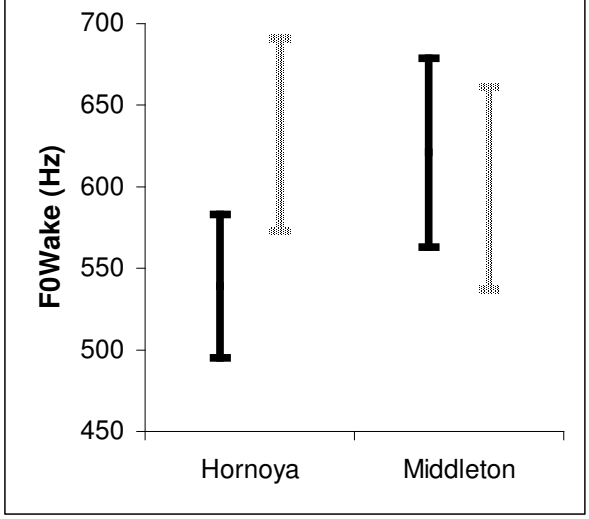


Figure 1 : F0Wake differences between males and females from the two populations. In black are the females, in grey the males (+/- standard deviation).

All temporal variables (except DurTiWk) were found to differ significantly between the two populations (see Table 3): Middleton individuals have a longer "ki", but a shorter silence between the "ki" and the "ti" (often absent, while always present for Hornøya kittiwakes), a shorter "ti", a shorter "wake" and finally a shorter call (i.e. DurIntKi) than Hornøya individuals (cf. Table 3 for test values). Middleton individuals distribute their energy in higher frequencies (Q25, Q50 and Q25B were significantly higher) and start their "wake" at a significantly higher frequency than Hornøya ones. Using principal components calculated with these significant variables, we were able to correctly classify 100% of the calls (26 coming from Hornøya, 83 from Middleton). Using temporal variables only, we still correctly classify 100% of the calls (5 variables), but the discrimination drops to 76% using frequency variables only (3 variables).

Parameters	Hornøya	Middleton island	CVb	CVw	PPC	Kruskal
DurKi (ms) (28-83)	44.7+/-25 (18-150)	69.5+/-16 (38-118)	34.08	39.8	0.86	34.58***
DurKiTi (ms) (28-83)	48.9+/-16 (14-93)	14.6+/-24 (0-91)	114.44	97.86	1.17	37.45***
DurTi (ms) (32-83)	84.5+/-28 (30-143)	64.5+/-17 (24-111)	32.04	29.85	1.07	16.10***
DurTiWk (ms) (32-83)	112.9+/-77 (38-372)	91.6+/-45 (0-248)	58.08	59.33	0.98	0.59 NS
DurWake (ms) (32-83)	474.3+/-234 (237-865)	178.7+/-38 (102-267)	59.09	25.06	2.36	67.07***
DurWakeBeg (ms) (17-74)	387.7+/-147 (138-659)	117.7+/-38 (48-199)	75.91	35.38	2.14	38.33***
DurIntKi (ms) (30-83)	932.4+/-115 (710-1165)	569.8+/-64 (425-727)	27.05	11.89	2.28	65.32***
F0WakeBeg (Hz) (32-83)	604+/-103 (450-857)	651+/-66 (478-824)	12.60	13.63	0.92	8.23**
F0Wake (Hz) (32-83)	598+/-82 (447-876)	609+/-65 (460-800)	11.60	12.28	0.94	0.90 NS
Q25 (Hz) (32-83)	2049+/-326 (1412-2625)	2534+/-439 (1130-3568)	19.39	16.71	1.16	29.79***
Q50 (Hz) (32-83)	3095+/-425 (2206-4532)	3431+/-429 (2472-4140)	13.57	13.19	1.03	13.40***
Q75 (Hz) (32-83)	4844+/-613 (3735-6246)	4718+/-929 (3550-9120)	17.97	16.25	1.11	2.78 NS
F0WakeB (Hz) (14-74)	1506+/-436 (485-1866)	1259+/-456 (360-2030)	35.49	32.89	1.08	3.54 NS
Q25B (Hz) (14-74)	2172+/-574 (1680-3480)	2811+/-633 (1550-3860)	24.58	24.76	0.99	9.84**
Q50B (Hz) (14-74)	3285+/-927 (1800-4930)	3572+/-570 (2490-7100)	18.26	22.37	0.82	2.53 NS
Q75B (Hz) (14-74)	5420+/-1027 (3790-6980)	6182+/-1881 (3090-12250)	29.61	24.90	1.19	1.81 NS

Table 3: Differences between Hornøya and Middleton kittiwake long-calls. For each parameter, the number of observations is given by the number in brackets (first number for Hornøya, second for Middleton). We give the mean +/- standard deviation (minimum-maximum) for each parameters and each population. PPC (potential of population coding) is the quotient of the coefficient of variation between populations (CVb) by the coefficient of variation intra-population (CVw). Its significance was tested by a Kruskal-Wallis test (last column, first number is the χ^2 and significance of the test follows using: ** if $p < 0.01$, *** if $p < 0.001$, NS = non significant)

None of the three variables found to code for gender in Hornøya (DurIntKi, F0Wake and F0WakeBeg, Aubin, et al. 2007) vary significantly between sexes in Middleton. In fact, F0Wake in males from Hornøya is not significantly different from the one in both males and females from Middleton (figure 1). Results are similar for F0WakeBeg.

Discussion

Consistently with Aubin, et al. 2007, our acoustic analysis shows a clear individual signature in the long-call. Discriminant analyses were able to classify most of the calls according to the year or to the season period, showing that individual signature might change over the season and over the year. However, most misclassified calls remain closer to calls uttered by the same individual at a different period than to other individuals' calls. Thus, individual signature changes over time, but seems stable enough to allow recognition, confirming a preliminary study realized over a 6 year period (cited in Wooller 1978). Individuals might certainly use other acoustic features not taken into account in our analyses, allowing them to correctly discriminate the others in all cases.

Surprisingly, and contrary to the results obtained by Aubin, et al. 2007 in Hornøya, we did not find any sexual dimorphism in Middleton population. Sex assessment by calls should be important for species with monomorphic adult plumage, and the strong sexual differences found in long-calls of Hornøya should be a common feature in all kittiwake populations. Since it is apparently not the case, Middleton adults may rely on other parameters, such as other calls or precise visual clues, to distinguish potential mates from competitors. Sexes are easily distinguishable according to morphometrical and behavioral measures in both populations (Baird 1994, Chardine 2002, Jodice, et al. 2000) and such gender differences might be stronger in Pacific than in Atlantic populations. Vocal parameters may then evolve free from any selective pressure, and sexual signature might disappear. However, sexual assessment is not always possible according to visual features only, particularly in foggy weather.

Middleton Island long-calls differed strongly from Hornøya's. Most of the temporal variables were different, Middleton's long-calls being much shorter than Hornøya's, only te "ki" part of the call being longer in Middleton. Some frequency variables also differed, Middleton adults starting their "wake" at a higher frequency and giving more energy to high frequency in the "wake" than Hornøya adults. A striking feature is the disappearance of the gender signature in the fundamental frequency of the long-calls of Middleton's kittiwakes. Thus, since Hornøya and Middleton populations are clearly distinguishable according to the long-call, these two types of long-calls could be seen as two different "dialects", a term usually restricted to geographic differences that arise due to learning. To our knowledge, this is the first example of geographic differences in non-oscine birds.

Dialects have long been studied in oscine birds (e.g. Chilton & Lein 1996, Searcy, et al. 2002) and song divergence is thought to be one of the driving causes of bird speciation (Irwin 2000, Slabbekoorn & Smith 2002). However, such passerines have the opportunity to learn the song from their parents, dialects being then transmitted from one generation to the next (Nelson & Marler 1994). In non-oscine birds such as black-legged kittiwakes, there is no evidence of long-call learning. Since acoustic structure of the long-call should thus depend on morphological constraints, acoustic divergences will reflect important differences in the vocal apparatus of the two populations. In addition, it is well known that when vocal signals differ between two distant populations, this provides a first clue that the genotypes of the birds in the populations differ too (Kroodsma 2004)

Indeed, Atlantic and Pacific populations differ in other variables that acoustic features. Pacific kittiwakes are on average heavier, have a longer culmen and wing than Atlantic's

(Sluys 1982), and differ in their wingtip patterns (Chardine 2002). Genetic differences (McCoy, et al. 2005) show also that these two populations have diverged for a long time; their status as two subspecies or two populations of the same species is still debated, since there are important overlaps in term of morphology between the two groups (Chardine 2002, Sluys 1982). All these differences in size and weight may explain a part of the differences in vocal features, and particularly in frequency, individuals having different resonance patterns and perhaps different syringal membrane lengths. However, this is difficult to predict, as differences in size do not systematically correlate with differences in song (Slabbekoorn & Smith 2000). Furthermore, there is little reason for morphology to explain differences in temporal variables. Such differences may perhaps come from a cryptic learning process, adults imitating the call from other conspecifics, or remembering the call from their parents. Calls uttered by chicks are very different from the adult ones, and are not easily comparable: cross-fostering experiments would be needed to elucidate if cryptic call learning occurs in a non-oscine bird such as kittiwakes.

Acoustic characteristics may also vary with the environment. Songbirds have been found to adapt their song to the resonance capacity of the environment (e.g. Tubaro & Segura 1995, Van Dongen & Mulder 2006) and such a process may be more general among birds. However, we do not have any indication that environment differ significantly, as the two studied populations leave in similar open areas.

Previous studies on kin recognition have shown that kittiwakes use the long-call to recognize their mates (Wooller 1978) and their parents (H. Mulard et al., unpubl. data). However, the level of response to kin calls is low in comparison to other Larids (e.g. Laughing gulls Beer 1969, Black-headed gulls Charrier, et al. 2001, or Slender-billed gulls Mathevon, et al. 2003), indicating that the selective pressure on the acoustic parameters of the long-call may be weak in kittiwakes. Thus, the call may evolve rapidly, and lead to the apparition of different dialects. Interestingly, morphological differences seem to evolve from Arctic Canada counterclockwise around the Arctic to the Pacific (Chardine 2002). Here we show acoustic distinctions between two very far located populations on this gradient, but further studies would be needed to see if close located populations are also distinguishable on acoustic features, and how vocal divergence evolve around the Arctic ocean. Furthermore, the temporal evolution of long-calls found in Middleton population may also indicate that the call in a non-oscine bird such as the kittiwake is sufficiently plastic to enable divergences on the long term. Since no genetic structure has yet been found in close populations such as Atlantic colonies (McCoy, et al. 2005), such measurement help to identify what among the

morphological, the behavioral, the acoustic and the genetic variables is the main source of divergence, and potentially of speciation, between seabirds populations.

Bibliography

Aubin, T. 2004. Penguins and their noisy world. *Anais da Academia Brasileira Ciências*, **76,** 279-283.

Aubin, T. & Jouventin, P. 2002. Localisation of an acoustic signal in a noisy environment: the display call of the king penguin *Aptenodytes patagonicus*. *Journal of Experimental Biology*, **205,** 3793-3798.

Aubin, T., Mathevon, N., Staszewski, V. & Boulinier, T. 2007. Acoustic communication in the kittiwake *Rissa tridactyla*: potential cues for sexual and individual signatures in long calls. *Polar Biology*.

Baird, P. H. 1994. Black-legged kittiwake. In: *The Birds of North America*, pp. 1-24: A. Poole and F. Gill, Editors.

Beer, C. G. 1969. Laughing gull chicks: recognition of their parents' voices. *Science*, **166,** 1030-1032.

Chardine, J. W. 2002. Geographic variation in the wingtip patterns of black-legged kittiwakes. *Condor*, **104,** 687-693.

Charrier, I., Mathevon, N., Jouventin, P. & Aubin, T. 2001. Acoustic communication in a black-headed gull colony: how do chicks identify their parents ? *Ethology*, **107,** 961-974.

Chilton, G. & Lein, M. R. 1996. Long-term changes in songs and song dialect boundaries of puget soung white-crowned sparrows. *Condor*, **98,** 567-580.

Danchin, E. 1987. The behaviour associated with the occupation of breeding site in the kittiwake gull *Rissa tridactyla*: the social status of landing birds. *Animal Behaviour*, **35,** 81-93.

Ellers, J. & Slabbekoorn, H. 2003. Song divergence and male dispersal among bird populations: a spatially explicit model testing the role of vocal learning. *Animal Behaviour*, **65,** 671-681.

Evans, R. M. 1970. Parental recognition and the 'mew call' in Black-billed gulls (*Larus bulleri*). *Auk*, **87,** 503.

Falls, J. B. 1982. Individual recognition by sounds in birds. In: *Acoustic communication in birds* (Ed. by Kroodsma, D. E. & Miller, E. H.), pp. 235-278. New York: Academic Press.

Gill, V. A. & Hatch, S. A. 2002. Components of productivity in black-legged kittiwakes *Rissa tridactyla*: response to supplemental feeding. *Journal of Avian Biology*, **33,** 113-126.

Grant, P. R. & Grant, B. R. 1997. Genetics and the origin of bird species. *Proceedings of the National Academy of Sciences USA*, **94,** 7768-7775.

Hutchinson, R. E., Stevenson, J. G. & Thorpe, W. H. 1968. The basis for individual recognition by voice in the Sandwich tern (*Sterna sandvicensis*). *Behaviour*, **32,** 150-157.

Irwin, D. E. 2000. Song variation in an avian ring species. *Evolution*, **54,** 998-1010.

Jodice, P. G. R., Lanctot, R. B., Gill, V. A., Roby, D. D. & Hatch, S. 2000. Sexing adult black-legged kittiwakes by DNA, behavior, and morphology. *Waterbirds*, **23,** 405-415.

Kroodsma, D. E. 2004. The diversity and plasticity of birdsong. In: *Nature's music* (Ed. by Marler, P. & Slabbekoorn, H.): Elsevier Academic Press.

Kroodsma, D. E. & Miller, E. H. 1996. *Ecology and evolution of acoustic communication in birds*. Ithaca, New York: Cornell University Press.

Mathevon, N., Charrier, I. & Jouventin, P. 2003. Potential for individual recognition in acoustic signals: a comparative study of two gulls with different nesting patterns. *Comptes-Rendus Biologies*, **326,** 329-337.

McCoy, K. D., Boulinier, T. & Tirard, C. 2005. Comparative host-parasite population structures: disentangling prospecting and dispersal in the Black-legged kittiwake *Rissa tridactyla*. *Molecular Ecology*, **14,** 2825-2838.

Nelson, D. A. & Marler, P. 1994. Selection-based learning in bird song development. *Proceedings of the National Academy of Sciences USA*, **91,** 10498-10501.

Price, T. D. 1998. Sexual selection and natural selection in bird speciation. *Philosophical Transactions of the Royal Society of London B*, **353,** 251-260.

Searcy, W. A., Nowicki, S., Hughes, M. & Peters, S. 2002. Geographic song discrimination in relation to dispersal distances in song sparrows. *American Naturalist*, **159,** 221-230.

Slabbekoorn, H. & Smith, T. B. 2000. Does bill size polymorphism affect courtship song characteristics in the African finch *Pyrenestes ostrinus*? *Biological Journal of the Linnean Society*, **71,** 737-753.

Slabbekoorn, H. & Smith, T. B. 2002. Bird song, ecology and speciation. *Proceedings of the Royal Society of London B*, **357,** 493-503.

Slabbekoorn, H. 2004. Singing in the wild: the ecology of bird song. In: *Nature's music, the science of birdsong* (Ed. by Marler, P. & Slabbekoorn, H.), pp. 178-205.

Sluys, R. 1982. Geographical variation of the kittiwake. *Rissa tridactyla*. *Le Gerfaut*, **72,** 221-230.

Tubaro, P. L. & Segura, E. T. 1995. Geographic, ecological and subspecific variation in the song of the rufous-browed peppershrike (*Cyclarhis gujanensis*). *Condor*, **97,** 792-803.

Van Dongen, W. F. D. & Mulder, R. A. 2006. Habitat density, song structure and dialects in the Madagascar paradise flycatcher *Terpsiphone mutata*. *Journal of Avian Biology*, **37,** 349-356.

Wooller, R. D. 1978. Individual vocal recognition in the kittiwake gull, *Rissa tridactyla* (L.). *Zeitschrift für Tierpsychology*, **48,** 68-86.

ARTICLE 2 : Experimental evidence of vocal recognition in young and adult black-legged kittiwakes

Hervé Mulard, Thierry Aubin, Joël F. White, Scott A. Hatch, Étienne Danchin

Will be submitted to *Behavioural Processes*

Abstract

Individual recognition is required for mate choice or parental care, as well as most social interactions and games. Not surprisingly so, its presence has been confirmed in many species. In birds, vocal cues appear to be the major components of recognition. However, vocal recognition seems to be surprisingly absent or limited in highly social species such as the black-legged kittiwake (*Rissa tridactyla*). It has been argued that in that cliff-nesting species individual recognition has been efficiently replaced by nest site recognition and specific constraints linked to the vertical location of the nest, the lack of evidence of parent-offspring recognition being thus generally interpreted as adaptive. Here we provide the first experimental evidence that chicks recognize their parents, this capacity being detectable as soon as 20 days after hatching, the earliest date that we tested. We also experimentally confirm mate recognition, and demonstrate that only a part of the long call is sufficient to elicit mate recognition, showing redundancy in individual signature. However, only about a third of the tested birds reacted to their mate's or parents' calls, and we were unable to document recognition among neighbours. We discuss this low reaction level in relation to the cliff-nesting habit as well as the life-history of this species, and compare our results to those on vocal recognition studies in other Larids.

Introduction

Most social interactions or games (e.g., the mechanisms of evolution of cooperation, mutualism or mating systems, see Bateson 1978, Emlen 1994, Hamilton & May 1977) assume the existence of individual recognition. Interactions between sexual mates are for example especially important in species with cooperative parental care. In monogamous long-lived species, mate recognition is also assumed for parental coordination. In species forming pair bonds over several reproductive episodes, mate recognition is also assumed to last over years. Parents further need to recognize their young to avoid misdirected parental care, and young need to recognize their parents to avoid infanticide or simply to solicit parental care.

Similarly, several hypotheses about the evolution of divorce implicitly assume the recognition of mates and neighbours (Cézilly, et al. 2000). Kin recognition is also a major element of inbreeding avoidance, etc.

Consequently, individual recognition has been the focus of many studies in various taxa. In birds, the stress has been mainly put on vocal recognition. Among Larids for example, chicks of laughing gulls (*Larus atricilla*, Beer 1969), black-headed gulls (*L. ridibundus*, Charrier, et al. 2001) and black-billed gulls (*L. bulleri*, Evans 1970) recognize their parents vocally. Black-headed and slender-billed gulls voices (*L. genei*, Mathevon, et al. 2003) have also been shown to be individually distinct.

In the cliff-nesting black-legged kittiwake (*Rissa tridactyla*), the question of vocal recognition remains surprisingly unresolved. If Wooller (1978) found evidence for vocal recognition between mates, Aubin *et al.* were unable to detect any recognition during incubation in Hornøya (Norway; pers. comm.). It is also unclear what part of the long-call contains the individual signature. Furthermore, in that species, parent-offspring recognition has rarely been studied: Cullen (1956) showed that adults do not recognize their chicks before 25 days of age, and Storey, et al. (1992) found that kittiwake chicks' call carry much weaker individual cues than closely related species such as the Herring gull (*L. argentatus*). Parent recognition by chicks has never been studied in this species.

In a previous study, we provide observational evidence that black-legged kittiwake chicks may recognize their parents at the time of their first flight (Mulard & Danchin, *submitted*). Here we report on an experimental study of mate, neighbour and parent/offspring recognition. Black-legged kittiwakes are strictly monogamous (Helfenstein, et al. 2004) and highly faithful to their mate across years (Coulson 1966, Naves, et al. 2006). Mate fidelity has further been experimentally shown to result from individual rather than nest site recognition (Fairweather & Coulson 1995). Despite the lack of evidence, we hypothesized that being long-lived (Cam, et al. 2002, Hatch, et al. 1993), kittiwakes recognize and memorize their mate, as well as their neighbours over several years.

Methods

Study population

Middleton Island (north-central Gulf of Alaska, 58°25' N, 146°19' W) supports a large population of black-legged kittiwakes (25 000 birds in 1999, Gill & Hatch 2002). We studied

kittiwakes nesting on an abandoned US Air Force radar tower enabling close observation and easy capture (Gill & Hatch 2002). The study plot was on vertical walls with uniform nest spacing and wooden ledge size. Nest sites were visited twice daily from early May to mid August 2006 to assess individual attendance and reproductive success. Chicks were marked at hatching and adults were banded using US metal and 4 coloured bands. Precise laying and hatching dates were thus obtained.

Recording and building playback samples

Long-calls (Cullen 1956, Danchin 1987, Tinbergen 1953, 1959, Wooller 1978, Wooller 1979), were recorded from individuals either during landing or while resting on the nest. A AKG D770 microphone, connected to a Marantz PMD670 recorder, was placed directly on the nest. Calls were thus recorded from less than 30 cm away. Sounds were modified using the CTWave32 software (Creative Technology Ltd), by silencing either the "kitti" or the "wake" part, without changing the general rhythm of the long-call. Sound tracks were broadcasted with a Marantz MA6100 and Audax AP080M4 loudspeakers. Every broadcasted track contained 10 repetitions of the "kitti-wake" (see Aubin et al. 2007 for the detailed sonogram and nomenclature of the different parts of the call). All playbacks were made in late July and early August (during the chick rearing period), using records made during the 25 days preceding the broadcasting.

Test of chick recognition capacities

Chicks were tested repeatedly at 20, 25 and 30 days of age. Older chicks were not tested because of risks of premature fledging. Chicks were put in the middle of a 2.5 m×65 cm table in the tower. Each side of the table had a loudspeaker, each call being broadcasted from a randomly chosen side of the table. During each session, we played-back successively up to 9 calls in random order: 2 unmodified parental calls (1 for each parent), 2 modified parental calls containing only the 'kitti' part, 2 modified parental calls containing only the 'wake' part, 2 unmodified calls coming from unknown adults, and 1 call coming from a neighbouring breeder. Calls coming from unknown adults (calls recorded at least 10 nests away from that of the tested chick) were chosen randomly. Each test call was separated from the next by at least 1 minute, the time to place the chick back in the middle of the setting and for it to calm down. A chick moving more than 40 cm toward the working loudspeaker and/or calling back in

response was recorded as reacting to the test call. When analyzing the data, a chick reacting to at least one of its 3 mother (father) calls (i.e. unmodified call, 'kitti' call and 'wake' call) was then recorded as reacting to its mother (father) (cases "all mother" and "all father" in Figure 1). Similarly, a chick reacting to at least one of the 6 parental calls was recorded as reacting to its parents (case "all parents" in Figure 1).

Test of breeder recognition capacities

Calls were played back to chick rearing individuals from an observation point circa 40m from the tower foot. Adult behaviour on 4-6 surrounding nests was video taped. We played back long-calls of every individual recorded in this local area (3 sorts of calls per individual: unmodified, or containing only the "kitti" or the "wake") and 6 unmodified calls of adults coming from another area (i.e. unknown individuals). All these calls were played back in random order every 20s, with calls coming from the same individual never played back at a time interval shorter than 5min, enabling the focal bird to calm down. Video tapes were analysed blindly. Reactions were recorded between the beginning of the broadcasting until 10 seconds after the end of each broadcasted sound track. When the adult did not stay completely immobile on the nest, we observed only two kinds of reactions: the adult either suddenly moved its head and looked towards the loudspeaker, and/or called back in response. When analysing the data, an adult reacting to at least one of its 3 mate calls (unmodified, 'kitti' or 'wake') was recorded as reacting to its mate (case "all mates" in Figure 2). We also recorded the behaviour of neighbours. For this last analysis, we only kept observations where the focal bird (i.e. the bird who heard its own call or the call of its mate) did not react, to control for the possibility of a reaction of neighbours to the focal bird's reaction.

Statistics

We used paired t-test (TTEST procedure, SAS® package, SAS Institute 1999) to compare reactions of a given individuals to different calls. For the trend in chick reaction, we used a GLM model (GLM procedure). Repeatability was analysed through correlation between the states observed at two different times (CORR procedure).

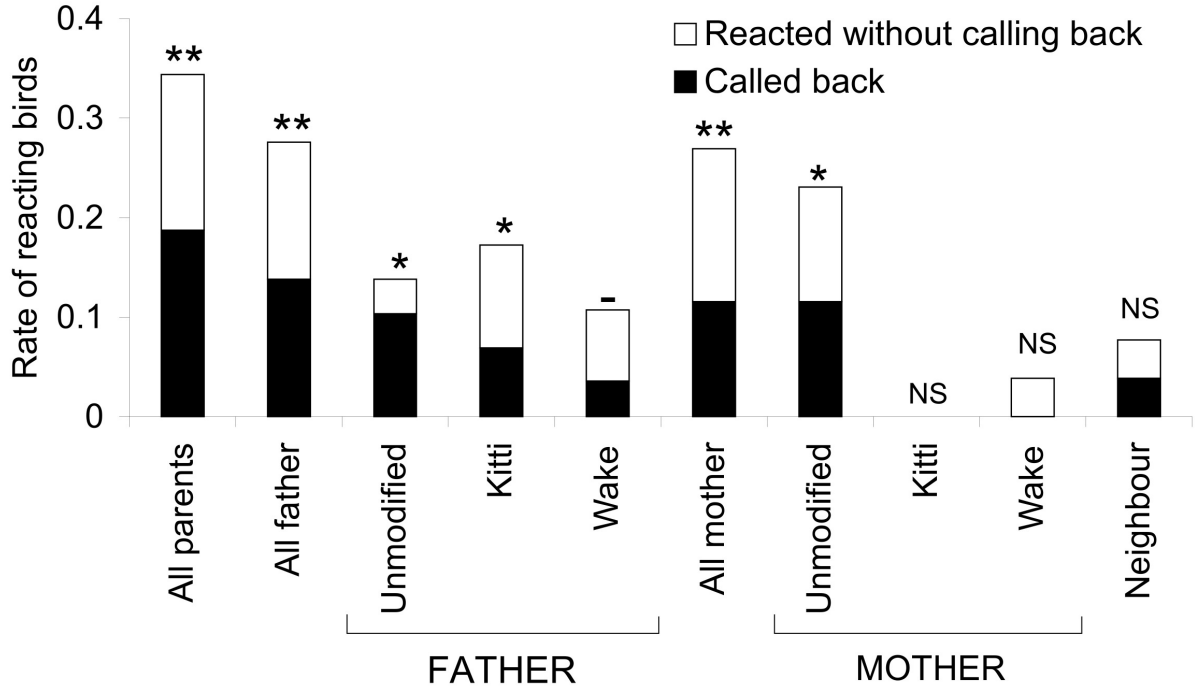


Figure 1: Chicks' reactions at 20 days old to parental calls. Each bar represents the total proportion of reacting chicks: in black when chicks called back in response, in white when chicks only weakly reacted, without calling back. For each parent, 3 different calls (1 unmodified and 2 modified) were broadcasted; if the chick react to at least one of these, then it was recorded as reacting to its parent, and the maximal level of reaction was kept to build the "all mother/all father/all parents" bars. Significance of paired t-test (between reaction to tested call and to unknown bird's call) is given by : NS for non significant, - for $p<0.1$, * for $p < 0.5$, ** for $p < 0.01$.

Results

Chick recognition capacities

35 chicks coming from 33 pairs were tested for recognition, but only 30 chicks were tested at least twice (because chick mortality is high on Middleton, Gill, et al. 2002, Roberts & Hatch 1993: 2 chicks died between 20 and 25 days old, 2 others between 25 and 30 days old; 3 chicks were tested only at 30 days old and 2 chicks were not tested at 25 days old). Chicks always reacted more or equally to parental calls than to calls coming from other adults. When results coming from different ages were pooled together, 19 chicks (54%) reacted more to parental calls (modified or not) than to unknown adults. Respectively, 11 chicks out of 32 (34%) reacted to parental calls at 20 days, 10 out of 28 (36%) at 25 days old,

and 12 out of 31 (39%) at 30 days old, showing a slight though non significant increase ($F = 0.2$, $p = 0.65$) of the level of reaction with age.

Chick behaviour to parental calls was repeatable: 47% (14 out of 30) of the chicks tested at least twice never reacted, and 33% reacted at least at two different ages (correlation analyses: between 20 and 25 days old: $r = 0.35$, $p = 0.06$; between 25 and 30 days old: $r = 0.42$, $p = 0.03$; between 20 and 30 days old: $r = 0.54$, $p = 0.003$). Thus, only results for 20 days old chicks are presented (Figure 1) since (1) they show the early recognition capacities of chicks and (2) they did not differ significantly from later tests.

Chicks reacted in a similar way to their mothers' or fathers' unmodified calls. Chicks also still recognize their fathers when only the "kitti" part of the long-call was played (paired t-test between unknown unmodified call and father's "kitti": $p < 0.05$), and tend to recognize their fathers when only the "wake" part was played (paired t-test: $p < 0.1$). However, we did not find any significant reaction when only parts of the mother's call were played (paired t-tests: $p > 0.1$). There were no correlation between reaction and the position of the call in the test session (chicks react for the first time at the 4-5th (ranging from the 1st to the 9th) broadcasted call on average).

Chicks did not react significantly more to neighbours than to unknown adults. In all play-backs, only two chicks called back to a neighbour call, and these two cases were highly particular. In the first, the chick also called back to its mother calls during the same session, and this behaviour might thus be due to an overreaction of the chick. In the second, the chick lost its father some days before the test and was let alone on the nest most of the time with the possibility to hear the voice of the neighbouring adult that we tested as it was breeding only 20 cm away.

D. Breeder recognition capacities

Adults never reacted to unknown individuals. Furthermore, out of 28 tested breeders, only 1 (4%) moved its head in response to its own unmodified call, whereas 9 adults (32%, see figure 2) responded to their mate's unmodified call, 7 of them even called back in response (25%). Thus, individuals reacted significantly more to their mate's calls than to calls of any other individual (paired t-test, $t = 3.58$, $p = 0.0013$, $n = 28$). There were no significant differences between males and females, as 4 out of 12 females and 5 out 16 males responded to their mate's calls.

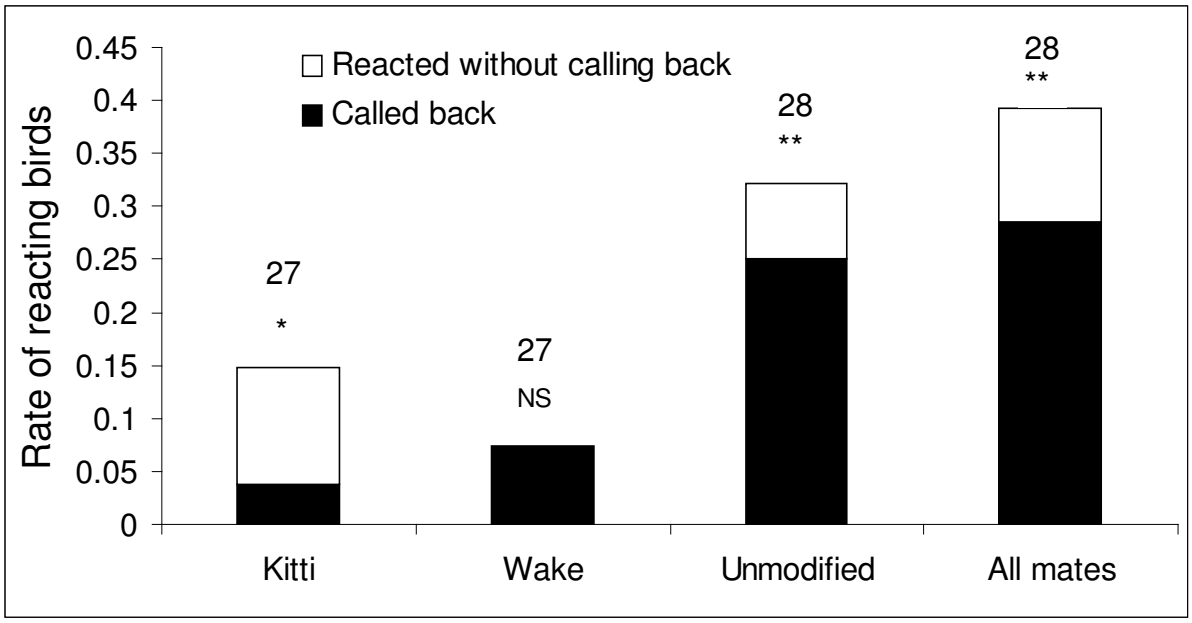


Figure 2: Adults' reactions to mate calls during chick rearing. Each bar represents the total proportion of reacting adults: in black when mates called back in response, in white when mates only reacted by head movements. For each adult, 3 different mate calls (1 unmodified and 2 modified) were broadcasted; if the adult reacted to at least one of these, then it was recorded as reacting to its mate (case "all mates"). The number of tested adults is given on each bar. Difference between reaction to mate versus unknown unmodified call has been tested and the significance of the paired t-tests are given by: NS for non significant, * for $p < 0.5$, ** for $p < 0.01$.

One individual was not tested for reaction to its mate's modified calls. Out of the 27 remaining tested birds, two individuals reacted to their mate's modified calls, although they did not react to unmodified calls. 4 adults (15%, 1 female and 3 males) reacted to calls containing only the "kitti" part. One of them called back in response. Two birds (7%, 1 male and 1 female) reacted and called back to calls containing only the "wake" part. Thus, adults reacted to their mates' calls that contained only the "kitti" part (paired t-test: $t = 2.13$, $p = 0.04$) but not to those that contained only the "wake" part ($t = 1.44$, $p = 0.16$).

Concerning the recognition of the voice of neighbours, we recorded 155 situations in which the focal bird did not react to its own or to its mate calls. Only one adult moved its head in response to a neighbour call, indicating that adult did not react significantly more to their neighbours' calls than to non-neighbour adults.

II. Discussion

Chicks reacted significantly more to their parents than to other adults. This study is the first, to our knowledge, to document parent recognition by young kittiwake chicks. Despite the fact that the experiment may stress the chicks, about a third of them did react as soon as 20 days of age, and this reaction was repeatable. This relatively weak reactivity may indicate either the effect of the stress from the experiment or a weak selective pressure on parent recognition by chicks in this species. Most kittiwakes in our population raise only one chick until fledging (Gill, et al. 2002, Roberts & Hatch 1993). Thus most chicks do not have to fight for food and parental care with siblings, and therefore they may not need to react immediately after their parents' landing. Alternatively, since parental attendance remains high before chicks are 20-25 days old (Roberts & Hatch 1993), selective pressure on chicks to recognize their parents may emerge only afterwards. However the latter explanation is inconsistent with the facts that 34% of the chicks reacted to their parents' calls at 20 days of age, and that this proportion did not increase significantly with time.

Our results further show that chicks are also able to recognize their parents' calls when only a part of it (and particularly the "kitti" part) is broadcasted. This suggests redundancy in individual signature that can be seen as facilitating the detection of individuals in the noisy environment of a kittiwake colony (Danchin & Nelson 1991). This also confirms a previous study (Aubin, et al. 2007) showing that individual signature was not coded particularly in one variable, but was carried by multiple vocal variables.

Our results confirm that adults are able to recognize their mates according to vocal cues (Wooller 1978). As for chicks, only a part of the call may be sufficient to elicit a reaction of the mate. However, only a third of the tested adults did react. This low proportion is consistent with the observation that most birds called back to their mates' pre-landing calls only less than one second before the actual landing, that is, when the mate was already very close. Only a low proportion of adults called back long before the landing of their mates, indicating that in most cases, vocal cues may not be sufficient to elicit a reaction. Visual and behavioural cues may thus also convey information about the identity of the incoming adult. Indeed, after landing in the presence of their mate, individuals perform a complex display including vocalizations and head movements (Baird 1994, Danchin 1987, Heath, et al. 1982) that may enable them to confirm their identities visually and behaviourally. Furthermore, fights involve also numerous vocalizations (calls, bow and moan, choking, etc.), that may have a role both in nest ownership and recognition. Wooller (1978) further indicated that

adults did not react if the sound was broadcasted from inside the building, suggesting that the origin of the sound may also matter and explain the overall weak reaction of adult kittiwakes to playbacks. Furthermore, breeder's reaction to the voice of their mate might be more obvious during pair formation, than during chick rearing. Accordingly, Wooller (1979) found a peak of vocalization at the beginning of the reproductive season, supporting the idea that vocalizations are important for individual recognition at this early stage. This is in agreement with the fact that pair formation essentially involves repeated arrival and departure in the kittiwake (Danchin & Nelson 1991).

Surprisingly enough, we were unable to detect any recognition between neighbours: neither adults, nor chicks, reacted differently to calls coming from close neighbours than to calls from more distant adults. Many social interactions and games assume individual recognition between interacting individuals and it would be surprising that individuals of a highly social species such as the kittiwake could not recognize numerous neighbours. Observations in natural conditions indeed strongly suggest that breeders do recognize their neighbours. Adults, for instance, often chase prospectors on neighbouring nests, suggesting that they do recognize non local individuals. However, prospectors usually land silently on nests (Danchin 1987, Danchin, et al. 1998) and neighbours may thus identify such adults as foreigners thanks to their behaviour, and not to their vocal signature. Alternatively, adults might recognize their neighbours but have no reason to react in a detectable way. Indeed, only squatters (i.e. individuals temporarily occupying a nest occupied by a pair, Monnat, et al. 1990) might endanger the focal individual's reproduction, as they may provoke high levels of disturbance (fights, harassments, etc. Cadiou, et al. 1994). Neighbours may thus not react detectably to their neighbours as long as they do not endanger their own breeding success, but this absence of reaction does not necessarily mean absence of recognition.

However, the level of reaction in Black-legged kittiwakes appears to be one of the lowest among Larids, a likely derived trait resulting from its cliff-nesting habit. For example, Mathevon, et al. (2003) showed that the potential for chick individual coding is less efficient in the nidiculous black-headed gull than in the nidifugous slender-billed gull. Most Larids studied so far have been shown to have accute mate recognition (e.g. in little terns, *Sterna albifrons*, Moseley 1979) or recognition of parents (e.g. in black-headed gulls, Charrier, et al. 2001; or laughing gulls, Beer 1969, Beer 1970). In some species, there might also be a change of recognition with age, chicks being able to recognize their parents only after fledging (e.g. in black-billed gulls, Evans 1970). In black-legged kittiwakes adults and chicks are highly

constrained on the nest, and the low level of reaction may thus be due to the lack of selective pressure on individual recognition.

The origin, function and evolution of songs and calls have been intensively studied in songbirds, but less is known about the role of calls in non-oscine birds. Calls appears to have evolved as recognition signals in most seabird species (e.g. Aubin & Jouventin 1998, Beer 1969, Charrier, et al. 2001, Evans 1970, Searby, et al. 2004). This feature has also been found in songbirds (e.g. Gentner & Hulse 1998), and these birds use both vocal behaviour to assess mate quality (e.g. Garamszegi, et al. 2004, Poesel, et al. 2006). Further studied are needed to know whether features of long-calls also function as signals of mate quality.

Acknowledgments

We would like to thank A. Lambrechts, M. Kryloff, M. du Toit, J. Kotzerka and C. Gouraud who helped during the experiments. Experiments were carried out in accordance to the American laws. This study was financed by a 4-year grant from the French Polar Institute Paul-Emile Victor ("Programme Arctique 429" 2004-2007), and was part of the French GDR2155 "Écologie comportementale".

Bibliography

Aubin, T. & Jouventin, P. 1998. Cocktail-party effect in king penguin colonies. *Proceedings of the Royal Society of London B*, **265,** 1665-1673.

Aubin, T., Mathevon, N., Staszewski, V. & Boulinier, T. 2007. Acoustic communication in the kittiwake *Rissa tridactyla*: potential cues for sexual and individual signatures in long calls. *Polar Biology*.

Baird, P. H. 1994. Black-legged kittiwake. In: *The Birds of North America*, pp. 1-24: A. Poole and F. Gill, Editors.

Bateson, P. P. G. 1978. Sexual imprinting and optimal outbreeding. *Nature*, **273,** 659-660.

Beer, C. G. 1969. Laughing gull chicks: recognition of their parents' voices. *Science*, **166,** 1030-1032.

Beer, C. G. 1970. On the responses of Laughing gull chicks (*Larus atricilla*) to the calls of adults I: Recognition of the voices of the parents. *Animal Behaviour*, **18,** 652-660.

Cadiou, B., Monnat, J.-Y. & Danchin, E. 1994. Prospecting in the kittiwake, *Rissa tridactyla*: different behavioural patterns and the role of squatting in recruitment. *Animal Behaviour*, **47,** 847-856.

Cam, E., Link, W. A., Cooch, E. G., Monnat, J.-Y. & Danchin, E. 2002. Individual covariation in life-history traits: seeing the trees despite the forest. *American Naturalist*, **159,** 96-105.

Cézilly, F., Dubois, F. & Pagel, M. 2000. Is mate fidelity related to site fidelity? A comparative analysis in Ciconiiforms. *Animal Behaviour*, **59,** 1143-1152.

Charrier, I., Mathevon, N., Jouventin, P. & Aubin, T. 2001. Acoustic communication in a black-headed gull colony: how do chicks identify their parents ? *Ethology*, **107,** 961-974.

Coulson, J. C. 1966. The influence of the pair-bond and age on the breeding biology of the kittiwake gull *Rissa tridactyla*. *Journal of Animal Ecology*, **35,** 269-279.

Cullen, E. 1956. Adaptations in the kittiwake to cliff-nesting. *Ibis*, **99,** 275-302.

Danchin, E. 1987. The behaviour associated with the occupation of breeding site in the kittiwake gull *Rissa tridactyla*: the social status of landing birds. *Animal Behaviour*, **35,** 81-93.

Danchin, E., Boulinier, T. & Massot, M. 1998. Conspecific reproductive success and breeding habitat selection: implications for the study of coloniality. *Ecology*, **79,** 2415-2428.

Danchin, E. & Nelson, J. B. 1991. Behavioral adaptations to cliff nesting in the kittiwake (*Rissa tridactyla*): convergences with the gannet (*Sula bassana*) and the black noddy (*Anous tenuirostris*). *Colonial Waterbirds*, **14,** 103-107.

Emlen, S. T. 1994. Benefits, constraints and the evolution of family. *Trends in Ecology & Evolution*, **9,** 282-285.

Evans, R. M. 1970. Parental recognition and the 'mew call' in Black-billed gulls (*Larus bulleri*). *Auk*, **87,** 503.

Fairweather, J. A. & Coulson, J. C. 1995. Mate retention in the kittiwake, *Rissa tridactyla*, and the significance of nest site tenacity. *Animal Behaviour*, **50,** 455-464.

Garamszegi, L. Z., Møller, A. P., Török, J., Michl, G., Péczely, P. & Richard, M. 2004. Immune challenge mediates vocal communication in a passerine bird: an experiment. *Behavioral Ecology*, **15,** 148-158.

Gentner, T. Q. & Hulse, S. H. 1998. Perceptual mechanisms for individual vocal recognition in European starlings, *Sturnus vulgaris*. *Animal Behaviour*, **56,** 579-594.

Gill, V. A. & Hatch, S. A. 2002. Components of productivity in black-legged kittiwakes *Rissa tridactyla*: response to supplemental feeding. *Journal of Avian Biology*, **33,** 113-126.

Gill, V. A., Hatch, S. A. & Lanctot, R. B. 2002. Sensitivity of breeding parameters to food supply in Black-legged kittiwakes *Rissa tridactyla*. *Ibis*, **144,** 268-283.

Hamilton, W. D. & May, R. M. 1977. Dispersal in stable habitat. *Nature*, **269,** 578-581.

Hatch, S. A., Roberts, B. D. & Fadely, B. S. 1993. Adult survival of Black-legged kittiwakes *Rissa tridactyla* in a Pacific colony. *Ibis*, **135,** 247-254.

Heath, J., Slater, A., Daniels, D. & Merrifield, S. 1982. Duration of Vocal Behavior During the Greeting Ceremony of the Kittiwake (Rissa-Tridactyla). *Behaviour Analysis Letters*, **2,** 221-225.

Helfenstein, F., Tirard, C., Danchin, E. & Wagner, R. H. 2004. Low frequency of extra-pair paternity and high frequency of adoption in Black-legged kittiwakes. *Condor*, **106,** 149-155.

Mathevon, N., Charrier, I. & Jouventin, P. 2003. Potential for individual recognition in acoustic signals: a comparative study of two gulls with different nesting patterns. *Comptes-Rendus Biologies*, **326,** 329-337.

Monnat, J.-Y., Danchin, E. & Estrella, R. R. 1990. Assessment of environmental quality within the framework of prospecting and recruitment: the squatterism in the Kittiwake. *Comptes-Rendus de l'Académie des Sciences de Paris*, **311,** 391-396.

Moseley, L. J. 1979. Individual auditory recognitino in the least tern (Sterna albifrons). *Auk*, **96,** 31-39.

Naves, L. C., Monnat, J.-Y. & Cam, E. 2006. Breeding performance, mate fidelity, and nest site fidelity in a long-lived seabird: behaving against the current? *OIKOS*, **115,** 263-276.

Poesel, A., Kunc, H. P., Foerster, K., Johnsen, A. & Kempenaers, B. 2006. Early birds are sexy: male age, dawn song and extrapair paternity in Blue tits, *Cyanistes* (formerly *Parus*) *caeruleus*. *Animal Behaviour*, **72,** 531-538.

Roberts, B. D. & Hatch, S. A. 1993. Behavioral ecology of Black-legged kittiwakes during chick rearing in a failing colony. *Condor*, **95,** 330-342.

Searby, A., Jouventin, P. & Aubin, T. 2004. Acoustic recognition in macaraoni penguins: an original signature system. *Animal Behaviour*, **67,** 615-625.

Storey, A. E., Anderson, R. E., Porter, J. M. & McCharles, A. M. 1992. Absence of parent-young recognition in kittiwakes: a re-examination. *Behaviour*, **120,** 302-323.

Tinbergen, N. 1953. *The herring gull' world*. London: Collins.

Tinbergen, N. 1959. Comparative studies of the behaviour of gulls (*Laridae*): a progress report. *Behaviour*, **15,** 1-70.

Wooller, R. D. 1978. Individual vocal recognition in the kittiwake gull, *Rissa tridactyla* (L.). *Zeitschrift für Tierpsychology*, **48,** 68-86.

Wooller, R. D. 1979. Seasonal, diurnal and area differences in calling activity within a colony of kittiwakes *Rissa tridactyla* (L.). *Zeitschrift für Tierpsychology*, **51,** 329-336.

ARTICLE 3: The role of parent-offspring interactions during and after fledging in the Black-legged Kittiwake

Hervé Mulard & Étienne Danchin

Submitted to Behavioural Processes

Abstract

Most bird species endure a high mortality at fledging, and selection should favour parental behaviour diminishing these costs. Post-fledging parental care varies greatly among species and is often linked to parent-offspring recognition. In the Black-legged Kittiwake (*Rissa tridactyla*), fledglings need to return to the natal nest to be fed by their parents until independence. Rejections of fledglings by non-parent adults may be fairly violent, and parents are expected to recognize and help their chicks at the time of first return. However, parent-offspring recognition before fledging has never been shown in kittiwakes. In this paper, we study the behaviour of both parents and chicks at fledging. We found that parents answered significantly more to their fledglings' calls than to those of others. Compared to silent juveniles, juveniles that called before landing were more likely to be accepted by their parents. No such pattern was observed with foreign juveniles, indicating that fledglings' voice may carry identity. Furthermore, fledglings found their way back to the natal nest faster when parents attended the natal nest and reacted to their offspring's calls than when they were absent or inactive. Such interactions may therefore diminish juvenile mortality at fledging.

Key words: black-legged Kittiwake | *fledging* | *parent-offspring recognition* | *post-fledging care* | Rissa tridactyla | *vocal signature*

Introduction

In many birds species, the usually low estimates of juvenile survival rates suggest that fledging constitutes a difficult stage (Parker, et al. 2003, Cam, et al. 2003). For example, mortality at fledging reaches 68% in wood thrush fledglings *Hylocichla mustelina* (Anders, et al. 1997) and 36% in brown thornbill fledglings *Acanthiza pusilla* (Green, & Cockburn 2001). Such high mortalities should favour parents expressing specific parental care at the time of fledging, a pattern that is observed in many bird species (e.g., Middleton, et al. 2007, Draganoiu, et al. 2005).

Post-fledging parental care varies greatly among bird species. In several species, juveniles become fully independent as soon as (or even before) they leave the nest. This is the case of Manx shearwaters (*Puffinus puffinus*), Gannets (*Sula bassana*) and most Procellariforms where the unique fledgling exceeds adult mass by up to 60% (Ydenberg 1989), and fledges by itself after a period of starvation that is often protracted after fledging. In the short-tailed albatross (*Phoebastria albatrus*), for instance, chicks fledge by themselves, soon after adults have deserted the colony (Hasegawa, & DeGange 1982). In other species however, there is a long post-fledging dependent period, during which parents progressively stop caring for their young. In Passerines, for instance, the brood often fledges simultaneously and leaves the nest definitively (*e.g.,* the House Wren *Troglodytes aedon*, Johnson, et al. 2004), with parents still providing care for 10 to 14 days until independence. In several species, the brood is divided between parents, each of the parents taking care of specific fledglings (Draganoiu, et al. 2005, Leedman, & Magrath 2003, Lessells 2002, Green, & Cockburn 2001, Slagsvold 1997). In blackbirds (*Turdus merula*) fledglings are fed at specific spots in the territory until full independence, but parents are apparently unable to recognize them and occasionally feed other juveniles (Edward 1985). In razorbills (*Alca torda*), fathers have a better vocal recognition of offspring than mothers, and juveniles leave follow the male parent only after fledging (Insley, et al. 2003).

In the Black-legged Kittiwake (*Rissa tridactyla*), fledglings have to return to the natal nest where parents feed them for about a fortnight (Danchin 1988a, 1988b, Cam, et al. 2003) a period we call 'post-fledging dependence'. This long period of post-fledging parental care appears to be vital for juvenile survival because first year survival is positively related to the length of post-fledging dependence in that species (Cam et al. 2003). When attempting to return for the first time, fledglings land apparently randomly on many nests, then enduring attacks by resident adults. Risks of offspring mortality at that stage should have favoured parent-offspring recognition, as well as specific behaviours directed to the returning chicks.

However, recurrent cross-fostering experiments on young chicks (before 25 days) showed the absence of offspring recognition before that age (Cullen 1956, Storey et al. 1992). The only evidence of individual recognition in this species concerns mates (Wooller 1978, 1979). However, chicks fledge at the age of 40 days on average (see results) which leaves the possibility for parent-offspring recognition to develop before fledging. Although kittiwakes behaviour has been intensively studied for many years (Cullen 1956, Coulson 1983, Danchin 1987, Danchin, et al. 1998, Cam, et al. 2003), fledging and post-fledging care have not been investigated. Here, we provide the first observational evidence for parent-offspring

recognition at fledging in that species and propose a hypothesis about the function of such parent-offspring interactions.

Methods

Studied population

The Black-legged Kittiwake (*Rissa tridactyla*) is one of the most common pelagic gulls in the northern portion of both the Atlantic and Pacific Oceans. It often winters in the vicinity of sea ice, and breeds colonially on vertical cliffs from April to the end of August. There can be one to three chicks hatching in sequence usually within two days. Cliff-nesting physically constrains chicks to stay on the nest until fledging.

The study population was in Cap Sizun (Brittany, France) where a breeding population has been monitored since 1979 (Danchin 1987, 1988a and b, Cadiou, et al. 1994, Danchin, et al. 1998, Cam, & Monnat 2000, Cam, et al. 2003). From 1979 to 2002, a total of 949 adults and 12246 chicks was individually marked with a unique code of 4 or 5 colour rings. Every year, colonies were visited at least once a week from February to mid June, and then daily until the end of August. This allowed us to determine egg laying date, hatching date, chick rank (i.e., the position of the chick in the hatching sequence of the brood) as well as first flight and return. Every year, nests with chicks older than 30 days were visited twice a day to estimate the date of first flight, first return and last observation at the colony from mid-June to the end of August. Data for breeder attendance presented here were obtained in 1995.

Observation of fledglings returns

Observations of first returns to the nest were performed by E.D. from 1983 to 1985, during specific observation bouts. Newly fledged juveniles were monitored daily during a total of 6400 nests/hours (57 fledglings from 46 nests in 1983, in 16 hours of observations; 100 fledglings from 80 nests in 51 hours of observations in 1984; 86 fledglings from 66 nests in 24 hours of observations in 1985) to record details of the first return to the nest. Observations were made from a vantage point less than 10 meters away from focal nests.

Observation focused on individually marked fledglings returning to their natal nest for the first time. Because the first absence usually lasts well above 24 hours our observation protocol (two visits per day) allowed us to ascertain that the chick was absent for the first

time. We recorded: (i) the sequence of visited sites (sites might be nests or any other landing area); (ii) juvenile behaviour before (silent versus calling) and after landing (aggression from or toward the fledgling, begging for food, calling); (iii) the reaction of the resident individual – adult or chick - (acceptance, rejection, feeding, leaving); (iv) the reaction of the parent at the focal-fledgling's natal nest (presence or absence of one or both parents, Bow and Moan, Long-Call, interest - head movements - towards the behaviour of the focal-fledgling, takeoffs and landings while calling); and (v) any other reaction of individuals on neighbouring sites (see Cullen 1956, Tinbergen 1959, Danchin 1987, 1988a and 1988b, for description and significance of displays associated with landing).

Observation bouts lasted 116 minutes on average (max: 534 minutes, min: 15 minutes) and focused on nests where fledglings were missing. There was no correlation between date and observation bout length ($F < 1.65$, $p > 0.21$ for every year), every fledgling being thus as likely to be observed during a long as in a short bout. If the fledgling returned to its nest during the observation bout, it was registered as a "Short Absence" and details of final landing were recorded. If the fledgling was still absent from its nest at the end of the observation bout, it was registered as a "Long Absence". The obvious imperfections of these two categories diminished our capacity to detect any pattern. Our analyses using these categories are thus conservative. When a fledgling was still away from its nest on the second day it was then recorded as ‘first absence’ in the first day, and as ‘subsequent absences’ in the following days. Again, this convention made our analyses conservative. Numerous previous observations had shown that first absences shorter than one day are rare.

Statistical analyses were made using the SAS® package (SAS Institute 1999). In order to correct for pseudo-replication, we used generalized mixed models for some analyses and report the Z statistics. All tests were two-tailed. We mainly used three dependent variables: the proportion of short versus long absences (Figure 2), the number of nests visited per absence (Figure 3) and the proportion of noisy versus silent landings (Figure 4). The proportion of short versus long absences did not vary significantly across years (taking all absences: $F_{2,181}= 0.2$, $p = 0.8$, $n = 182$; taking only first absences $F_{2,64}= 2$, $p = 0.15$, $n = 65$). For each absence date, the number of visited nests did not vary significantly across years (for example for absences on day 0: $F_{2,64}= 1.21$, $p = 0.3$, $n = 65$; absences on day 1: $F_{2,21}= 0.98$, $p = 0.39$, $n = 22$; etc.). Similarly, the proportion of silent landings did not vary between 1984 and 1985 (taking all absences: $F_{1,178}= 0.57$, $p = 0.45$, $n = 179$; taking only first absences: $F_{1,83}= 3.4$, $p = 0.07$, $n = 84$; sample size regarding this aspect of chicks behaviour was very

low in 1983 and not sufficient to allow comparisons with other years). Thus, data from all years were pooled in further analyses.

Results

Parental attendance at fledging

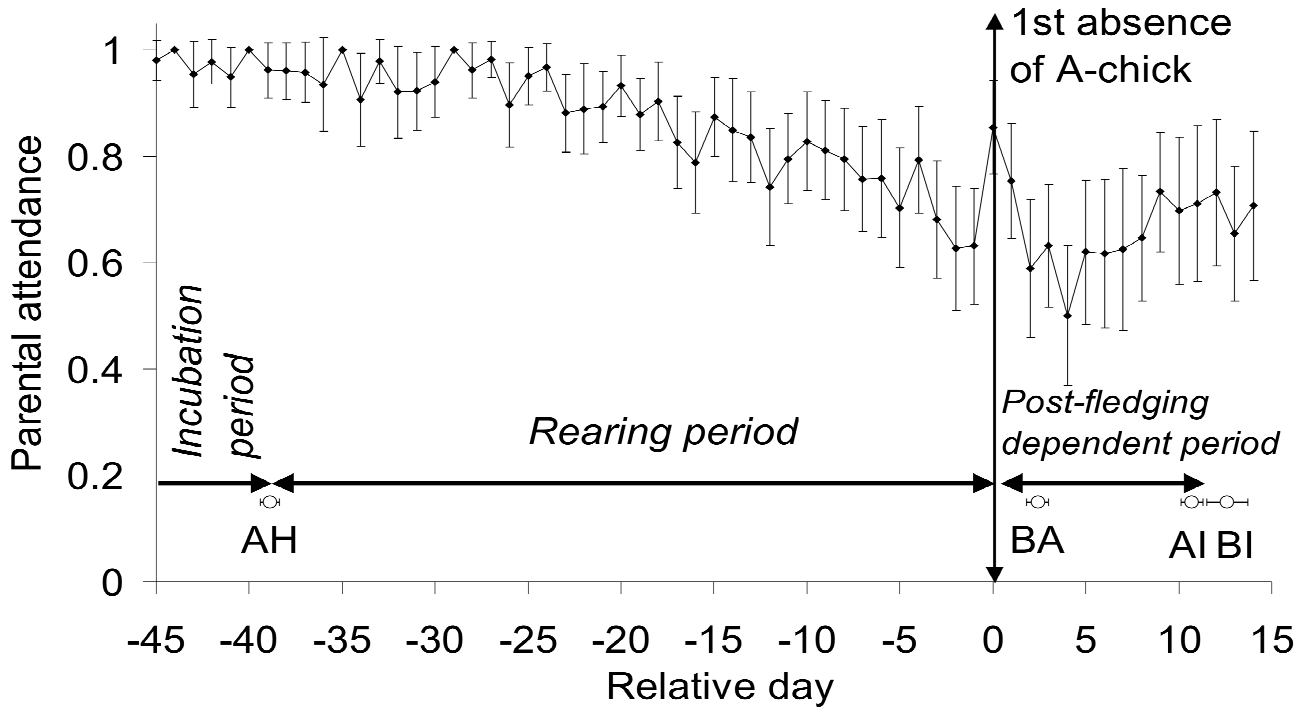


Figure 1: Parental attendance from 45 days before to 13 after the fledging of the first chick.
Data from 1995 (197 chicks coming from 8 different adjacent cliffs). Relative day means the observation Julian day minus the Julian day of the first flight of the first chick. Parental attendance is the proportion of nests occupied by at least one breeder. Data (parental attendance, hatching, first flight of the chicks, last observation of the first and the second chick) are given as mean ± the confidence interval for α=0.05. Main life-history events are abbreviated as: AH = "A-chick hatches"; BA = "first absence of B-chick (if existing)"; AI = "independence of A-chick"; BI = "independence of B-chick".

Fledglings' first absences (i.e., fledging) occurred approximately 40 days after hatching (mean ± 95% confidence interval = 39.7 ± 1.7 days, n = 197 in 1995; Figure 1). After their first return, they were fed regularly on the nest during approximately eleven days (10.7 ± 0.6 in 1995, n = 197). In almost 30 years of studies, we never saw fledglings being fed by their

parents outside the natal nest. The only exception was that of a relatively old banded chick that fell out from its isolated nest to a ledge 1.5 meter below and that was fed until fledging by its marked parents. Thus returning to the nest appeared to be important for post-fledging survival.

Parental attendance (at least one parent) was close to 100% until chicks were about 15 days old (Figure 1). It then declined progressively until fledging. First returns occurred when parental attendance was of about 65% on average. However, parental attendance increased significantly for two days immediately after the first absence of the first chick (attendance in day –1 and 0 differed, $t_{134} = 3.45$, $p = 0.0007$; attendance in day 0 and 1 did not differ, $t_{120} = 1.81$, $p = 0.07$; but attendance between day 0 and 2 differed, $t_{114} = 3.8$ $p = 0.0002$, parental attendance being higher on day 0 and 1 than before or after; see Figure 1). After two days, parental attendance came back to values similar to before fledging and remained at about 60% for 15 days after fledging (Figure 1).

Second chicks usually fledged 2.4 ± 0.6 days after the first chick. Parental attendance was thus significantly higher during the first return of the first chick than that of the second or third ones ($p = 0.0002$ between day 0 and day 2).

Absence duration and parental activity

Short first absences (see Methods for definition) were more frequent when at least one parent was on the nest (Figure 2, cases "Active" plus "Inactive") than when both parents were absent (cases "Absent" plus "Squatters"; Figure 2, $\chi^2_1 = 4.9$, $p = 0.027$, $n = 62$). Short first absences were more common in the presence of active (parents reacting to the chick, even calling back, $n = 29$) than inactive (parents oblivious to the chick, $n= 9$) parents (Figure 2, $\chi^2_1 = 12.5$, $p = 0.0004$, $n = 62$).

Short absences were less frequent in first than subsequent returns (45% and 82% respectively, $t_{180} = 5.4$, $p < 0.0001$). Absence duration in subsequent returns still depended on parental attendance ($F_{1,37} = 4.9$, $p = 0.033$, $n = 120$; Generalized mixed model including fledgling identity as a random factor: Z=1.5, $p = 0.062$) but no longer on parental activity. Furthermore, parents reacted more to their young's call during first than subsequent return ($F_{1,72} = 2.2$, $p = 0.15$, $n = 114$; Generalized mixed model with fledgling identity as a random factor: $Z = 1.9$, $p = 0.031$). Thus, in subsequent returns juveniles did not seem to have any difficulty in finding their way back, and experienced fledglings typically waited for their parents' return to come back directly to their nest to beg for food immediately.

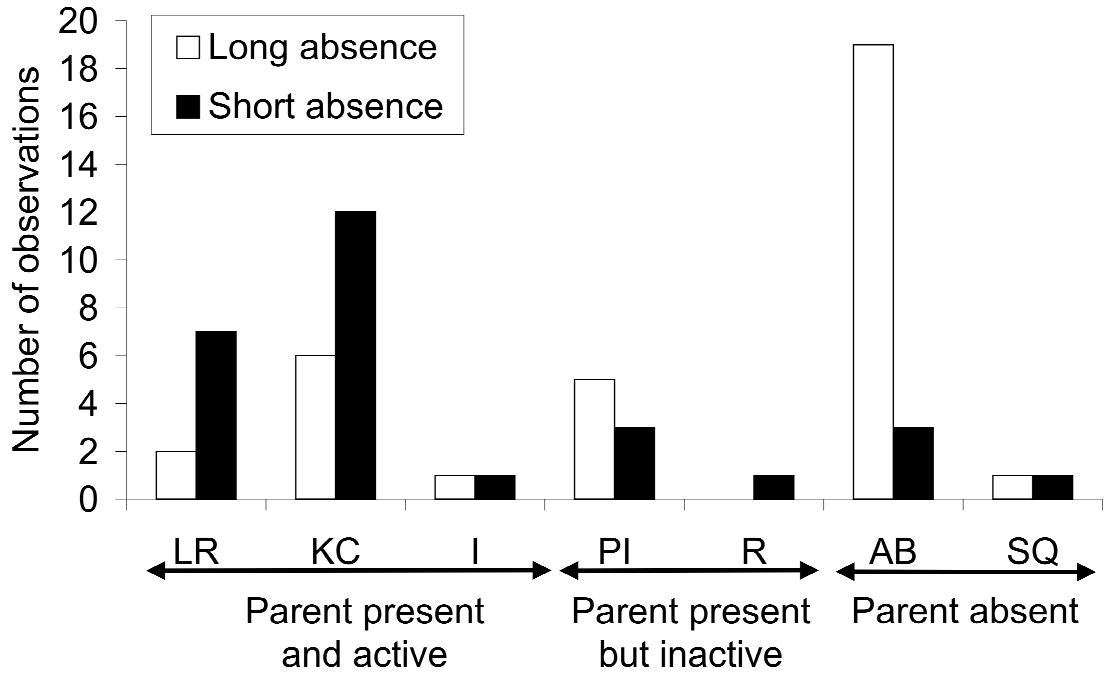


Figure 2: Distribution of long (white bars) and short (black bars) first absences according to parental behavior.

Long first absences are those in which the first return of the newly fledged juvenile occurred after the first observation bout following the departure of the juvenile. Short ones are those for which the first return occurred during the first observation bout after the first absence of the juvenile. Parental activity. LR: parent(s) took off and landed on the nest while uttering the long-call; KC: parent(s) kittiwaked and/or chocked spontaneously on the nest; I: parent(s) reacted to the behavior of its young without calling; PI: parent(s) present but did not react in any way; R: parent(s) present but rejected it when it landed on the nest; AB: parent absent; SQ: parent absent and the nest occupied by a squatter.

Parent-offspring recognition during post-fledging dependence

Chicks landed on:	Another nest			Natal nest					
Nest was occupied by:	An adult or another chick			At least one parents			A squatter		
	Chick did not call before	Chicks called before	Total	Chick did not call before	Chicks called before	Total	Chick did not call before	Chicks called before	Total
Adult does not react	0	0	0	2	31	33	0	2	2
Adult reacts by:									
- Accepting the chick	*4*	*1*	*5*	*6*	*46*	*52*	*0*	*1*	*1*
- Rejecting the chick	*61*	*14*	*75*	*3*	*2*	*5*	*4*	*14*	*18*
- Leaving the nest	*3*	*3*	*6*	*1*	*4*	*5*	*0*	*2*	*2*
Total of reactions	68	18	86	10	52	62	4	17	21
Total	**68**	**18**	**86**	**12**	**83**	**95**	**4**	**19**	**23**

Table 1: Reaction of adults attending a nest when a chick landed on their nest according to the chick's landing behavior

We gathered 129 observations (involving 50 chicks) when the focal fledgling called (either from another nest or while flying) with at least one parent attending the natal nest. Parents reacted in 86 cases (67%), and called significantly more often in reply than did other adults, (40% vs. 5% respectively, $t_{256} = 7.4$, $p < 0.0001$). Focal fledglings did not always reply to their parents' calls, but in 6 cases, parental calls were directly followed by the first return of the focal fledgling that obviously changed its trajectory after its parent(s) called.

Foreign fledglings were rejected by the nest occupant in 87% of the cases (75 of 86 cases, in 56 cases by an adult, in 19 cases by a chick, Table 1), and accepted in 4 cases. In the last 6 cases, the nest occupant left the nest immediately after the landing of the juveniles. Such occupants were probably squatters (Danchin, 1988a, Monnat, et al. 1990, Cadiou, et al. 1994). We also recorded 23 returns (Table 1) to natal nests that were occupied by known squatters. 18 (78%) of these juveniles were rejected, two provoked the departure of the squatter, and one managed to stay after having been hit violently. Finally, among 95 cases of landings on the natal nest in the presence of at least one parent, 62 triggered parental reaction (Table 1). In 52 cases parents performed a full greeting display (kittiwake and downward and upward chocking), or fed the returning juvenile. In 5 cases they rejected their juvenile (the

parent eventually accepted the juvenile in 3 instances), and in the 5 last cases the parent left the nest. Adults thus recognized their fledglings in 91% of the cases (52 out of 57 without including cases when adults left the nest). Thus, fledglings are significantly less rejected by their parents than by other adults ($\chi^2_1 = 133.3$, $p < 0.0001$).

When landing at natal nests, fledglings that called were significantly more likely to be accepted than silent ones ($\chi^2_1 = 8.1$, $p = 0.0045$) on the natal nest. This pattern was not found on foreign nests ($\chi^2_1 = 0.023$, $p = 0.88$), suggesting that foreign adults rejection was independent of juvenile landing behaviour. This suggests that juvenile calls carry an individual signature.

Fledgling behaviour: number of sites visited and occurrence of pre-landing calls

The mean number of sites visited was significantly greater during first returns than subsequent ones (Figure 3, $t_{124} = 7.6$, $p < 0.0001$). The number of nest visited by juveniles that successfully returned to their nest on the first day varied greatly (on average 7.7 +/- 6.1, ranging from 1 to 23, $n = 31$). The percentage of silent landings was affected by the status of

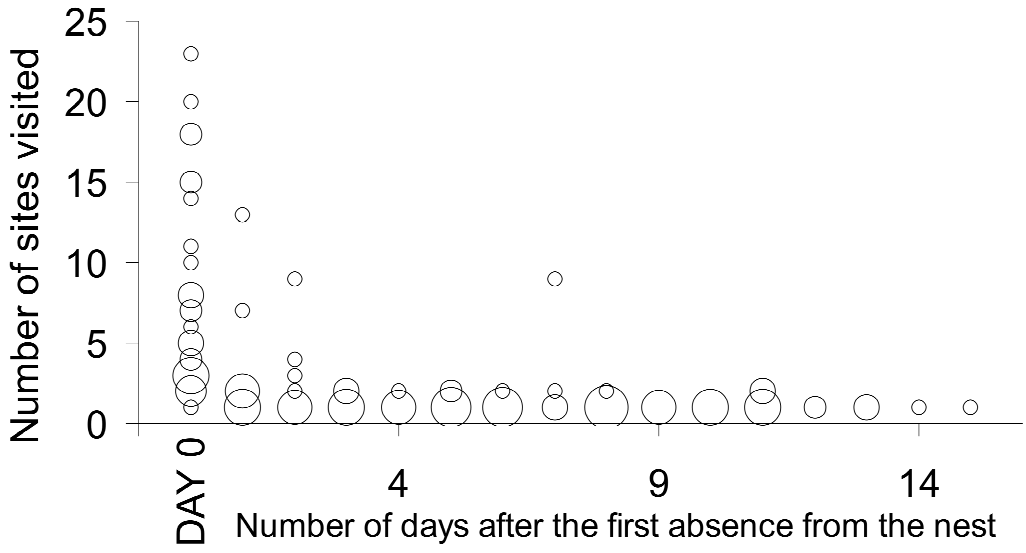


Figure 3: Number of sites visited during the first and subsequent returns to the natal nest. Only successful attempts ($n = 126$) are shown here. Day 0 is the day of the first absence, and the size of the circles is proportional to the number of observations.

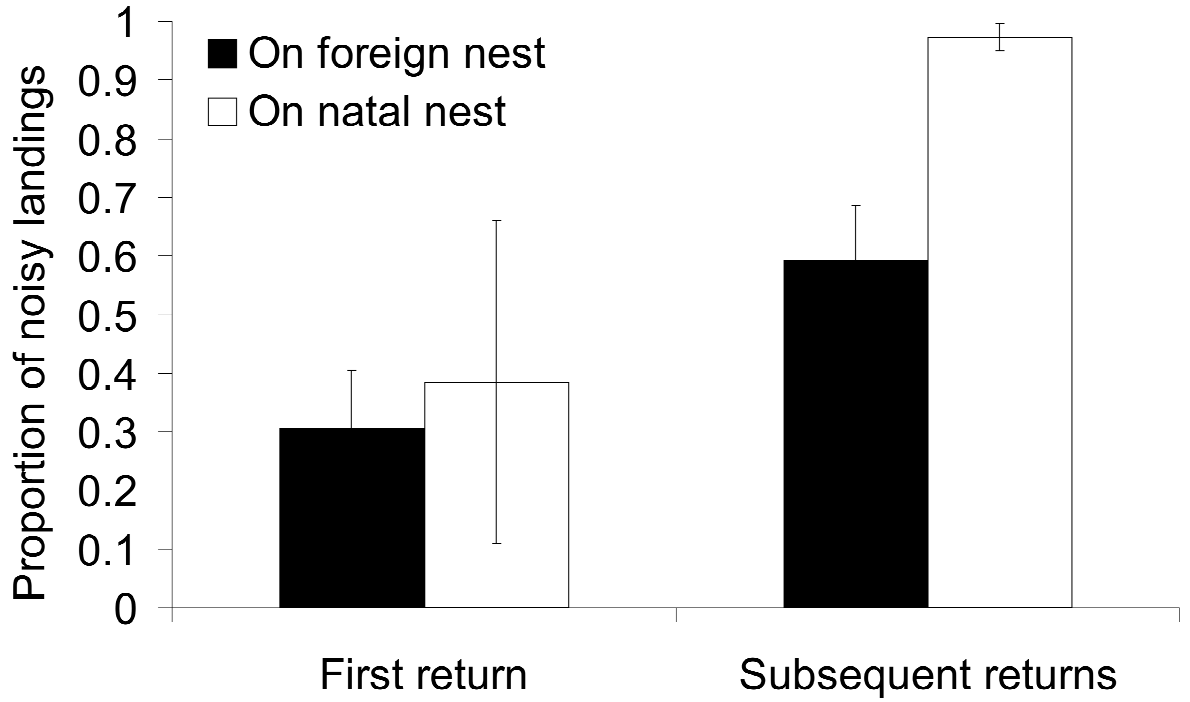


Figure 4: Mean proportion of noisy landings according to return (first versus subsequent) and nest (natal versus foreign) status. The status of the return ($F_{1,417} = 38.6$, $p < 0.0001$), the status of the nest ($F_{1,417} = 19.1$, $p < 0.0001$) and the interaction between the two ($F_{2,417} = 5.6$, $p = 0.0187$) were significant (n = 652 landings displayed by 228 different fledglings; SAS MIXED Procedure with fledgling as random parameter: $p < 0.0001$).

the return (first versus subsequent, $F_{1,417} = 38.6$, $p < 0.0001$), the status of the nest (natal versus foreign, $F_{1,417} = 19.0$, $p < 0.0001$) and the interaction between both parameters ($F_{2,417} = 5.6$, $p = 0.019$, 652 landings displayed by 228 different fledglings; Mixed model with fledgling identity as random factor: $Z = 4.68$, $p < 0.0001$, Figure 4).

Discussion

Our methods and conventions have obvious imperfections. In particular our definition of short and long absences is very crude. However, these imperfections are unlikely to be responsible for the detected patterns. In fact, they mainly diminish our power to detect patterns, and our analyses are thus conservative. This implies that reported patterns are biologically sound but probably more important than estimated here.

Parent-offspring recognition in kittiwakes

The existence of the post-fledging dependence period suggests the existence of some parent-offspring recognition avoiding misdirected parental care. All the previous studies failed to show such recognition in kittiwakes before the age of 25 days (Cullen 1956, Storey, et al. 1992). Our study thus provides the first evidence for parent-offspring vocal recognition in that species at the time of fledging when chicks are on average 40 days old. Parents reacted more than foreign adults to their juvenile's calls. In several observations, reacting parents were unable to see their fledgling, suggesting that vocal recognition is involved in kin recognition. This is supported by our observation that (i) fledglings were rejected more often by their own parents after silent than noisy landings, and (ii) the fact that this pattern was not observed when landing near foreign adults. Whatever the mechanisms involved, our result suggest that recognition is not perfect because erroneous rejection or acceptance were observed.

Adoption is frequent in many bird species (Carter, & Spear 1986, Plissner, & Gowaty 1988, Morris, et al. 1991, Holley 2000). It also occurs at a detectable rate in kittiwakes (Roberts, & Hatch 1994, Helfenstein, et al. 2004) in spite its cliff-nesting habit. In that species, most adoptions occur during the first ten days after hatching, adoption of fledglings remaining exceptional (Danchin, pers. obs.). Thus the trade-off between costs of efficient parent-offspring recognition and costs of adoption also exists in that species. If the costs of taking care of a foreign juvenile are lower than those of ejecting its own ones, then adults are expected to show intermediate level of recognition as we observed (Riedman 1982, Avital, et al. 1998).

Our results suggest that parent-offspring recognition exists in kittiwakes but develops late. Such delayed development may result from the strong constraints on chick movements imposed by cliff-nesting. Recognition only develops just before fledging, a stage when parental care may be misdirected. Parents have the opportunity to learn their offspring's voice when chicks flap wings on the nest while uttering their characteristic harsh long call during the ten days before fledging.

Our results further suggest that parent-offspring vocal recognition helps fledglings to find their way back to their natal nest, a stage that may affect fitness. Recognition of parents by chicks has already been demonstrated in Larids (Beer 1969, 1970a, 1970b, 1979, Evans 1970, Charrier, et al. 2001). However, evidence that chicks are recognized by parents (Buckley, & Buckley 1972, Miller, & Emlen 1975) is rarer and is thought to be limited to periods of

parental investment on mobile offspring. Our study fits this view, since parents sometimes adopt young chicks, but appear to recognize their offspring and reject foreigners after fledging. Play-back experiments would be necessary to demonstrate that vocalizations are the basis of parent-offspring recognition at the time of fledging in kittiwakes.

Post-fledging parental care may also influence fitness either immediately or on the long-term. Previous studies have documented the importance of the post fledging period of dependence for juvenile survival rates in kittiwakes (Cam, et al. 2003). Juveniles with longer dependent periods show higher local survival rates during the first winter, and higher reproductive success as adults (Cam, et al. 2003). Here we show that the duration of the first absence may also be influenced by parental attendance and activity, suggesting that parents actively help their offspring to return to the nest. Juveniles taking a long time to return may be attacked by other adults and more exposed to predation. Post-fledging parental care owing to parent-offspring recognition may therefore diminish the energetic costs of the first return, and affect post-fledging survival and thus fitness.

Change in parent-offspring interactions after the first return

Behavior of newly fledged juveniles changed from first to subsequent returns to the nest. During their first flight, juveniles visited numerous nests randomly, demonstrating their capability to land on any kind of nest. Their difficulty was thus mainly in locating their natal nest, which they have never seen from outside before. Newly fledged juveniles are often silent, while during subsequent flights, juveniles usually fly directly to their nest and call before landing as adults do (Danchin 1987 and 1988a). The reason why newly fledged juveniles remain silent during their first return attempt is unclear because selection should favor juveniles capable of calling at that time. In fact, adult kittiwakes show the same pattern. Individuals landing on an unknown site are much more often silent than those landing on their own nest, the latter usually uttering a pre-landing call (Danchin 1987 and 1988a). This suggests that pre-landing calls are typical of confident individuals (Danchin 1987 and 1988a).

In parallel, parental behavior changes in two ways around fledging. They first react to the absence of their chick by staying on the nest thus increasing their attendance. They also become more reactive to the voice of their offspring. These two components however disappear in only three days, implying that parents are much less likely to be present at the time of the return of their second offspring. This reduced post-fledging parental care to second fledglings, combined with sibling aggression and reduced food availability throughout

development (Roberts, & Hatch 1994), may explain the long-term fitness consequences of hatching rank in that species (Cam, et al. 2003). Post-fledging parental care may therefore play a crucial role in individual fitness and population demography.

Most species experiences high mortality rate before recruitment, but few studies have focused on the early stages after fledging in order to understand what selective pressures may constrain fledgling survival. This study suggests that parent-offspring interactions may influence fledging success and, in turn, affect offspring survival. In kittiwakes the nest seems to be the "meeting point" for parents and offspring, a function that is mediated by parent-offspring vocal interactions at the time of the first return. Parent-offspring recognition is common in Larids (Storey, et al. 1992, Tinbergen 1959, Charrier, et al. 2001, Beer 1970a), which suggests that it is an ancestral feature in this taxon. Cliff-nesting in kittiwakes seems to have led to the disappearance of parent-offspring recognition (Cullen 1956, Storey, et al. 1992) except at the time of fledging, a moment when it may impact fitness significantly. This led to the evolution of an unusual pattern of nest use where the nest is still used for a fortnight after fledging, while in most bird species the nest is deserted definitively by both breeders and juveniles immediately after fledging.

Acknowledgments

We thank Richard H. Wagner and Fabrice Helfenstein who gave constructive comments on earlier drafts of the manuscript. Financial support was provided by a grant from the French ministry of national education to H. Mulard. Field studies were cared for in accordance to the regulations of the French ministry of Environment.

References

Anders, A. D., Dearborn, D. C., Faaborg, J. & Thompson III, F. R. 1997. Juvenile survival in a population of Neotropical migrant birds. *Conserv. Biol.*, **11,** 698-707.

Avital, E., Jablonka, E. & Lachmann, M. 1998. Adopting adoption. *Anim. Behav.*, **55,** 1451-1459.

Beer, C. G. 1969. Laughing gull chicks: recognition of their parents' voices. *Science*, **166,** 1030-1032.

Beer, C. G. 1970a. On the responses of Laughing gull chicks (*Larus atricilla*) to the calls of adults I: Recognition of the voices of the parents. *Anim. Behav.*, **18,** 652-660.

Beer, C. G. 1970b. On the responses of Laughing gull chicks (*Larus atricilla*) to the calls of adults II: Age changes and responses to different types of calls. *Anim. Behav.*, **18,** 661-677.

Beer, C. G. 1979. Vocal communication between Laughing gull parents and chicks. *Behaviour*, **70,** 118-146.

Buckley, F. G. & Buckley, P. A. 1972. The breeding ecology of Royal terns *Sterna (Thalasseus) maxima maxima*. *Ibis*, **114,** 344-359.

Cadiou, B., Monnat, J.-Y. & Danchin, E. 1994. Prospecting in the kittiwake, *Rissa tridactyla*: different behavioural patterns and the role of squatting in recruitment. *Anim. Behav.*, **47,** 847-856.

Cam, E. & Monnat, J.-Y. 2000. Apparent inferiority of first-time breeders in the kittiwake: the role of heterogeneity among age classes. *J. An. Ecol.*, **69,** 380-394.

Cam, E., Monnat, J.-Y. & Hines, J. E. 2003. Long-term fitness consequences of early conditions in the kittiwake. *J. An. Ecol.*, **72,** 411-424.

Carter, L. R. & Spear, L. B. 1986. Costs of adoption in western gulls. *Condor*, **88,** 253-256.

Charrier, I., Mathevon, N., Jouventin, P. & Aubin, T. 2001. Acoustic communication in a black-headed gull colony: how do chicks identify their parents ? *Ethology*, **107,** 961-974.

Coulson, J. C. 1983. The changing status of the Kittiwake *Rissa tridactyla* in the British Isles, 1969-1979. *Bird Study*, **30,** 9-16.

Cullen, E. 1956. Adaptations in the kittiwake to cliff-nesting. *Ibis*, **99,** 275-302.

Danchin, E. 1987. The behaviour associated with the occupation of breeding site in the kittiwake gull *Rissa tridactyla*: the social status of landing birds. *Anim. Behav.*, **35,** 81-93.

Danchin, E. 1988a. Rôle des facteurs comportementaux dans les mécanismes de régulation des populations d'oiseaux coloniaux. Cas de la mouette tridactyle (*Rissa tridactyla*). Paris: Université Pierre et Marie Curie.

Danchin, E. 1988b. Social interactions in Kittiwakes colonies: social facilitation and/or favourable social environment. *Anim. Behav.*, **36,** 443-451.

Danchin, E., Boulinier, T. & Massot, M. 1998. Conspecific reproductive success and breeding habitat selection: implications for the study of coloniality. *Ecology*, **79,** 2415-2428.

Draganoiu, T. I., Nagle, L., Musseau, R. & Kreutzer, M. 2005. Parental care and brood division in a songbird, the black redstart. *Behaviour*, **142,** 1495-1514.

Edward, P. J. 1985. Brood division and transition to independance in Blackbirds, *Turdus merula*. *Ibis*, **127,** 42-59.

Evans, R. M. 1970. Parental recognition and the 'mew call' in Black-billed gulls (*Larus bulleri*). *Auk*, **87,** 503.

Green, D. J. & Cockburn, A. 2001. Post-fledging care, philopatry and recruitment in brown thornbills. *J. An. Ecol.*, **70,** 505-514.

Hasegawa, H. & DeGange, A. R. 1982. The Short-tailed albatross *Diomedea albatrus*, its status, distribution and natural history. *Am. Birds*, **36,** 806-814.

Helfenstein, F., Tirard, C., Danchin, E. & Wagner, R. H. 2004. Low frequency of extra-pair paternity and high frequency of adoption in Black-legged kittiwakes. *Condor*, **106,** 149-155.

Holley, A. J. F. 2000. Ring-billed gull adoption: what arms race? *Anim. Behav.*, **60,** F15-F16.

Insley, S. J., Paredes, R. & Jones, I. L. 2003. Sex differences in razorbill *Alca torda* parent-offspring vocal recognition. *J. Exp. Biol.*, **206,** 25-31.

Johnson, L. S., Rauch, R. L. & Dellone, S. N. 2004. The process and causes of fledging in a cavity-nesting passerine bird, the house wren (*Troglodytes aedon*). *Ethology*, **110,** 693-705.

Leedman, A. W. & Magrath, R. D. 2003. Long-term brood division and exclusive parental care in a cooperatively breeding passerine. *Anim. Behav.*, **65,** 1093-1108.

Lessells, C. M. 2002. Parentally biased favouritism: why should parents specialize in caring for different offspring? *Phil. Trans. R. Soc. London B*, **357,** 381-403.

Middleton, H. A., Green, D. J. & Krebs, E. A. 2007. Fledgling begging and parental responsiveness in American dippers (Cinclus mexicanus). *Behaviour*, **144,** 485-501.

Miller, D. E. & Emlen, J. T. J. 1975. Individual chick recognition and family integrity in the Ring-billed gull. *Behaviour*, **52,** 124-143.

Monnat, J.-Y., Danchin, E. & Estrella, R. R. 1990. Assessment of environmental quality within the framework of prospecting and recruitment: the squatterism in the Kittiwake. *C.R. Acad. Sc. Paris*, **311,** 391-396.

Morris, R. D., Woulfe, M. & Wichert, G. D. 1991. Hatching synchrony, chick care and adoption in the common tern: can disadvantaged chicks win? *Can. J. Zool.*, **68,** 661-668.

Parker, N., Cam, E., Lank, D. B. & Cooke, F. 2003. Post-fledging survival of marbled murrelets *Brachyramphus marmoratus* estimated with radio-marked juveniles in Desolation Sound, British Columbia. *Mar. Ornithol.*, **31,** 207-212.

Plissner, J. A. & Gowaty, P. A. 1988. Evidence of reproductive error in the adoption of nestling Eastern bluebirds (*Sialis sialis*). *Auk*, **105,** 575-578.

Riedman, M. L. 1982. The evolution of alloparental care and adoption in mammals and birds. *Quat. Rev. Biol.*, **57,** 405-435.

Roberts, B. D. & Hatch, S. A. 1994. Chick movements and adoption in a colony of Black-legged kittiwakes. *Wilson Bull.*, **106,** 289-298.

Slagsvold, T. 1997. Brood division in birds in relation to offspring size: sibling rivalry and parental control. *Anim. Behav.*, **54,** 1357-1368.

Storey, A. E., Anderson, R. E., Porter, J. M. & McCharles, A. M. 1992. Absence of parent-young recognition in kittiwakes: a re-examination. *Behaviour*, **120,** 302-323.

Tinbergen, N. 1959. Comparative studies of the behaviour of gulls (*Laridae*): a progress report. *Behaviour*, **15,** 1-70.

Wooller, R. D. 1978. Individual vocal recognition in the kittiwake gull, *Rissa tridactyla* (L.). *Z. Tierpsychol.*, **48,** 68-86.

Wooller, R. D. 1979. Seasonal, diurnal and area differences in calling activity within a colony of kittiwakes *Rissa tridactyla* (L.). *Z. Tierpsychol.*, **51,** 329-336.

Ydenberg, R. C. 1989. Growth-mortality trade-offs and the evolution of juvenile life histories in the Alcidae. *Ecology*, **70,** 1494-1506.

ARTICLE 4: Mating pattern and various fitness costs of genetic similarity in a monogamous bird

Hervé Mulard, Etienne Danchin, Sandra L. Talbot, Andrew M. Ramey, Scott A. Hatch, Joël F. White, Fabrice Helfenstein & Richard H. Wagner

Will be submitted to *Molecular Ecology*

Abstract

Evidence of multiple genetic criteria of mate choice is rapidly accumulating in numerous taxa. Females may choose mates that are genetically dissimilar, or extra-pair partners that are more genetically compatible than their social males, in order to increase the heterozygosity of their offspring. While most studies testing these predictions are of genetically polygamous species, few studies have addressed genetically monogamous species in which mate choice is mutual. Here we used microsatellite loci to assess individual global heterozygosity and pair genetic similarity in a socially and genetically monogamous long-lived seabird, the black-legged kittiwake *Rissa tridactyla.* We found that pairs were more genetically dissimilar than expected by chance. We also identify fitness costs of breeding with genetically similar individuals: (i) genetically similar pairs hatched fewer chicks than dissimilar pairs, and (ii) homozygous offspring grew slower (in term of weight, tarsus or wing length) and were more likely to die before 25 days old than heterozygous offspring. This is the first evidence, to our knowledge, of a genetically monogamous species showing a mating pattern in accordance to a strategy to avoid the fitness costs of breeding with a genetically similar individual.

Introduction

Many species exhibit non-random mating patterns and numerous characteristics may influence mate choice. Although most studies have focused on morphological and behavioral traits (e.g. Burley & Foster, 2006, Johnsen, et al. 2005, Poesel, et al. 2006, Rantala, et al. 2006), there is rapidly growing evidences of multiple genetic criteria of mate choice (reviewed in Neff & Pitcher, 2005 and Mays & Hill, 2004). For example, females might choose males with specific alleles or males with high heterozygosity (Bonneaud, et al. 2006), which may increase the resistance of offspring to parasites (Penn, et al. 2002, Westerdahl, et al. 2005). Alternatively, females might choose males with compatible genes. The main

driving forces of mate choice would then be to maintain equilibrium between co-adapted genes (Cohen & Dearborn, 2004) or, alternatively, to enhance the genetic variability of offspring (e.g. Thuman & Griffith, 2005, Olsson, et al. 2003, Freeman-Gallant, et al. 2003, Blomqvist, et al. 2002, Landry, et al. 2001, Tregenza & Wedell, 2000).

In species with biparental care, both sexes are choosy (Trivers 1972, Jones & Hunter, 1993). Blomqvist *et al.* (2002) reported that both sexes in socially monogamous birds obtain extra-pair fertilizations when mates are genetically similar. In blue tits (*Parus caeruleus*), females acquire extra-pair fertilizations that enhance the heterozygosity and fitness of their offspring (Foerster, et al. 2003). In the superb starling (*Lamprotornis superbus*), the benefits of extra-pair copulation may differ according to the genetic similarity of the extra-pair parner (Rubenstein 2007). However, few studies have focused on the mating patterns in genetically monogamous species, where extra-pair fertilization is by definition inexistent.

In black-legged kittiwake (*Rissa tridactyla*), a long-lived monogamous seabird in which extra-pair fertilization are absent (Helfenstein, et al. 2004), mate choice is likely a critical life-history decision. Kittiwakes also exhibit high inter-annual fidelity (Coulson & Thomas, 1985, Fairweather & Coulson, 1995). Given the genetic monogamy and low divorce rates of this long-lived species, mate choice may profoundly affect reproductive success throughout an individual's lifetime.

To examine whether mating pattern in kittiwakes is influenced by genetic criteria, individual heterozygosity and genetic similarity of mates were assessed by microsatellite markers. Microsatellites are generally assumed to be neutral genetic markers, and have been widely used to estimate relatedness, individual heterozygosity and population inbreeding (e.g., Coulson, et al. 1999, Coltman & Slate 2003, Hansson 2004, Höglund, et al. 2002). If heterozygosity at certain selected loci enhances fitness (Penn, et al. 2002, Landry, et al. 2001), heterozygosity at microsatellite loci may be a good surrogate of the overall genetic quality of an individual, especially in wild species where little is known about genes under selection. For breeding pairs observed in 2003 and 2004 in a kittiwake colony on Middleton Island, Alaska, we tested three hypotheses, namely that breeders are paired with: (1) the most heterozygous mates ("good genes as heterozygosity" hypothesis, Landry, et al. 2001), (2) genetically dissimilar mates in order to increase the genetic variability of offspring ("genetic similarity avoidance" hypothesis; Blomqvist, et al. 2002, Bensch, et al. 1994, Brown 1996), (3) genetically similar mates in order to preserve the link between locally co-adapted genes ("genetic similarity preference" hypothesis; Cohen & Dearborn, 2004, Kokko & Ots, 2006). Hypothesis 1 predicts that highly heterozygous males mate with genetically heterozygous

females, since only high quality females will have access to high quality males. Thus, we expect to find a positive correlation between male and female heterozygosity (Bonneaud, et al. 2006), and paired individuals should be more heterozygous than unpaired ones. Hypothesis 2 predicts that the observed mean genetic similarity between pair members will be lower than expected through random matings. Hypothesis 3 is the converse of Hypothesis 2 and predicts that mates share more alleles than expected by chance.

A second aim of the study was to test the fitness effects of breeding with a genetically similar individuals. First, we looked for correlations between genetic quality of the pairs and fitness components such as clutch size and hatching success (Kempenaers, et al. 1996, Van de Casteele, et al. 2003). Second, as genetically similar pairs are more likely to give birth to homozygous offspring than dissimilar pairs, we also test for correlations between chick heterozygosity and their survival or their growth, evaluated through repeated measures of the weight, the tarsus length and the wing length of chicks throughout their development.

Materials and methods

Study species and population

We conducted our study on Middleton Island (Gulf of Alaska, 58°25' N, 146°19' W, May-July 2003-2006). This island supports a large declining population of black-legged kittiwakes (from 166,000 birds in 1981 to fewer than 25,000 in 1999, Gill & Hatch, 2002). We studied kittiwakes nesting on the edges of an abandoned U.S. Air Force radar tower that has been modified to enable close observations and easy captures. The study plot is characterized by vertical walls and uniform nest spacing, with breeders nesting on wooden ledges built specifically for cliff-nesting seabirds (Gill & Hatch, 2002).

Nest sites were observed twice daily from mid-May to late-August to assess individual attendance and reproductive success. We arrive on the field site too late to assess the arrival date of adults. At each visit, we recorded the individual color band combination of birds in attendance, the stage of nest completion, the behavior of adults in the form of incubating, nest building and copulating which were used to confirm pair identity, and presence of eggs and chicks. Each chick was marked at hatching and banded at 25 days old. Adults were banded using coded U.S. metal and four colored bands. We could thus precisely determine laying and hatching dates of focal pairs, as well as the hatching rank of every chick (that is, its hatching position in the brood, recorded as A-chicks for first hatched, and B-chicks for second hatched

chicks). Copulation behavior of every pair was also followed in order to determine the sex of every paired adult; for unpaired adult, the sex was assumed according to morphological values such as bill length, tarsus length, bill width and head+bill length (see Jodice, et al. 2000 for more details about these methods). We sampled blood from breeding and non-breeding individuals in 2003-2004 in order to analyze mating pattern. In 2005, we only sampled pairs with chicks, in order to analyze the fitness costs of homozygosity, and chicks were bled at hatching. Blood was kept in a preservation buffer (Longmire, et al. 1988) that allows storage of samples for long periods without refrigeration. Chicks were also weighed and measured (for wing and tarsus length) every 5 days from hatching to 25 days old.

Genetic analysis

DNA extraction

Whole genomic DNA was extracted from each blood sample using a "salting out" protocol described in Medrano, et al. (1990), modified by substituting Pronase E for Protease K and incubating at 37°C in the lysis phase, and substituting 0.7 volumes of 2-propanol in place of 2 volumes of ETOH in the DNA precipitation phase. Genomic DNA extractions were quantified using fluorometry and diluted to 50ng/µL working solutions.

PCR and electrophoresis

Samples were genotyped at 10 microsatellite loci. Loci K6, K16, K31, K32, K67 and K71 were first described in the black-legged kittiwake (Tirard, et al. 2002, see Table 1); RBG20, RBG27, RBG29 and RBG39 were developed for the red-billed gull, *Larus novaehollandiae scopulinus* (Given, et al. 2002, see Table 1). Three additional loci (K56, Tirard, et al. 2002) RBG18 and RBG13 (Given, et al. 2002) were also tested: the two first were found to be unreliable (see the quality controls below), and the third was monomorphic in our population.

The forward primer in each primer pair was synthesized with a modified 19- to 20-bp universal tail (M13F, M13R or SP6) added to the 5' end of the oligonucleotide (Oetting, et al. 1995). We used a complementary fluorescently labeled (IRD700 or IRD800) primer, identical to the specific tail used to modify the forward primer, to detect alleles at each loci. We carried out amplifications in a final volume of 10 µL that contained 1 µL DNA extract, 0.2 mM

Locus	*Repeated motif*	*Allele Sizes*	*No. of alleles*	*No. of ind.*	*Hexp*	*Hobs*	*p-value*	*Genebank Accession No.*
K6	$(AC)_4T(TA)_{12}$	*111-139*	*15*	*593*	*0.86*	*0.82*	*N.S.*	*AY083596*
K16	$(TG)_4(TA)_8(GA)_{10}$	*151-187*	*13*	*591*	*0.86*	*0.72*	*< 0.0001*	*AY083597*
K31	$(TG)_{13}$	*176-225*	*26*	*580*	*0.88*	*0.87*	*N.S.*	*AY083598*
K32	$(GA)_2(GT)_{12}$	*116-188*	*35*	*596*	*0.90*	*0.90*	*N.S.*	*AY083599*
K67	$(CA)_2(TA)_9$	*135-147*	*7*	*463*	*0.48*	*0.43*	*N.S.*	*AY083601*
K71	$(AC)_{11}$	*143-159*	*7*	*593*	*0.65*	*0.69*	*N.S.*	*AY083602*
RBG20	$(GT)_{13}$	*186-199*	*10*	*572*	*0.68*	*0.67*	*N.S.*	*AY091849*
RBG27	$(GT)_{12}$	*207-223*	*9*	*593*	*0.73*	*0.71*	*N.S.*	*AY091851*
RBG29	$(GT)_{13}$	*151-169*	*9*	*584*	*0.65*	*0.63*	*0.005*	*AY091853*
RBG39	$(AC)_{11}$	*180-190*	*6*	*597*	*0.55*	*0.50*	*N.S.*	*AY091852*

Table 1 : Summary of the ten microsatellite loci. K6, K16, K31, K32, K67 and K71 were first described in the Black-legged kittiwake (Tirard, et al. 2002), whereas RBG20, RBG27, RBG29 and RBG39 were sequenced from Red-billed gull, *Larus novaehollandiae scopulinus* (Given, et al. 2002). H_{exp} and H_{obs} are the expected and observed heterozygosity computed by GENEPOP, and we give also the p-value the Hardy-Weinberg equilibrium test (after Bonferroni correction for multiple tests).

dNTPs, 0.1 mg BSA, 1X PCR buffer (Perkin Elmer Cetus I ; PE Biosystems, Forest City, California), 10.0 pmoles forward and reverse unlabeled primers, 1.0 pmole fluorescently labeled primer, and 0.2 units of Taq polymerase. PCR reactions began at 94ºC for 90 seconds, and continued with 40 cycles each of 94 C for 15s, 50ºC for 15s and 74ºC for 30 s.

Amplification products were separated on a 48-well 25-cm 6% polyacrylamide gel on a LI-COR 4200 LR automated sequencer, using Base ImagIR™ (LI-COR, Inc., Lincoln, Nebraska). Allele sizes for specific samples at each locus were determined relative to the M13 phage single nucleotide ladder. These samples were later used as internal size standards to score new genotypes, using Gene ImagIR™ 4.05 software (Scanalytics, Inc., Fairfax, Virginia). For quality control purposes, we reprocessed a minimum of 10% of the samples for all markers; two markers (K56, Tirard, et al. 2002, and RBG18 Given, et al. 2002) gave various scores at each PCR and were thus discarded.

Statistical analyses

Genetic Diversity and Tests of Equilibrium

Mean number of alleles (A) and observed and expected heterozygosities (H_O and H_E) were calculated in GENEPOP Version 3.1 (Raymond & Rousset, 1995). We also used this program to test genetic disequilibria and deviation from Hardy-Weinberg equilibrium (Markov chain parameters: 10,000 dememorization steps, 100 batches, and 5,000 iterations per batch). These different tests guided final marker selection.

Genetic similarity indices

We used two different indices to assess genetic similarity between pair members. The first is the Queller & Goodnight (1989) index, which has been predominantly used in previous studies (e.g. Amos, et al. 2001, Hansson 2004, Yu, et al. 2001). To estimate the actual probability of producing a homozygous offspring, we also computed a new and more direct index, named hereafter "the probability of producing homozygous offspring" (*Phm*). For each locus *l*, given the genotype of the two pair members, this probability is:

$$Phm_{xy}(l)=\frac{(s_{ac}+s_{ad}+s_{bc}+s_{bd})}{4}$$

following Lynch & Ritland's (1999) notation, where s_{ij} is a Boolean factor equal to 1 if alleles *i* and *j* are similar (*i* and *j* standing for any other letter), and 0 otherwise. An index based on such probabilities was first proposed by Mathieu *et al.* (1990), but has never been used to our knowledge. Belkhir *et al.* (2002) showed that when the number of alleles per loci is low, such indices have a lower variance in their estimations than the Queller & Goodnight index, something indeed interesting in our study where some loci are not highly variable (see Table 1).

To obtain a value for *Phm* across all loci, a weighted average of the $Phm_{xy}(l)$ is calculated using the formula proposed by Mathieu *et al.* (1990):

$$Phm_{xy}=\frac{\sum_l \frac{1}{p_l} Phm_{xy}(l)}{\sum_l \frac{1}{p_l}}$$

where p_l is the probability of an individual being homozygous by chance at locus *l*.

Individual heterozygosity

We used three different indices to assess individual global heterozygosity: the direct heterozygosity *H* (proportion of heterozygous loci in a given individual), the standardized heterozygosity *SH* and the intern relatedness *IR*, all described in Amos *et al.* (2001). All these indices were calculated using the same program computed in Delphi (Borland Delphi 5.0, ©1983, 1989 Inprise Corporation; the program is available on request from the corresponding author).

Monte-Carlo analyses

Genetic indices and Monte-Carlo bootstraps were performed using a program computed in Delphi (Borland Delphi 5.0, ©1983, 1989 Inprise Corporation) that was previously tested for no significant differences with Queller & Goodnight estimations obtained from the IDENTIX software (Belkhir, et al. 2002).

For each given sample of individuals, we re-mated the adults according to their sex 10,000 times (i.e. 10,000 bootstraps). These bootstraps gave a set of values that we used as the distribution under the assumption of random mating (in further analyses, we only give the mean of the genetic indices of all these bootstraps). The observed values of the genetic indices were then tested for significant departure from the expected distribution obtained by the bootstraps. When bootstrapping, we used only adults seen in the considered year, and in each bootstrap we randomly made the same number of pairs that had been genotyped in the population using all alive adults, as they were all considered as potentially available for pairing. Allelic frequencies were calculated for this sub-sample of individuals. For Hypothesis 2, that mates are genetically less similar than expected by chance, statistical significance was assessed by the proportion of the bootstraps having a mean genetic index lower than the observed mean.

We statistically tested Hypothesis 3, that mates share more alleles than expected by chance, by assessing the proportion of the bootstrap values with a mean genetic similarity index higher than the observed mean (i.e. 1 minus the p-value calculated under Hypothesis 2). By bootstrapping on observed individuals and not on randomly created individuals generated via allelic frequencies, we might also expect to correct for biases due to linkage disequilibria or genotyping errors. Indeed, poorly genotyped individuals had the same probability of being included in observed pairs as in bootstrapped pairs.

General statistics

General statistical analyses were made using SAS® package (©SAS Institute Inc.1999, Cary, NC, USA). To analyze the correlations between the two indices of genetic similarity, as well as the correlations between male and female heterozygosity, we used a GLM (Generalized Linear Model) procedure. To analyze the links between reproductive success (number of eggs laid and the number and proportion of eggs hatched) and genetic variables, we used a GENMOD procedure since the dependant variables never showed more than three levels; this procedure allows to fit generalized linear models to discrete variables. For each of

these three levels, the genetic variables and the residuals from the GENMOD procedure were normally distributed. Similarly, we analyzed the correlations between chick's survival and heterozygosity using GENMOD procedures, since both chick's survival (alive or dead) and rank (first-hatched or second-hatched) only show two levels. For each rank and each survival value, the residuals from the GENMOD procedure were normally distributed.

For the analyses of growth variable (weight, tarsus or wing length), we have repeated measures (each chick was measured every 5 days), so we conduct MIXED procedures, that is, generalized linear models with chick's identity as random parameter in order to correct for pseudo-replication. We made mixed models with tarsus length, wing length or weight as dependent variables, chick identity as random parameter, and age (A), age*age (A^2), heterozygosity (H) and chick's rank (R) as explaining variables. We add A^2 because morphologic variables do not evolve linearly with age. For each growth variable and each heterozygosity index, we run several models and kept only the one with the lowest AIC value. Given that the three growth variable are correlated, and given that the different indices of heterozygosity are also correlated, the structure of the model with the lowest AIC was always the same, in the form of Growth variable = A + H + R + AH + AR + HR + AHR + A^2 + Chick identity as random parameter.

Results

A total of 645 adults were genotyped in the Middleton population from 2003 to 2006. The number of alleles per locus varied from 6 to 35. After correction for multiple tests, two loci appeared to be out of Hardy-Weinberg equilibrium: K16 ($p < 0.0001$) and RBG29 ($p = 0.005$). Because of its lack of variability, K67 has only been genotyped for paternity analysis in a part of the samples. Thus, for each analysis, we present the results obtained using all loci, or excluding K16, K67 and RBG29 which are designated the "OHW" loci, for "out of Hardy-Weinberg equilibrium". Only K31 and K32 were genetically linked ($p < 0.05$ after correction for multiple tests. As our genetic similarity analyses are based on bootstrapping on the multilocus genotypes (such that linkages between alleles are conserved for every bootstrap) and not on alleles, both loci were kept. We also conducted every allelic-based analysis with either all loci or without "OHW" loci and K31.

348 genotyped adults were monitored in 2003 and 2004: 241 were seen alive in 2003 and 289 in 2004. Adults formed 74 pairs in 2003, and 72 in 2004; the remaining adults for each

years correspond to unpaired adults or adults paired with non-genotyped mates. All these adults were used in the bootstrap analyses as they were alive in the considered year and thus potentially available for pairing. Some of these adults (and pairs) were seen during more than one year.

As expected, Phm_{xy} and r_{xy} are closely, but far from perfectly, correlated (coefficient of determination $r^2 = 0.71$, 100 pairs observed in 2003-2005, $p < 0.0001$). Based on the 2005 samples, we looked at correlations between genetic similarity of pairs and heterozygosity of offspring. Both indices of genetic similarity were highly correlated to offspring heterozygosity (82 chicks, $p < 0.007$ for all correlations between the indices of genetic similarity and the three indices *H*, *SH* and *IR* of offspring heterozygosity), confirming that genetically similar pairs are indeed more likely to give birth to homozygous offspring than dissimilar pairs.

Mating pattern and genetics

To correct linkage between loci, OHW loci and K31 were excluded from the calculation of *H*, *SH* and *IR*, but results were not different when including all loci. Male *H* was not correlated with female *H* for any of the analyzed years (r ranging from 0.021 to 0.10, p ranging from 0.38 to 0.86). Results were the same for *SH* ($0.02 \leq r \leq 0.13$, $0.26 \leq p \leq 0.87$) and *IR* ($0.05 \leq r \leq 0.10$, $0.38 \leq p \leq 0.67$). Unpaired individuals were also not significantly less heterozygous than paired individuals (for all indices: in 2003, $t_{2,241} < 1.93$, $p > 0.06$; in 2004, $t_{2,241} < 1.25$, $p > 0.21$).

For each year and for both indices (Phm_{xy} and r_{xy}), we ran 10,000 bootstraps using the observed individuals. For each run, we used either a calculation across all loci, or excluding OHW loci (Table 2). Results produced from r_{xy} were qualitatively similar but weaker than from Phm_{xy}. Using r_{xy}, there was no significant difference between observed and random pairs in 2003 with all loci (see Table 2) but a significant difference when excluding OHW loci from the calculations. Results were not significant in 2004. Using Phm_{xy}, we observed significant difference between observed and simulated pairs in 2003 and in 2004.

	r *(Queller & Goodnight index)*			
	Both years	***Stable pairs***	***2003***	***2004***
Pairs Adults	*92* *348*	*58* *348*	*74* *241*	*72* *289*
All loci	*-0.0353* *-0.00065* ***0.031***	*-0.0321* *-0.00061* *0.092*	*-0.0297* *0.0013* *0.060*	*-0.0299* *-3.75E-5* *0.083*
Without OHW loci	*-0.0314* *-0.00051* *0.068*	*-0.0213* *-0.00065* *0.22*	*-0.037* *0.00076* ***0.048***	*-0.016* *0.0013* *0.25*
	Phm *(prob. of producing a homozygous offspring)*			
All loci	*0.184* *0.199* ***0.016***	*0.182* *0.199* ***0.029***	*0.181* *0.196* ***0.021***	*0.184* *0.201* ***0.018***
Without OHW loci	*0.172* *0.189* ***0.026***	*0.171* *0.189* ***0.048***	*0.162* *0.184* ***0.005***	*0.174* *0.191* ***0.043***

Table 2: Difference between observed and simulated means for two relatedness indexes. For each case, we give the number of pairs used, and the number of genotyped adults alive in the considered year; we made 10,000 bootstraps to calculate the simulated mean and the p-value, each bootstrap consisting on randomly making *P* different pairs using the adults available. In each case, first line is the observed mean in the population, second line is the simulated mean. Last line is the p-value calculated as the proportion of bootstraps having a mean lower than the observed mean. Significant p-value are in bold. The first column contains the results for both years combined (with one observation per pair, even if the pairs were observed during different consecutive years), the second contains the results for all years combined using only pairs breeding together at both years (with again one observation per pair).

Given the very low divorce rate (only 7% among the genotyped pairs between 2003 and 2004), we were unable to compare divorced pairs to faithful pairs. Correlatively, most pairs were identical during consecutive years. To avoid pseudo-replication, we performed an analysis comparing random bootstraps to pairs observed during 2003 and/or 2004 using each pair only once (Table 2, column "all pairs"). We still obtained significant results with Phm_{xy}, and weaker results with r_{xy}. Results were similar when taking only pairs of birds breeding together at both years (considered as "stable pairs", Table 2).

It can be argued that the correlation we found may be due to only a subset of certain loci linked to fitness loci (e.g., Lieutenant-Gosselin & Bernatchez 2006), so we did also the analysis on 2003 pairs without OHW loci (that is, our most significant result) and re-run the simulations taking away one locus. We still found significant or close to significant non-

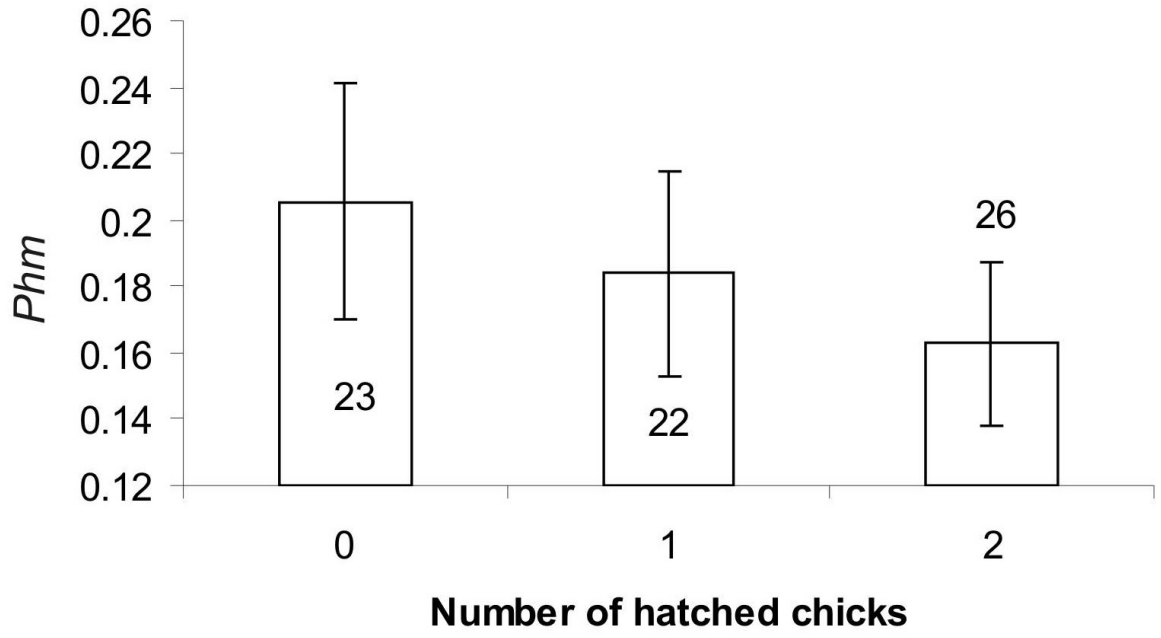


Figure 1: Relationship between number of hatched chicks and *Phm* in 2003. Here *Phm* was calculated without OHW loci ($\chi^2_{69} = 4.03$, p = 0.045, n = 71), but results are similar with all loci.

random mating patterns (*Phm* index: p < 0.008 if we discarded K31, K71, RBG20, RBG27 or RBG39; p = 0.026 if we discarded K6; p = 0.066 if we discarded K32), indicating that the pattern described here is not due to the contribution one locus alone to the calculation of the multilocus estimate.

Hypothesis 3 predicts that mates share more alleles than expected by chance. This means that the p-value for Phm_{xy} should be more than 0.95, which we did not find (Table 2).

Reproductive success and genetic similarity

We only used 2003 data to determine the number of eggs laid, and the number and proportion of eggs hatched, because the reproductive success in 2004 was modified by other experiments. Genetic similarity indices were computed without OHW loci and K31. The number of eggs laid was not correlated with Phm_{xy} ($\chi^2_{72} = 0.01$, p = 0.91, n = 74), or with $\hat{r}_{xy}$ ($\chi^2_{72} = 0.077$, p = 0.38, n = 74). For pairs that laid eggs, the number of hatched chicks was not correlated with r_{xy} ($\chi^2_{69} = 2.16$, p = 0.14, n = 71) but was correlated with Phm_{xy} ($\chi^2_{69} = 4.0$, p = 0.045, n = 71, see Figure 1). Similarly, hatching rate was not correlated with r_{xy} ($\chi^2_{69} = 0.79$, p = 0.37, n = 71) but was correlated with Phm_{xy} ($\chi^2_{69} = 4.0$, p = 0.045, n = 71). Results were similar when the indices where calculated over all loci.

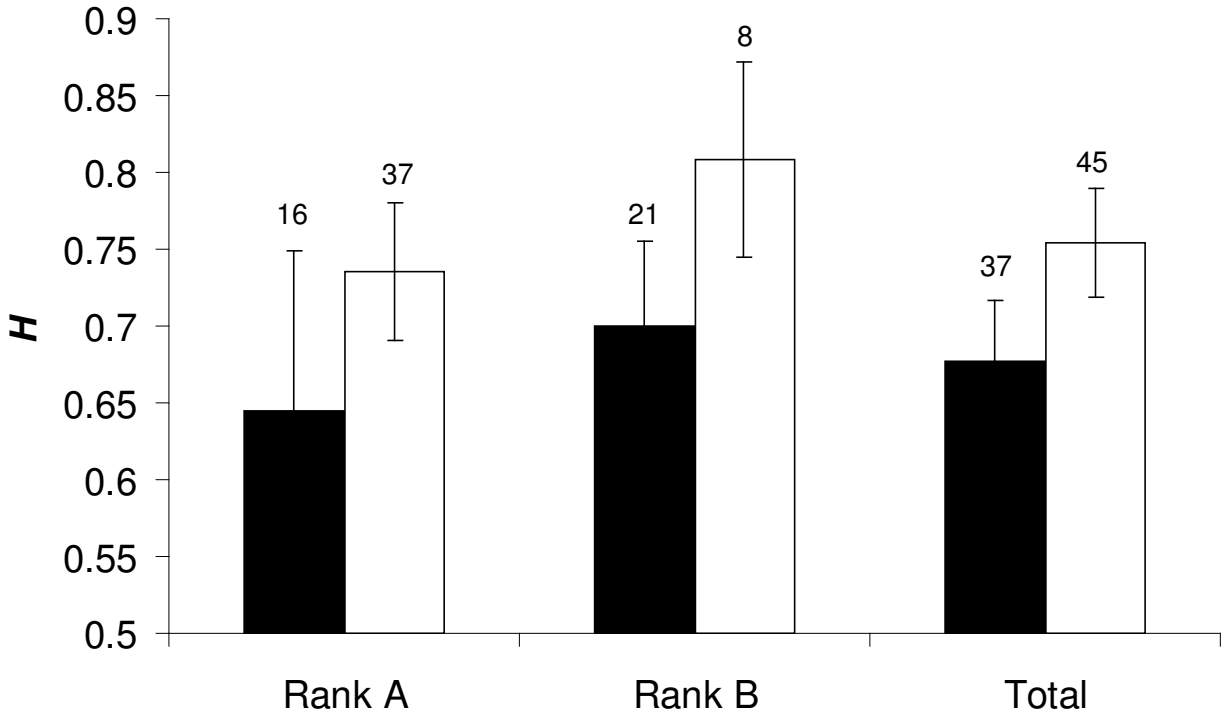


Figure 2: Relationship between heterozygosity and chick survival in 2005. We only show here the correlation between chick survival at 25 days old and chick heterozygosity estimated by the *H* index calculated over all loci. The model show that A-chicks being also more likely to survive ($\chi^2 > 12$, $p < 0.001$), but more interestingly, heterozygous chicks are also more likely to survive ($\chi^2 > 7.0$, $p < 0.0081$); however, this relationship is non-significant if heterozygosity is calculated without OHW loci and K31.

Offspring survival and heterozygosity

In 2005, we were able to assess survival of 82 chicks at 25 days old. This survival rate was highly correlated to chick's rank at hatching ($\chi^2 > 12$, $p < 0.001$ in all further analyses), but not to the co-variable rank*heterozygosity ($p > 0.6$). This co-variable was therefore eliminated from the models, and in all further models, we will only give the statistics associated to chick's heterozygosity. When taking heterozygosity indices calculated over all loci, chick's survival was positively correlated to heterozygosity (*H*: $\chi^2 > 7.0$, $p < 0.0081$, see Figure 2; *SH*: $\chi^2 > 5.9$, $p < 0.015$; *IR*: $\chi^2 > 6.1$, $p < 0.014$). However, this correlations become non-significant when heterozygosity indices were computed without OHW loci and K31 (*H*: $\chi^2 > 3.6$, $p < 0.06$; *SH*: $\chi^2 > 2.5$, $p < 0.11$; *IR*: $\chi^2 > 3.3$, $p < 0.07$).

Offspring growth and heterozygosity

In Table 3, we only show the p-value associated to the parameters of the model with the lowest AIC (for each index of heterozygosity). Wing length and weight show similar results whatever the index used; both strongly depend on the interaction A*H, indicating that homozygous individuals will grow slower than heterozygous individuals. The significance of the triple interaction A*H*R indicates that the slope of the interaction A*H is greater for B-chicks than A-chicks; however, only 8 B-chicks survived until 25 days old (see Figure 2), and the significance of this parameter is therefore to confirm. For tarsus length, the dependence on A*H seems smaller, and disappear when heterozygosity indices were calculated without OHW loci and K31. Thus, heterozygosity only margely affect tarsus growth.

Explained variable	*Index used*	*Parameters of the model with the lowest AIC*									*AIC*
		A	*H*	*R*	*A*H*	*A*R*	*H*R*	*A*H*R*	*A²*	*Chick as random*	
W*eight*	**H**	***NS***	***NS***	***NS***	*****	*****	***NS***	*****	**	*****	***3281.1***
	SH	*	*NS*	*NS*	****	***	*NS*	**	***	****	*3282.6*
	IR	****	*NS*	*NS*	****	***	*NS*	***	***	****	*3283.4*
	H'	***	*NS*	*NS*	****	***	*NS*	*	***	****	*3284.8*
	SH'	****	*NS*	*NS*	****	*	*NS*	*	***	****	*3285.9*
	IR'	****	*NS*	*NS*	***	*	*NS*	*NS*	***	****	*3289.2*
Wing	**H**	***NS***	***NS***	***NS***	*****	*****	***NS***	*****	*****	*****	***2619.7***
	SH	*NS*	*NS*	*NS*	****	***	*NS*	**	****	****	*2620.8*
	IR	****	*NS*	*NS*	***	*	*NS*	***	****	****	*2621.2*
	H'	*NS*	*NS*	*NS*	***	**	*NS*	*	****	****	*2621.7*
	SH'	*NS*	*NS*	*NS*	****	*	*NS*	*	****	****	*2621.6*
	IR'	****	*NS*	*NS*	***	*NS*	*NS*	*	****	****	*2626.8*
Tarsus	**H**	*****	***NS***	***NS***	**	**	***NS***	*	*****	*****	***2762.2***
	SH	****	*NS*	*NS*	*	*	*NS*	*NS*	****	****	*2764.7*
	IR	****	*NS*	*NS*	*	*	*NS*	**	****	****	*2763.7*
	H'	****	*NS*	*NS*	*NS*	*	*NS*	*	****	****	*2765.2*
	SH'	****	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	****	****	*2767.2*
	IR'	****	*NS*	*NS*	*NS*	*NS*	*NS*	*	****	****	*2766.3*

Table 3: Significance of the parameters associated to the models explaining the evolution of weight, wing and tarsus length. For each morphometric variable (weight, tarsus or wing), we run several models; only the models with the lowest AIC value for a given heterozygosity index are shown here, and they are all on the same form: A+H+R+AH+HR+AHR+A² with chick identity as random. Parameters are : A (age), H (heterozygosity, estimated by the index in the column "Index used") and R (hatching rank, a factor of 2 levels). For heterozygosity indices: *H*, *SH* and *IR* are calculated over all loci, while *H'*, *SH'* and *IR'* are calculated without OHC loci and K31. P-value associated to each parameters of the models are given by: NS = non significant ; * if $p < 0.05$; ** if $p < 0.01$; *** if $p < 0.005$; **** if $p < 0.001$.

Discussion

Our main objective was to describe the correlation between genetic variables and mate choice in a strictly monogamous species. To assess genetic similarity between pair members, we designed the *Phm* index of genetic similarity, which seems to give stronger and more consistent results than Queller & Goodnight index in our system. It also appears to be more robust to potentially disequilibrated loci, a property of some value to the many studies where it is difficult to select perfectly equilibrated loci. However, performances of genetic similarity indices usually depends on the set of loci used (Van de Casteele, et al. 2001), and further studies would be needed to determine what index will more accurately estimate the heterozygosity of the offspring (independently to its pedigree) depending on each data configuration.

We found that individuals were not mated randomly with respect to genetic similarity in both years. Observed pairs had a lower probability of producing homozygous offspring and less genetic similarity than expected. Our findings are consistent with the "genetic similarity avoidance" hypothesis, and fail to support the "good genes as heterozygosity", and the "genetic similarity preference" hypotheses. This is, to our knowledge, the first evidence of a long-lived and genetically monogamous species avoiding pairing with genetically similar mates. Non-random mating with respect to genetic similarity has also previously been reported in three shorebird species (Blomqvist, et al. 2002), ruffs (*Philomachus pugnax*, Thuman & Griffith, 2005), sand lizards (*Lacerta agilis,* Olsson, et al. 2003), mice (Roberts & Gosling, 2003) or humans (Milinski & Wedekind, 2001). Other species behave consistently with the good-genes hypothesis (e.g. house sparrows *Passer domesticus*, Bonneaud, et al. 2006, and Seychelles warblers *Acrocephalus sechellensis*, Richardson, et al. 2005), or with the genetic similarity preference hypothesis (e.g. pied flycatcher *Ficedula hypoleuca*, Rätti, et al. 1995, and great frigatebirds *Fregata minor*, Cohen & Dearborn, 2004), while other species display no significant patterns of genetic criteria of mate choice (e.g. great reed warbler *Acrocephalus arundinaceus*, Westerdahl 2004).

We showed also different deleterious effects of genetic similarity and homozygosity. As expected (Coltman & Slate 2003), the correlations we found were weak but taken together, they gave interesting insights on the fitness costs of breeding with a genetically similar individual. First, genetically similar pairs hatched fewer offspring and therefore have a lower hatching success than dissimilar pairs, confirming previous studies (Kempenaers, et al. 1996, Van de Casteele, et al. 2003) showing effects of inbreeding on egg hatchability. Hatching

success was found to correlate negatively with the genetic similarity of the pair. This pattern could be due to at least two non-exclusive effects: 1) since both sexes incubate, cooperation between them may be maximized when the genetic quality of the pair is high, and 2) the overall quality of heterozygous offspring may be higher, therefore increasing the success of early development. Given the apparently weak relationships between measures of reproductive success and genetic similarity, further evaluations of these hypotheses are warranted. Second, homozygous offspring are less likely to reach 25 days old than heterozygous ones. Again this pattern may be explained by different effects: 1) heterozygous individuals may be more able to defend themselves again pathogens (e.g., Penn, et al. 2002), such attacks being likely important early in life, and 2) homozygous offspring are more likely to come from genetically similar pairs that may be not very cooperative during rearing, a time when chicks are highly dependent from parental care. Third, we found that homozygous chicks grow slower than heterozygous chicks, a pattern that may also explain the cost in survivorship: chicks growing slower are in worse condition than fast growing chicks, and are thus less likely to defend themselves again pathogens and environmental variations.

The use of multilocus estimates of heterozygosity and genetic similarity has been recently debated. Studies (Lieutenant-Gosselin & Bernatchez 2006, Tiira, et al. 2005, Hansson & Westerberg 2002) argued that global heterozygosity-fitness correlations may be driven by certain selected loci, that may be directly linked to fitness loci in the local chromosomal vicinity. That does not seem to be the case in our study. Indeed, the correlation between genetic similarity and mate choice remains significant or very close to significance even if we exclude randomly one locus, and we found also the same patterns when including potentially biased loci. Since the number of microsatellites we used (7-10) is low, given that the number of loci needed to achieve an accurate estimation of individual global heterozygosity may be very large (e.g., Cooper, et al. 1999). We try three additional loci that did not give reliable data, and no other loci in kittiwakes or close related species has been published so far. However, the fact that we found effects of genetic similarity and homozygosity on different components of fitness (mate choice, hatching success, offspring growth and survival) is a good indication that genetic similarity is indeed highly costly, and therefore selected against, in this population.

Little is known about the process of mate choice in kittiwakes, and the pattern we describe here may come from different histories. First it could be due to a passive process, if for example failing pairs are more likely to be genetically similar and to divorce than successful pairs. In our population, it seems rather unrealistic, since the rate of divorce after

reproductive failure is low and the correlation between reproductive success and genetic similarity is weak. Another explanation may comes from birds pairing with individuals from the same part of a structured population over time. This explanation seems also unlikely, since no genetic structure has yet been found in kittiwakes (McCoy, et al. 2005). A third explanation would be that adults are actively looking for genetically dissimilar mates. Given the high fitness costs of genetic similarity (lower hatching success and lower offspring fitness), this hypothesis may be the likelier, since it would allow individuals to reach the most suitable mate faster than any passive process. However, to be tested, it would need mate choice experiments that are rather difficult to undertake with such long-lived cliff-nesting seabirds, but may be feasible on smaller birds showing the same kind of pattern. We would also need to compare pairs that divorced to pairs that stayed together, and therefore we have started a long-term blood sampling protocol in order to increase the sample size of individuals with known pre- and post-divorce mate.

The only mate choice experiments showing an active choice of genetically dissimilar mate come from mammals studies. For example, genetic similarity might be correlated with phenotypic characters such as olfactory cues in humans (Wedekind 2002), lemurs (Knapp, et al. 2006) and mice (Yamazaki, et al. 1990). Although the use of smell by many birds is still controversial, Antarctic prions have been shown to recognize their mate through odors (*Pachiptila desolata*, Bonadonna & Nevitt, 2004). Genetically driven odors have not been shown in birds, but we have observed various behaviors that may allow kittiwakes to recognize and choose their mate according to odor. Olfactory individual recognition may thus be an important feature in this species that warrants further investigation.

In conclusion, black-legged kittiwakes are paired with individuals that are genetically dissimilar, a pattern in concordance with a strategy to avoid the potential costs of producing homozygous progeny such as a decrease in hatching success or the ability of these offspring to respond to changing environmental conditions. Future studies on kin and mate recognition might enable us to find phenotypic cues used by birds to assess the genetic similarity of their counterparts, and thus to choose the most genetically compatible mates.

Acknowledgements

We thank Kevin Sage and Judy Gust for their help in the lab, as well as several assistants for their help in the field. Karen McCoy gave valuable comments on earlier drafts of this

article. Experiments were carried on in accordance with American regulations and under permits from the U.S. Fish and Wildlife Service (MB789758-2) and the U.S. Department of Agriculture (43860). This study was financed by a four year grant from the French Polar Institute Paul-Emile Victor ("Programme Arctique 429" 2004-2007), a PICS (Projet International de Coopération Scientifique) for French-Austrian cooperation, and by the U.S. Geological Survey, Alaska Science Center. This project is part of the French GDR2155 "Écologie comportementale".

References

Amos, W., Worthington Wilmer, J., Fullard, K., Burg, T. M., Croxall, J. P., Bloch, D. & Coulson, T. 2001. The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society of London B*, **268,** 2021-2027.

Belkhir, K., Castric, V. & Bonhomme, F. 2002. IDENTIX, a software to test for relatedness in a population using permutation methods. *Molecular Ecology Notes*, **2,** 611-614.

Bensch, S., Hasselquist, D. & Von Schantz, T. 1994. Genetic similarity between parents predicts hatching failure nonincestuous inbreeding in the Great reed warbler? *Evolution*, **48,** 317-326.

Blomqvist, D., Andersson, M., Küpper, C., Cuthill, I. C., Kis, J., Lanctot, R. B., Sandercock, B. K., Székely, T., Wallander, J. & Kempenaers, B. 2002. Genetic simimarity between mates and extra-pair parentage in three species of shorebirds. *Nature*, **419,** 613-615.

Bonadonna, F. & Nevitt, G. A. 2004. Partner-specific odor recognition in an Antarctic seabird. *Science*, **306,** 835.

Bonneaud, C., Chastel, O., Federici, P., Westerdahl, H. & Sorci, G. 2006. Complex MHC-based mate choice in a wild passerine. *Proceedings of the Royal Society of London B*, **273,** 1111-1116.

Brown, J. L. 1996. A theory of mate choice based on heterozygosity. *Behavioral Ecology*, **8,** 60-65.

Burley, N. T. & Foster, V. 2006. Variation in female choice of mates: condition influences selectivity. *Animal Behaviour*, **72,** 713-719.

Cohen, L. B. & Dearborn, D. C. 2004. Great frigatebirds, *Fregata minor*, choose mates that are genetically similar. *Animal Behaviour*, **68,** 1229-1236.

Coltman, D. W. & Slate, J. 2003. Microsatellite measures of inbreeding: a meta-analysis. *Evolution*, **57,** 971-983.

Cooper, G., Amos, W., Bellamy, R., Ruby Siddiqui, M., Frodsham, A., Hill, A. V. S. & Rubinsztein, D. C. 1999. An empirical exploration of the $(\delta\mu)^2$ genetic distance for 213 human microsatellite markers. *American Journal of Human Genetics*, **65,** 1125-1133.

Coulson, J. C. & Thomas, C. S. 1985. Changes in the biology of the kittiwake Rissa tridactyla: a 31-year study of a breeding colony. *Journal of Animal Ecology*, **54,** 9-26.

Coulson, T. N., Albon, S. D., Slate, J. & Pemberton, J. M. 1999. Microsatellite loci reveal sex-dependent responses to inbreeding and outbreeding in red deer calves. *Evolution*, **53,** 1951-1960.

Fairweather, J. A. & Coulson, J. C. 1995. Mate retention in the kittiwake, *Rissa tridactyla*, and the significance of nest site tenacity. *Animal Behaviour*, **50,** 455-464.

Foerster, K., Delhey, K., Johnsen, A., Lifjeld, J. T. & Kempenaers, B. 2003. Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature*, **425,** 714-717.

Freeman-Gallant, C. R., Meguerdichian, M., Wheelwright, N. T. & Sollecito, S. V. 2003. Social pairing and female mating fidelity predicted by restriction fragment length polymorphism similarity at the major histocompatibility complex in a songbird. *Molecular Ecology*, **12,** 3077-3083.

Gill, V. A. & Hatch, S. A. 2002. Components of productivity in black-legged kittiwakes *Rissa tridactyla*: response to supplemental feeding. *Journal of Avian Biology*, **33,** 113-126.

Given, A. D., Mills, A. & Baker, J. 2002. Isolation of polymorphic microsatellite loci from the red-billed gull (*Larus novaehollandiae scopulinus*) and amplification in related species. *Molecular Ecology Notes*, **2,** 416-418.

Hansson, B. 2004. Marker-based relatedness predicts egg-hatching failure in great reed warblers. *Conservation Genetics*, **5,** 339-348.

Hansson, B. & Westerberg, L. 2002. On the correlation between heterozygosity and fitness in natural populations. *Molecular Ecology*, **11,** 2467-2474.

Helfenstein, F., Tirard, C., Danchin, E. & Wagner, R. H. 2004. Low frequency of extra-pair paternity and high frequency of adoption in Black-legged kittiwakes. *Condor*, **106,** 149-155.

Höglund, J., Piertney, S. B., Alatalo, R. V., Lindell, J., Lundberg, A. & Rintamäki, P. T. 2002. Inbreeding depression and male fitness in black grouse. *Proceedings of the Royal Society of London B*, **269,** 711-715.

Jodice, P. G. R., Lanctot, R. B., Gill, V. A., Roby, D. D. & Hatch, S. 2000. Sexing adult black-legged kittiwakes by DNA, behavior, and morphology. *Waterbirds*, **23,** 405-415.

Johnsen, A., Delhey, K., Schlicht, E., Peters, A. & Kempenaers, B. 2005. Male sexual attractiveness and parental effort in Blue tits: a test of the differential allocation hypothesis. *Animal Behaviour*, **70,** 877-888.

Jones, I. L. & Hunter, F. M. 1993. Mutual sexual selection in a monogamous seabird. *Nature*, **362,** 238-239.

Kempenaers, B., Adriaensen, F., Van Noordwijk, A. J. & Dhondt, A. A. 1996. Genetic similarity, inbreeding and hatching failure in blue tits: are unhatched eggs infertile? *Proceedings of the Royal Society of London B*, **263,** 179-185.

Knapp, L. A., Robson, J. & Waterhouse, J. S. 2006. Olfactory signals and the MHC: a review and a case study in *Lemur catta*. *American Journal of Primatology*, **68,** 568-584.

Kokko, H. & Ots, I. 2006. When not to avoid inbreeding. *Evolution*, **60,** 467-475.

Landry, C., Garant, D., Duchesne, P. & Bernatchez, L. 2001. 'Good genes as heterozygosity': the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). *Proceedings of the Royal Society of London B*, **268,** 1279-1285.

Lieutenant-Gosselin, M. & Bernatchez, L. 2006. Local heterozygosity-fitness correlations with global positive effects of fitness in threespine stickleback. *Evolution*, **60,** 1658-1668.

Longmire, J. L., Lewis, A. K., Brown, N. C., Buckingham, J. M., Clark, L. M., Jones, M. D., Meincke, L. J., Meyne, J., Ratliff, R. L., Ray, F. A., Wagner, R. P. & Moyzis, R. K. 1988. Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family Falconidae. *Genomics*, **2,** 14-24.

Lynch, M. & Ritland, K. 1999. Estimation of pairwise relatedness with molecular markers. *Genetics*, **152,** 1753-1766.

Mathieu, E., Autem, M., Roux, M. & Bonhomme, F. 1990. Epreuves de validation dans l'analyse de structures génétiques multivariées: comment tester l'équilibre panmictique? *Revue de Statistique Appliquée*, **38,** 47-66.

Mays, H. L. & Hill, G. E. 2004. Choosing mates: good genes versus genes that are a good fit. *Trends in Ecology & Evolution*, **19,** 554-559.

McCoy, K. D., Boulinier, T. & Tirard, C. 2005. Comparative host-parasite population structures: disentangling prospecting and dispersal in the Black-legged kittiwake *Rissa tridactyla*. *Molecular Ecology*, **14,** 2825-2838.

Medrano, J. F., Aasen, E. & Sharrow, L. 1990. DNA extraction from nucleated red blood cells. *Biotechniques*, **8,** 43.

Milinski, M. & Wedekind, C. 2001. Evidence for MHC-correlated perfume preferences in humans. *Behavioral Ecology*, **12,** 140-149.

Neff, B. D. & Pitcher, T. E. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Molecular Ecology*, **14,** 19-38.

Oetting, W. S., Lee, H. K., Flanders, D. J., Wiesner, G. L., Sellers, T. A. & King, R. A. 1995. Linkage analysis with multiplexed short tandem repeat polymorphisms using infrared fluorescence and M13 taied primers. *Genomics*, **30,** 450-458.

Olsson, M., Madsen, T., Nordby, J., Wapstra, E., Ujvari, B. & Wittsell, H. 2003. Major histocompatibility complex and mate choice in sand lizards. *Proceedings of the Royal Society of London B*, **Supplement,** S1-S3.

Penn, D. J., Damjanovich, K. & Potts, W. K. 2002. MHC heterozygosity confers a selective advantage against multiple-strain infections. *Proceedings of the National Academy of Sciences USA*, **99,** 11260-11264.

Poesel, A., Kunc, H. P., Foerster, K., Johnsen, A. & Kempenaers, B. 2006. Early birds are sexy: male age, dawn song and extrapair paternity in Blue tits, *Cyanistes* (formerly *Parus*) *caeruleus*. *Animal Behaviour*, **72,** 531-538.

Queller, D. C. & Goodnight, K. F. 1989. Estimating relatedness using genetic markers. *Evolution*, **43,** 258-275.

Rantala, M. J., Eriksson, C. J. P., Vainikka, A. & Kortet, R. 2006. Male steroid hormones and female preference for male body odor. *Evolution and Human Behavior*, **27,** 259-269.

Rätti, O., Hovi, M., Lundberg, A., Tegelström, H. & Alatalo, R. V. 1995. Extra-pair paternity and male characteristics in the pied flycatcher. *Behavioral Ecology and Sociobiology*, **37,** 419-425.

Raymond, M. & Rousset, F. 1995. GENEPOP (ver. 1.2): a population genetics software for exact test and ecumenicism. *Heredity*, **86,** 248-249.

Richardson, D. S., Komdeur, J., Burke, T. & Von Schantz, T. 2005. MHC-based patterns of social and extra-pair mate choice in the Seychelles warbler. *Proceedings of the Royal Society of London B*, **272,** 759-767.

Roberts, S. C. & Gosling, L. M. 2003. Genetic similarity and quality interact in mate choice decisions by female mice. *Nature Genetics*, **35,** 103-106.

Rubenstein, D. R. 2007. Female extrapair mate choice in a cooperative breeder: trading sex for help and increasing offspring heterozygosity. *Proceedings of the Royal Society of London B*, **274,** 1895-1903.

Thuman, K. A. & Griffith, S. C. 2005. Genetic similarity and the nonrandom distribution of paternity in a genetically highly polyandrous shorebird. *Animal Behaviour*, **69,** 765-770.

Tiira, K., Laurila, A., Enberg, K., Piironen, J., Aikio, S., Ranta, E. & Primmer, C. R. 2005. Do dominants have higher heterozygosity? Social status and genetic variation in brown trout, Salmo trutta. *Behavioral Ecology and Sociobiology*, **59,** 657-665.

Tirard, C., Helfenstein, F. & Danchin, E. 2002. Polymorphic microsatellites in the Black-legged kittiwake *Rissa tridactyla*. *Molecular Ecology Notes*, **2,** 431-433.

Tregenza, T. & Wedell, N. 2000. Genetic compatibility, mate choice and patterns of parentage: Invited review. *Molecular Ecology*, **9,** 1013-1027.

Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual selection and the descent of man, 1871-1971* (Ed. by Campbell, B.), pp. 136-179. Chicago, IL: Aldine Publishing Company.

Van de Casteele, T., Galbusera, P. & Matthysen, E. 2001. A comparison of microsatellite-based pairwise relatedness estimators. *Molecular Ecology*, **10,** 1539-1549.

Van de Casteele, T., Galbusera, P., Schenck, T. & Matthysen, E. 2003. Seasonal and lifetime reproductive consequences of inbreeding in the great tit *Parus major*. *Behavioral Ecology*, **14,** 165-174.

Wedekind, C. 2002. The MHC and body odors: arbitrary effects caused by shifts of mean pleasantness. *Nature*, **31,** 237.

Westerdahl, H. 2004. No evidence of an MHC-based female mating preference in great reed warblers. *Molecular Ecology*, **13,** 2465-2470.

Westerdahl, H., Waldenström, J., Hansson, B., Hasselquist, D., Von Schantz, T. & Bensch, S. 2005. Associations between malaria and MHC genes in a migratory songbird. *Proceedings of the Royal Society of London B*, **272,** 1511-1518.

Yamazaki, K., Beauchamp, G. K., Imai, Y., Bard, J., Phelan, S. P., Thomas, L. & Boyse, E. A. 1990. Odortypes determined by the major histocompatibility complex in germfree mice. *Proceedings of the National Academy of Sciences USA*, **87,** 8413-8416.

Yu, H.-T., Liao, Y.-Y. & Kao, C.-H. 2001. Relatedness structure and individual identification in a semi-fossorial shrew (Soricidae: *Anourosorex squamipes*) - an application of microsatellite DNA. *Zoological Studies*, **40,** 226-232.

ARTICLE 5: Sequence variation in the MHC Class II DRB1-like gene of four colonial seabirds

Hervé Mulard, Christian Barnabé & Karen McCoy

Will be submitted to *Immunogenetics*

Abstract

Genes of the Major Histocompatibility Complex have been found to influence many life history traits and their characterization is therefore of major interest for both ecological and behavioural questions. However, little is known about the evolution of these genes in birds, especially seabirds. Here we sequenced the exon 2, intron 2 and exon 3 of the MHC Class II DRB1-like locus in four seabird species from the sub-order Charadrii: the black-legged kittiwake, the Atlantic puffin, the common guillemot and the razorbill. The number of functional loci seems low in all species. We found no indication of more than one locus in kittiwakes and puffins. However guillemots and razorbills showed two loci, distinguishable by inserts in the flanking region of exon 2. We found complete lineage sorting in all species, but the evolutionary relationships among guillemot sequences were unresolved. The alignment of alleles with others from the Ciconiiform family supported the monophyly of the sub-order Charadrii. There was a general trend for higher rates of non-synonymous to synonymous substitutions in exon 2 compared to the other regions, and particularly in codons involved in the Peptide Binding Region. In addition we found no evidence that alleles of the MHC Class II were geographically distinct in kittwakes from different ocean basins (Atlantic and Pacific), despite their sub-species status. Both of these observations support an influence of balancing selection on the maintenance of these sequences. The high variability of these genes supports their potential role in individual recognition and thus as significant genes to study when examining seabird life history decisions.

Key words: MHC Class II; Charadrii; balancing selection; phylogeny; geographical variation

Introduction

Genes of the Major Histocompatibility Complex (MHC) have been found to influence many life history traits, and their characterization is therefore not only of major importance for understanding the evolution of vertebrate immunity (e.g., Danchin et al. 2004), but also for answering numerous ecological and behavioural questions in relation to the dynamics of natural populations (reviewed in Piertney and Oliver 2006). For example, there is a general consensus that heterozygosity at MHC genes should be beneficial as it should enhance the capacity of an individual to defend against various pathogens (Bonneaud et al. 2004a, McClelland et al. 2003, Penn et al. 2002, , Wedekind et al. 2004, Westerdahl et al. 2005). Indeed, MHC genotypes have been found to influence mate choice in several vertebrate species, with individuals typically choosing mates that differ in their MHC genotype (Bonneaud et al. 2006, Ekblom et al. 2004, Landry et al. 2001, Penn and Potts 1998a, Richardson et al. 2005, Wedekind and Füri 1997, Wegner et al. 2003). Evidence now suggests that this selection may be based on odours, as different MHC genotypes are associated with different detectable smells (Milinski and Wedekind 2001, Penn and Potts 1998b). As MHC genotypes are highly variable, the link between odour and genotype may also be used in kin recognition (e.g., Penn 2002, Wedekind and Penn 2000) and thus, be at the origin of a plethora of individual life history decisions (e.g., whether to disperse or remain in the local population).

MHC genes have been extensively sequenced in humans (The MHC Sequencing Consortium 1999) and other mammals (e.g., Knapp et al. 2006), along with various fishes (e.g. Olsén et al. 1998, Reusch et al. 2001) and reptiles (e.g. Olsson et al. 2003). In birds, Galliforms (Shiina et al. 2004, Zoorob et al. 1990) and Passeriforms have received most attention (Bonneaud et al. 2004b, Edwards et al. 1995, Westerdahl 2003), but recent studies have also focused on the Spheniscidae (Kikkawa et al. 2005, Tsuda et al. 2001). These studies have revealed very different patterns among birds. While all birds show a relatively compact genome compared to mammals, passerines tend to have many copies of MHC genes and pseudogenes are abundant, whereas galliforms typically show few copies and no pseudogenes (Hess and Edwards 2002). So far, little is known about MHC organisation and variation in the sub-order Charadrii (including seabirds and waders). MHC diversity may be particularly important in this group as most species are colonial, and therefore tend to be highly exposed

to parasites (e.g., Møller et al. 2001). They also tend to be long-lived with strong inter-annual mate fidelity, meaning that mate choice is a key life history trait for these species.

Here, we explore variation at the MHC class II B genes in four seabird species, the black-legged kittiwake (*Rissa tridactyla*), the Atlantic puffin (*Fratercula arctica*), the common guillemot (*Uria aalge*) and the razorbill (*Alca torda*). We first examine the number of functional loci in these species. We then analyse the variation at the exon 2 and the peptide binding region (PBR) of this gene class to determine the potential role of balancing selection in maintaining alleles at both inter-specific and inter-populational levels. Finally, we consider the potential use of MHC genotypes as individual and population-level markers for ecological studies.

Material and methods

Sampling

All birds were sampled in their colonies between 1999 and 2003 (see Table 1 for locations). On Helgoland (Germany), a growing feather was taken from chicks sampled on the nest and was placed in 70-90% ethanol. For all other samples, a blood sample of approximately 0.5 ml was taken from the left ulnar vein of the birds using a sterile syringe rinsed with heparin. Blood samples were then stored in 1.5 ml tubes containing a storage buffer (Tris-EDTA or Longmire buffer) and were kept at ambient temperature until DNA extraction.

Genetic analyses

DNA was extracted from blood or feather tips using Qiagen DNEasy Blood and Tissue Extraction kit (Qiagen, Courtaboeuf, France). The MHC region was then amplified using either the primers LP1 5'-TGGGGCCCTACTGGTGGCACT-3' and LP2 5'-CGATCCCCGTCAGCATGTTGC-3' (Kikkawa et al. 2005) or the primers Pen1 5'-AACGGCACCGAGCGGGTGAGGT-3' and Pen4 5'-CCCGTAGTTGTGTTGGCAG-3' (Tsuda et al. 2001). These primers were originally designed for penguins. Pen1 and Pen4 amplify a section of 198bp inside the exon 2 of the MHC Class II – DRB1-like region (where the peptide binding region is located), and LP1 and LP2 amplify a section of approximately 1200bp containing exon 2, intron 2, exon 3 and the flanking regions of both exons (Figure 1).

All PCR reactions were conducted in 50µL wells, containing approximatively 100-150ng of

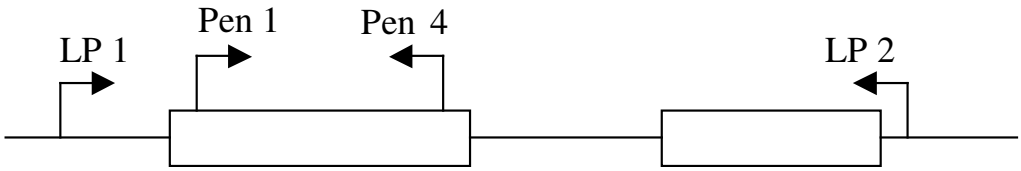


Figure 1 : Map of the MHC-Class II B region showing primer locations.
The codons involved in the PBR are found within the exon 2.

genomic DNA.

Samples from 14 black-legged kittiwakes, 5 Atlantic puffins, 5 razorbills, and 3 common guillemots were amplified using primers LP1/LP2 (Table 1). Each reaction was conducted with 1mM dNTP, 5mM $MgCl_2$, 2µM of each primer, 2µL Promega 5X buffer and 0.75 U Promega Taq polymerase (Promega, Madison, U.S.A.). The samples were then incubated at 94°C for 1min, then during 30 cycles, at 94°C for 1min, 57°C for 1min and 72°C for 1min 30sec. The final elongation step was 72°C for 3min. The total PCR product was then run on a 0.6% agarose gel. Electrophoretic bands of the expected size were extracted from the gel using Qiagen Gel Extraction kit and inserted in PGEM-TEasy plasmids. Ligation reactions were conducted in 5µL containing 25ng of the PGEM-TEasy plasmids, 1.5 units of T4 DNA ligase, 2.5µL of T4 2X buffer, and the amplified DNA (1.5ng for Pen1/Pen4, and 12ng for LP1/LP2). Ligation tubes were incubated at 4°C overnight. The next day, 1µL of each ligation reaction was incubated at 4°C during 20min with 25µL of JM109-high efficiency bacteria (Promega, Madison, U.S.A). Bacteria were then transformed at 42°C during 50sec and 4°C during 2min. Bacteria grew at 37°C during 3 hours, and were then dispatched on Petri dishes containing LB-Agar, ampicilline (50µg/mL), 20mM of Isopropyl β-D-1-thiogalactopyranoside (IPTG) and 0.15% of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (XGal). Petri dishes were incubated at 37°C overnight. Two to four white colonies were selected per individual; inserts were extracted using Qiagen Spin Miniprep kit (Qiagen, Courtaboeuf, France) and sent for sequencing (Genome Express, Meylan, France) using various primers (LP1, LP2, M13, T7, M13Rev or SP6). All clones were sequenced in both directions to obtain an unambiguous sequence. All chromatographs were re-read in order to correct for base-calling errors.

The primers Pen1/Pen4 were used on 10 black-legged kittiwake samples. For these amplifications, each reaction was conducted with 1mM dNTP, 5mM $MgCl_2$, 1µM of Pen1 and 2µM of Pen4, 2µL of Promega 5X buffer and 0.75 U Promega Taq polymerase. The samples were incubated at 94°C for 1min, then during 30 cycles, at 94°C for 1min, 54°C for 1min and 72°C for 1min 15sec. The final elongation step was at 72°C for 3min. These PCR products were sent for direct sequencing in both directions (Genome Express, Meylan, France). A pre-analysis conducted by SSCP (Single Strand Conformation Polymorphisms) gel electrophoresis on 40 kittiwake individuals showed that these two primers never amplified more than two alleles in this species. Thus, by comparing the direct sequence of individuals and the sequence obtained by cloning (described above), we were able to resolve the sequence of the second allele.

Statistics and phylogeny

Sequences were analysed using MEGA version 4 software (Tamura et al. 2007). When two or more sequences obtained from the same individual varied by less than 5 nucleotides, we considered that these sequences represented the same allele; different alleles of the same individual always varied to a much greater degree. In such cases, we only kept the sequence with the lowest number of single nucleotide polymorphisms (SNPs, that is, sites that differ in only one sequence while constant in all others). Such SNPs could be due to errors of the Taq polymerase during the PCR reaction. The same logic was applied to sequences obtained from different individuals that differed by less than 5 nucleotides. As possible PCR errors should affect all sequences in the same way, they reduce the power of our tests, making them more conservative.

Phylogenetic trees were obtained using the Neighbor-Joining method. When not stated otherwise, trees were obtained with 1000 bootstrap replicates, a Kimura 2 parameters model, pairwise deletion of gap/missing data, and a uniform rate of substitutions among sites. We first constructed a tree for the entire sequence of the exon 2-intron 2-exon 3 region in order to see if there was complete lineage sorting among seabird species. We then built a tree to look at the phylogenetic relationships among published MHC-Class II B sequences of various bird species using only the exon 2 region. Finally, we built a tree of kittiwake allele sequences of the exon 2 region (limited to the sequence obtained by Pen1/Pen4) in order to examine the potential geographic structure of these alleles. We only show results obtained by the neighbor-joining method, as trees obtained by other methods (maximum parcimony,

minimum evolution, UGPMA or maximum likelihood) did not differ significantly. Rates of synonymous (d_S) and non-synonymous (d_N) substitutions were calculated using the method of Nei and Gojobori (1986) with a Jukes-Cantor correction. Standard errors were calculated on 1000 bootstrap replicates.

Results

Exon 2-intron 2-exon 3

We obtained 12 sequences from 14 black-legged kittiwakes, 6 sequences from 5 Atlantic puffins, 5 sequences from 5 razorbills and 5 sequences from 3 common guillemots. Sampling locations and genbank accession codes for sequences are given in Table 1.

Number of functional loci

In kittiwakes and puffins, we found no evidence that LP1/LP2 amplified more than one locus, that is, we never found more than one band per individual on electrophoresis gels. Moreover, it seems that LP1/LP2 and Pen1/Pen4 amplified the same locus; indeed, for individuals analysed with both set of primers (KT26612 and KT605), the sequence of the alleles amplified by LP1/LP2 were in accordance with the sequence obtained by direct sequencing of the individual with Pen1/Pen4. In line with this and the preliminary screening of kittiwakes using SSCP (see methods), allele diversity in all species was low among clones of the same individual (i.e., different clone sequences often revealed the same allele).

In guillemots and razorbills, gel electrophoreses showed two distinct bands of approximately 1200 (named Kitt, Razo, Guil and Puff) and 1500 bp (named GuilBis and RazoBis). We only managed to obtain the sequence of the 1500 bp band for one clone (GuilBis1); sequence analysis showed that it was also an MHC sequence and that the size difference was due to differences in the flanking region of exon 2. We obtained only partial sequences of the 1500bp band from razorbills (RazoBis1); the size (660bp) and the sequence of the flanking region was closer to that of GuilBis1 than to the other razorbill sequences (see Figure 2 of the Supplemental material for the complete alignment). This suggests that the duplication of the locus may have occurred before the split between razorbills and guillemots (see discussion). Due to poor resolution in the exon 2 and intron 2 regions of the razorbill sequence, we were not able to include it in further analyses. As the analysis of the GuilBis1

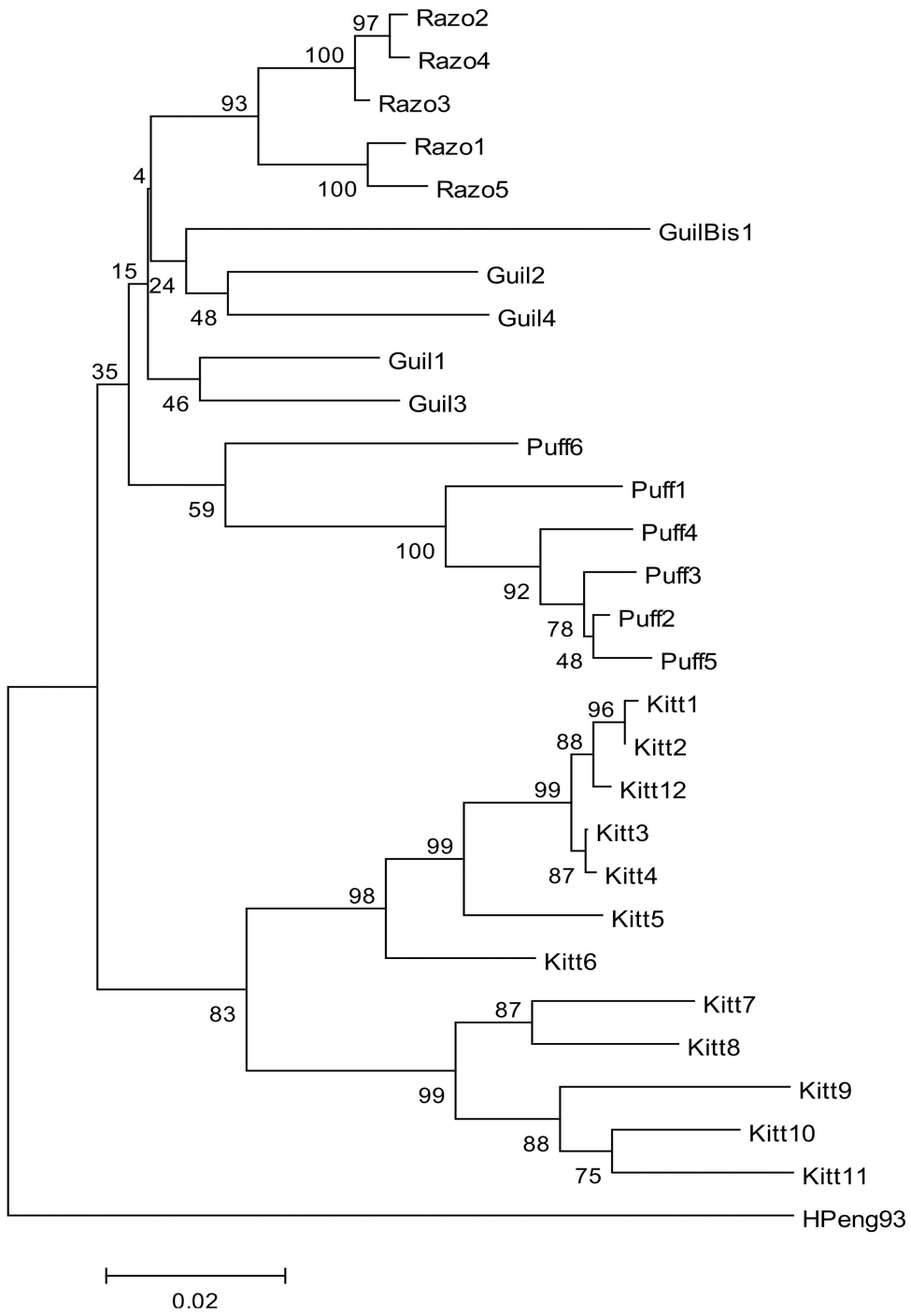


Figure 2 : Neighbor-joining tree of all sequences containing exon 2, intron 2 and exon 3. Numbers on branches indicate percentage of bootstrap support calculated after 1000 replications. A Humboldt Penguin (Hpeng93, genbank n°AB154393) sequence was used as an outgroup. This tree is similar to the one obtained by parcimony (not shown).

sequence revealed neither stop-codons nor indels in the coding regions (exon 2 and 3), this 1500bp sequence might also be functional in these species.

Phylogeny

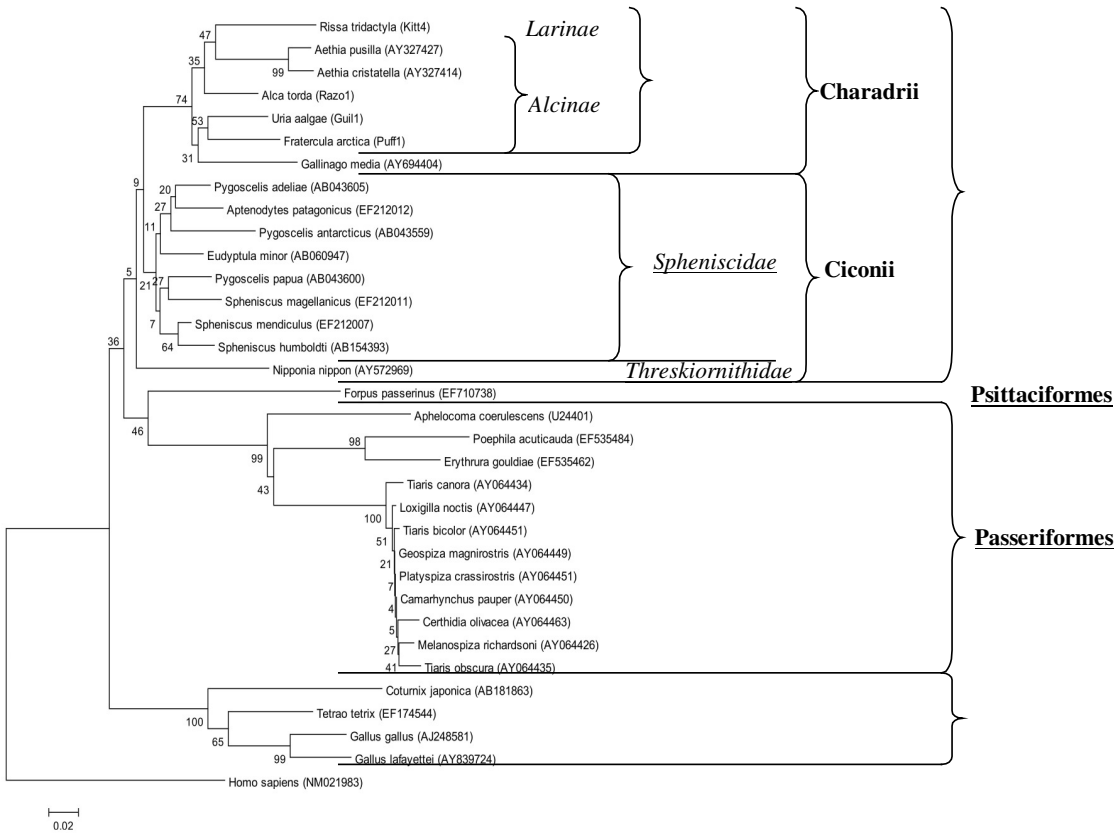


Figure 3 : Neighbor-joining tree of the exon 2 in Ciconiiformes, Passeriformes, Psittaciformes and Galliformes. This tree was obtained by the Maximum Composite Likelihood model; the root of the Ciconiiformes differed slightly in trees obtained by other methods, but never with bootstrap support. Accession numbers of Genbank sequences are given on the figure. Sub-families (in italics), families (in underlined italics), sub-orders (in bold) and orders (in underlined bold) are given according to Sibley & Ahlquist (1991). *Homo sapiens* was used as an outgroup.

A phylogenetic tree of the entire exon2-exon3 sequence (Figure 2) showed that all kittiwakes cluster together, forming a distinct clade from the other species. Among the Alcids (Puffins, Guillemots and Razorbills), results are more ambiguous. Although razorbill and puffin sequences are clustered by species (well supported in the case of razorbills), the phylogeny of guillemot sequences is unresolved. Bootstrap analyses did not give reliable values (< 50%) for these nodes. Furthermore, the position of the Puff1 sequence in the tree, supported here by a low bootstrap value (59%) may differ with the method used (i.e., when using the maximum likelihood method, this sequence was mixed with guillemot sequences). Interestingly, kittiwake sequences from Atlantic colonies clustered together with sequences from the Pacific colony (Middleton), and one allele (Kitt3) was shared between the two ocean basins.

Figure 3 represents the phylogenetic tree obtained when aligning our exon 2 sequences with others published on Genbank (accession numbers on figure). All taxonomic delineations follow those of Sibley and Ahlquist (1991). The sub-order Charadrii is found to be monophyletic with a bootstrap value (BV) of 74%. However, within the sub-order, the phylogeny is unresolved. Similarly, although the Passeriforme and Galliforme clades are strongly supported, the phylogeny of the Ciconiiformes is unresolved.

Polymorphism of the exon 2

In exon 2, we found that the rate of synonymous substitutions (d_S) tended to be lower than the rate of non-synonymous substitutions (d_N) in all species except razorbills (see Table 2). For this species, the number of polymorphic sites was rather small, indicating a low diversity of MHC alleles, at least in the studied population. Interestingly, 7 of the 8 usually conserved amino acids (Brown et al. 1993) were present in all sequences. The eighth amino acid (nucleotides 491-493, see supplemental material Figure 1) showed a mutation from proline to serine in 12 kittiwake sequences; this codon is part of the peptide binding region (PBR). When taking into account only codons involved in the PBR (Brown et al. 1993), the difference d_N-d_S was significant overall (mean +/- standard error = 0.168 +/- 0.076)., that is, non-synonymous substitutions were more frequent suggesting that diversifying selection is acting on the PBR. However, when we considered the PBR in each species separately, this difference was only significant in kittiwakes (0.170 +/- 0.058 in kittiwakes, 0.051 +/- 0.060 in puffins, 0.021 +/- 0.033 in razorbills, and 0.070 +/- 0.082 in guillemots). In all species (except razorbills), d_N and d_S rates were 1.5 to 3.9 times higher in exon 2 than in exon 3. The rate of synonymous substitutions in intron 2 and exon 3 tended always to be higher than the rate of non-synonymous substitutions, indicating that, contrary to exon 2, balancing selection is not occurring in exon 3.

	Kittiwake (n = 12; n = 22 for Pen-sequences)				Puffin (n = 6)			Razorbill (n = 5)			Guillemot (n = 4*)		
Region	Ex-2	Ex-2 (Pen)	In-2	Ex-3	Ex-2	In-2	Ex-3	Ex-2	In-2	Ex-3	Ex-2	In-2	Ex-3
Nb of variable nucleotides	65	46	26	23	37	26	16	16	3	12	45	16	14
Nb of SNPs	18	5	10	4	28	12	16	5	1	2	35	15	6
Proportion of polymorphic sites	0.24	0.29	0.13	0.08	0.14	0.14	0.06	0.06	0.02	0.04	0.17	0.08-0.09	0.05
d_S	0.074 +/- 0.020	0.088 +/- 0.027	-	0.049 +/- 0.018	0.042 +/- 0.016	-	0.027 +/- 0.011	0.038 +/- 0.017	-	0.031 +/- 0.016	0.092 +/- 0.03	-	0.041 +/- 0.019
d_N	0.101 +/- 0.016	0.117 +/- 0.024	-	0.035 +/- 0.011	0.061 +/- 0.013	-	0.017 +/- 0.006	0.032 +/- 0.011	-	0.023 +/- 0.009	0.104 +/- 0.02	-	0.027 +/- 0.01
$d_N - d_S$	0.027 +/- 0.027	0.029 +/- 0.039	-	- 0.014 +/- 0.023	0.018 +/- 0.019	-	- 0.009 +/- 0.013	- 0.007 +/- 0.016	-	- 0.008 +/- 0.018	0.011 +/- 0.031	-	- 0.014 +/- 0.022

Table 2: Polymorphism of different MHC class II B regions in seabirds. Rates of synonymous (d_S) and non-synonymous (d_N) substitutions were calculated using the method of Nei and Gojobori (Nei and Gojobori 1986) with a Jukes-Cantor correction. Standard errors were calculated on 1000 bootstrap replicates. Ex-2 (exon 2) consists of 270 bp, whereas Ex-2 (Pen) on includes only 159 bp of this exon. The length of intron 2 (In-2) was variable (193 bp in kittiwakes, 181-203 bp in puffins, 189-205 bp in razorbills and 179-204 bp in guillemots). The analyses of variable sites was thus performed only on sites present in all sequences from a given species. The length of Exon 3 (Ex-3) was 282 bp in all sequences. n refers to the number of sequences included in the analysis.

* **GuilBis1 excluded**

Partial exon 2 sequences and geographic structure

We additionally obtained 13 sequences (some of them being shared across individuals) using the Pen1/Pen4 primers. Comparison of sequences obtained by these primers and LP1/LP4 showed that they amplified the same region, but that Pen1/Pen4 contained only 59% of the total exon 2. A phylogenetic tree for this partial exon 2 of kittiwakes (Figure 4) was constructed by pooling sequences obtained by both primer sets. Sequences did not cluster according to the primers used confirming again that both sets of primers amplified the same region.

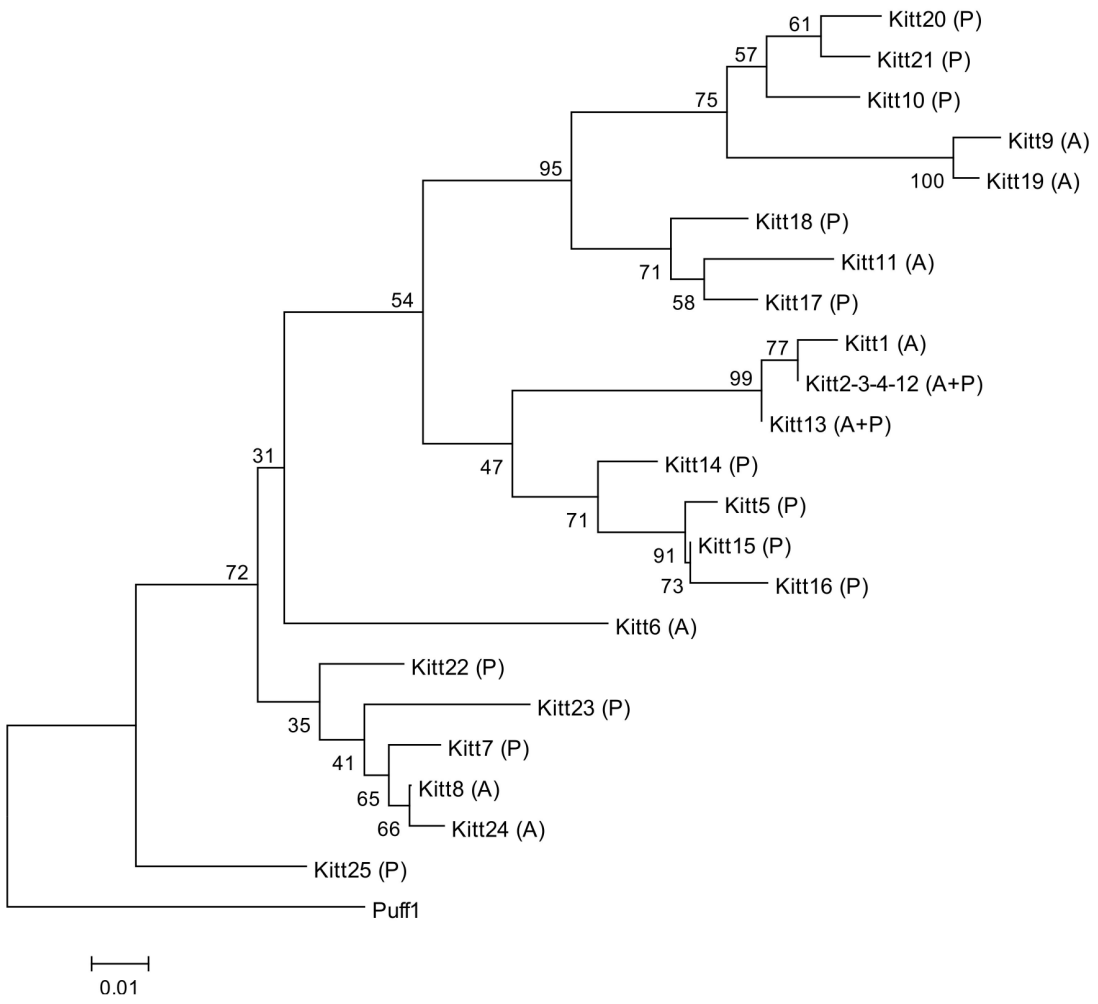


Figure 4 : Neighbor-joining tree of all partial exon 2 sequences in kittiwakes. Numbers on branches indicate percentage of bootstrap support calculated after 1000 replications. Puff1 was used as an outgroup. The origins of sequences (A for Atlantic, and P for Pacific) are indicated on the figure. Alleles do not clustered by ocean basin, and some (e.g., Kitt3, Kitt13) are identical across ocean basins.

We obtained 22 different sequences of the partial exon 2: 9 sequences from the 13 Atlantic individuals, and 15 sequences from the 9 Pacific individuals, with 2 sequences shared between Atlantic and Pacific kittiwakes (Kitt3, Kitt13). Sequence diversity therefore seems to be twice as high in the Pacific than in the Atlantic. In addition, the rate of synonymous substitutions was significantly higher in the Pacific colony than in Atlantic colonies (0.135 +/- 0.028 vs 0.067 +/- 0.026), while the rate of non-synonymous substitutions did not vary (0.106 +/- 0.023 vs 0.095 +/- 0.031). As a consequence, the difference between non-synonymous and synonymous mutations was only significant in Atlantic populations (Atlantic d_N-d_S = 0.068 +/- 0.039; Pacific d_N-d_S =0.010 +/- 0.041).

Discussion

Whereas we did not find evidence for more than one locus of MHC class II B in kittiwakes and puffins, at least two different loci may be present in guillemots and razorbills. Based on the sequence similarity, this duplication may have occurred before the split between razorbills and guillemots. This would fall in line with the current phylogeny of this group in which puffin divergence predates that of guillemots and razorbills (Moum, et al. 1994).

Despite this duplication, the overall number of Class II B loci in seabirds is still rather small compared to Passeriformes (see for example Wittzell et al. 1999) and seems to be more similar to that of a distantly related species, the great snipe (*Gallinago media*, Ekblom et al. 2003). In addition, we detected no frame-shifts or stop-codons in our sequences suggesting that all sequences presented here may be expressed (i.e., there was no evidence of pseudogenes). The structural organisation of MHC class II B genes in seabirds therefore seems to more closely resemble that found in the Galliformes than the Passeriformes (Hess and Edwards 2002).

Analyses of the entire Class II B sequence (exon2-intron2-exon3) among different seabird species showed that all species clustered together, except the guillemot. For the latter, the evolutionary relationships among alleles were unresolved, with low bootstrap values and long branches. Guillemot alleles were found close to the razorbill lineage on the tree which corresponds with their phylogenetic relatedness (Moum, et al. 1994). Overall, our results differ from other studies where lineage sorting was found to be incomplete (e.g., Gentoo penguin *Pygoscelis papua* or Little penguin *Eudyptula minor*, Tsuda et al. 2001); the type of selection acting on these alleles may therefore differ among different seabirds.

When looking at the global phylogeny of the exon 2, the monophyly of the sub-order Charadrii is well supported, but the evolutionary history of the Ciconiiformes (the order to which Charadrii belongs) is far from resolved. In general, branches are longer in Ciconiiformes than in Passeriformes. This could be for several reasons. First, the MHC Class II B genes might evolve faster in Ciconiiformes compared to the Passeriformes due to differences in mutation rates, population sizes, etc. It could also be that the radiation of the Ciconiiformes predates that of the Passeriformes. In this case, we would expect congruent trees at neutral markers; a quick survey of available sequences of Cytochrome oxidase I in Ciconiiformes and Passeriformes suggests that this is not the case (unpublished data). Finally, the type of selection on the MHC genes may be fundamentally different in the two groups.

Indeed, Ciconiiformes contain many colonial and long-lived species, whereas passeriformes typically include short-lived, territorial birds. These differences in life histories may result in different patterns of selection in the two groups (Bergelson et al. 2001, Grenfell et al. 2004). Detailed studies at the within species level of both groups will be required to differentiate among these hypotheses.

Some evidence of balancing selection was found in the peptide binding region (PBR) of kittiwakes. This was not clear in the other species, even though trends in this direction were apparent in puffins and guillemots; non-synonymous substitutions were typically more frequent than synonymous ones in exon 2 and substitution rates were higher in exon 2 than in exon 3. Contrasting results in the razorbill may be linked to the low polymorphism of these sequences. However, as of yet, we do not know whether this low polymorphism is an attribute of this species or simply due to low within-colony variation at the MHC (only individuals from a single colony were considered here). More generally, our results fall in line with those of other analyses that show balancing selection in the exon 2 of MHC Class II B genes (e.g. Aguilar et al. 2004, Ekblom et al. 2003) and highlight the potential importance of these immunological genes for seabirds.

There was no clear distinction between Pacific and Atlantic alleles in kittiwakes based on partial exon 2 sequences. Similar alleles (and two identical) were shared between ocean basins. This contrasts with patterns found in microsatellite markers and mitochondrial DNA which show a clear divergence between Atlantic and Pacific populations (Friesen et al. 2007, McCoy et al. 2005). Indeed, populations from the two ocean basins are considered to represent two sub-species based on morphological (Chardine 2002, Sluys 1982) and genetic data (Friesen et al. 2007). Our results suggest that the maintenance of alleles across ocean basins is due to balancing selection and not simply incomplete lineage sorting. As neutral mutations and diversity seem to be higher in Pacific populations, these results further suggest a Pacific origin of Atlantic alleles. Therefore, evidence of MHC geographic structure within and between ocean basins, if it exists, may reside more in the allele frequencies than in their sequence. Population-level studies within each ocean basin will be required to determine whether population structure exists and whether the MHC Class II B locus could be of use in conservation efforts (e.g., colony assignments after oil spills, Riffaut et al. 2005; determining management units, Friesen et al. 2007).

In addition to their role in the immune system, MHC genes have been shown to be associated with various behaviours, such as mate choice and individual recognition (Bonneaud et al. 2006, Ekblom et al. 2004, Landry et al. 2001, Penn and Potts 1998a,

Richardson et al. 2005, Wedekind and Füri 1997, Wegner et al. 2003), with the phenotypic expression of the MHC genotype being associated with olfactory cues (Milinski and Wedekind 2001, Penn and Potts 1998b). Although the use of smell is still controversial in birds (Warden et al. 1936, Prosser 1950), evidence of olfactive abilities is accumulating (reviewed in Lambrechts and Hossaert-McKey 2006 and Roper 1999). The use of MHC-linked odours to assess mate quality may therefore be more general than previously thought and thus, the factors acting on the evolution of these essential gene classes may be more complex than those associated with immune defence alone.

Acknowledgments

We would like to thank Thierry Boulinier, Étienne Danchin and O. Hueppop for help with sampling. All sampling complied with the laws of the countries concerned (U.S.A., Iceland, France, United Kingdom, Germany and Norway). We also thank Céline Arthanau, Patrick Durand, Nathalie Charbonnel, and Max Galen for technical avis and assistance, and Yannis Michalakis for analytical discussions. This study was financed by grants from the French Polar Institute Paul Emile Victor ("Programme Arctique n°429" and "Programme Parasito-arctique n°333"), the Bureau des Ressources Génétiques and the Agence Nationale de la Recherche (ANR-06-JCJC-0095-01).

References

Aguilar A, Roemer G, Debenham S, Binns M, Garcelon D, and Wayne RK (2004) High MHC diversity maintained by balancing selection in an otherwise genetically monomorphic mammal. *Proceedings of the National Academy of Sciences USA 101:* 3490-3494.

Bergelson J, Kreitman M, Stahl EA, and Tian D (2001) Evolutionary dynamics of plant R-genes. *Science 292:* 2281-2285.

Bonneaud C, Chastel O, Federici P, Westerdahl H, and Sorci G (2006) Complex MHC-based mate choice in a wild passerine. *Proceedings of the Royal Society of London B 273:* 1111-1116.

Bonneaud C, Mazuc J, Chastel O, Westerdahl H, and Sorci G (2004a) Terminal investment induced by immune challenge and fitness traits associated with major histocompatibility complex in the House sparrow. *Evolution 12:* 2823-2830.

Bonneaud C, Sorci G, Morin V, Westerdahl H, Zoorob R, and Wittzell H (2004b) Diversity of MHC class I and IIB genes in House sparrows (*Passer domesticus*). *Immunogenetics 55:* 855-865.

Brown JH, Jardetzky TS, Gorga JC, Stern LJ, Urban RG, Strominger JL, and Wiley DC (1993) Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature 364:* 33-39.

Chardine JW (2002) Geographic variation in the wingtip patterns of black-legged kittiwakes. *Condor 104:* 687-693.

Danchin E, Vitiello V, Vienne A, Richard O, Gouret P, McDermott MF, and Pontarotti P (2004) The major histocompatibility complex origin. *Immunological Reviews 198:* 216-232.

Edwards SV, Wakeland EK, and Potts WK (1995) Contrasting histories of avian and mammalian MHC genes revealed by class II ß sequences from songbirds. *Proceedings of the National Academy of Sciences USA 92:* 12200-12204.

Ekblom R, Grahn M, and Höglund J (2003) Patterns of polymorphism in the MHC Class II of a non-passerine bird, the Great snipe (*Gallinago media*). *Immunogenetics 54:* 734-741.

Ekblom R, Saether SA, Grahn M, Fiske P, Kålås JA, and Höglund J (2004) Major histocompatibility complex variation and mate choice in a lekking bird, the Great snipe (*Gallinago media*). *Molecular Ecology 13:* 3821-3828.

Friesen VL, Burg TM, and McCoy KD (2007) Mechanisms of population differenciation in seabirds. *Molecular Ecology 16:* 1765-1785.

Grenfell BT, Pybus OG, Gog JR, Wood JLN, Daly JM, Mumford JA, and Holmes EC (2004) Unifying the epidemiological and evolutionary dynamics of pathogens. *Science 303:* 327-332.

Hess CM and Edwards SV (2002) The evolution of the major histocompatibility complex in birds. *BioScience 52:* 423-431.

Kikkawa EF, Tsuda TT, Naruse T, Sumiyama D, Fukuda M, Kurita M, Murata K, Wilson RP, Le Maho Y, Tsuda M, Kulski JK, and Inoko H (2005) Analysis of the sequence variations in the *MHC DRB1*-like gene of the endangered Humboldt penguin (*Spheniscus humbodlti*). *Immunogenetics 57:* 99-107.

Knapp LA, Robson J, and Waterhouse JS (2006) Olfactory signals and the MHC: a review and a case study in *Lemur catta*. *American Journal of Primatology 68:* 568-584.

Lambrechts MM and Hossaert-McKey M (2006) Olfaction, volatile compounds and reproduction in birds. *Acta Zoologica Sinica 53S:* 284-287.

Landry C, Garant D, Duchesne P, and Bernatchez L (2001) 'Good genes as heterozygosity': the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). *Proceedings of the Royal Society of London B 268:* 1279-1285.

McClelland EE, Penn DJ, and Potts WK (2003) Major histocompatibility complex heterozygote superiority during coinfection. *Infection & Immunity 71:* 2079-2086.

McCoy KD, Boulinier T, and Tirard C (2005) Comparative host-parasite population structures: disentangling prospecting and dispersal in the Black-legged kittiwake *Rissa tridactyla*. *Molecular Ecology 14:* 2825-2838.

Milinski M and Wedekind C (2001) Evidence for MHC-correlated perfume preferences in humans. *Behavioral Ecology 12:* 140-149.

Moum, T., Johansen, S., Enkstad, K. E. & Piatt, J. F. 1994. Phylogeny and evolution of the auks (subfamily Alcinae) based on mitochondrial DNA sequences. *Proceedings of the National Academy of Sciences USA*, **91,** 7912-7916.

Møller AP, Merino S, Brown CR, and Robertson RJ (2001) Immune defense and host sociality: a comparative study of swallows and martins. *American Naturalist 158:* 136-145.

Nei M and Gojobori T (1986) Simple method for estimating the number of synonymous and nonsynonymous nucleotide substitutions. *Molecular Biology and Evolution 3:* 418-426.

Olsén KH, Grahn M, Lohm J, and Langefors Å (1998) MHC and kin discrimination in juvenile Arctic charr, *Salvelinus alpinus* (L.). *Animal Behaviour 56:* 319-327.

Olsson M, Madsen T, Nordby J, Wapstra E, Ujvari B, and Wittsell H (2003) Major histocompatibility complex and mate choice in sand lizards. *Proceedings of the Royal Society of London B Supplement:* S1-S3.

Penn DJ (2002) The scent of genetic compatibility: sexual selection and the major histocompatibility complex. *Ethology 108:* 1-21.

Penn DJ, Damjanovich K, and Potts WK (2002) MHC heterozygosity confers a selective advantage against multiple-strain infections. *Proceedings of the National Academy of Sciences USA 99:* 11260-11264.

Penn DJ and Potts WK (1998a) MHC-disassortative mating preferences reversed by cross-fostering. *Proceedings of the Royal Society of London B 265:* 1299-1306.

Penn DJ and Potts WK (1998b) Untrained mice discriminate MHC-determined odors. *Physiology & Behavior 63:* 235-243.

Piertney SB and Oliver MK (2006) The evolutionary ecology of the major histocompatibility complex. *Heredity 96:* 7-21.

Prosser CL (1950) Comparative animal physiology. W.S. Saunders Co., Philadelphy.

Reusch TBH, Häberli MA, Aeschlimann PB, and Milinski M (2001) Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature 414:* 300-302.

Richardson DS, Komdeur J, Burke T, and Von Schantz T (2005) MHC-based patterns of social and extra-pair mate choice in the Seychelles warbler. *Proceedings of the Royal Society of London B 272:* 759-767.

Riffaut L, McCoy KD, Tirard C, Friesen VL, and Boulinier T (2005) Population genetics of the Common guillemot *Uria aalge* in the North Atlantic: geographic impact of oil spills. *Marine Ecology Progress Series 291:* 263-273.

Roper TJ (1999) Olfaction in birds. *Advances in the Study of Behavior 28:* 247-332.

Shiina T, Shimizu S, Hosomichi K, Kohara S, Watanabe S, Hanzawa K, Beck S, Kulski JK, and Inoko H (2004) Comparative genomic analysis of two avian (quail and chicken) MHC regions. *Journal of Immunology 172:* 6751-6763.

Sibley CG and Ahlquist JE (1991) Phylogeny and classification of birds: A study in molecular evolution. Yale University Press.

Sluys R (1982) Geographical variation of the kittiwake. *Rissa tridactyla*. *Le Gerfaut 72:* 221-230.

Tamura K, Dudley J, Nei M, and Kumar S (2007) *MEGA4*: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution:* 10.1093/molbev/msm092.

The-MHC-Sequencing-Consortium (1999) Complete sequence and gene map of a human major histocompatibility complex. *Nature 401:* 921-923.

Tsuda TT, Tsuda M, Naruse T, Kawata H, Ando A, Shiina T, Fukuda M, Kurita M, Le Maho Y, Kulski JK, and Inoko H (2001) Phylogenetic analysis of penguin (*Spheniscidae*) species based on sequence variation in MHC Class II genes. *Immunogenetics 53:* 712-716.

Warden CJ, Jenkins TN, and Warner LH (1936) Comparative psychology. *Vertebrates*, Ronald Press, New York.

Wedekind C and Füri S (1997) Body odour preferences in men and women: do they aim for specific MHC combinations or simply heterozygosity? *Proceedings of the Royal Society of London B 264:* 1471-1479.

Wedekind C and Penn D (2000) MHC genes, body odours, and odour preferences. *Nephrology Dialysis Transplantation 15:* 1269-1271.

Wedekind C, Walker M, Portmann J, Cenni B, Müller R, and Binz T (2004) MHC-linked susceptibility to a bacterial infection, but no MHC-linked cryptic female choice in Whitefish. *Journal of Evolutionary Biology 17:* 11-17.

Wegner KM, Kalbe M, Kurtz J, Reusch TBH, and Milinski M (2003) Parasite selection for immunogenetic optimality. *Science 301:* 1343.

Westerdahl H (2003) Avian MHC: variation and selection in the wild.,, Lund University, Lund.

Westerdahl H, Waldenström J, Hansson B, Hasselquist D, Von Schantz T, and Bensch S (2005) Associations between malaria and MHC genes in a migratory songbird. *Proceedings of the Royal Society of London B 272:* 1511-1518.

Wittzell H, Madsen T, Westerdahl H, Shine R, and Von Schantz T (1999) MHC variation in birds and reptiles. *Genetica 104:* 301-309.

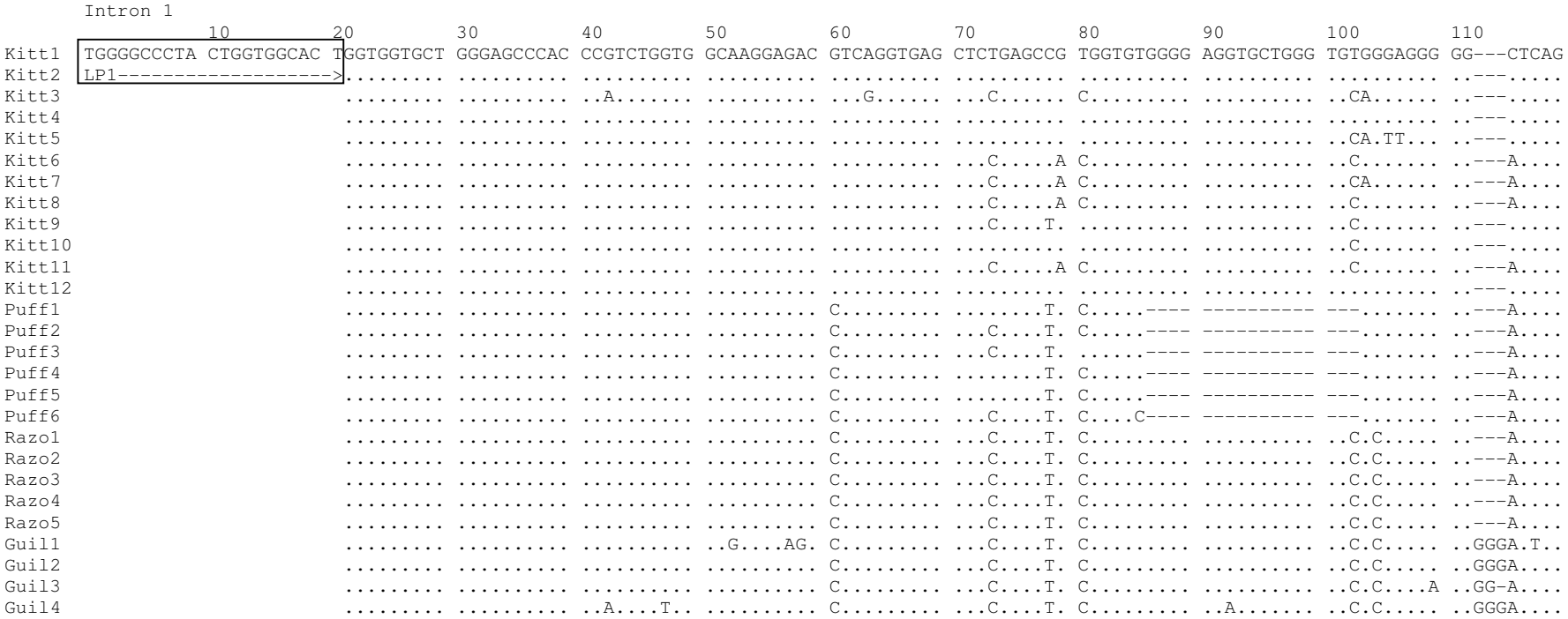
Zoorob R, Béhar G, Kroemer G, and Auffray C (1990) Organization of a functional chicken class II B gene. *Immunogenetics 31:* 179-187.

Supplemental material

Figure 1: Sequence alignments (excluding the 1500bp sequences) with the primer positions (LP1/2, Pen1/4), and the limits of exons and introns indicated.

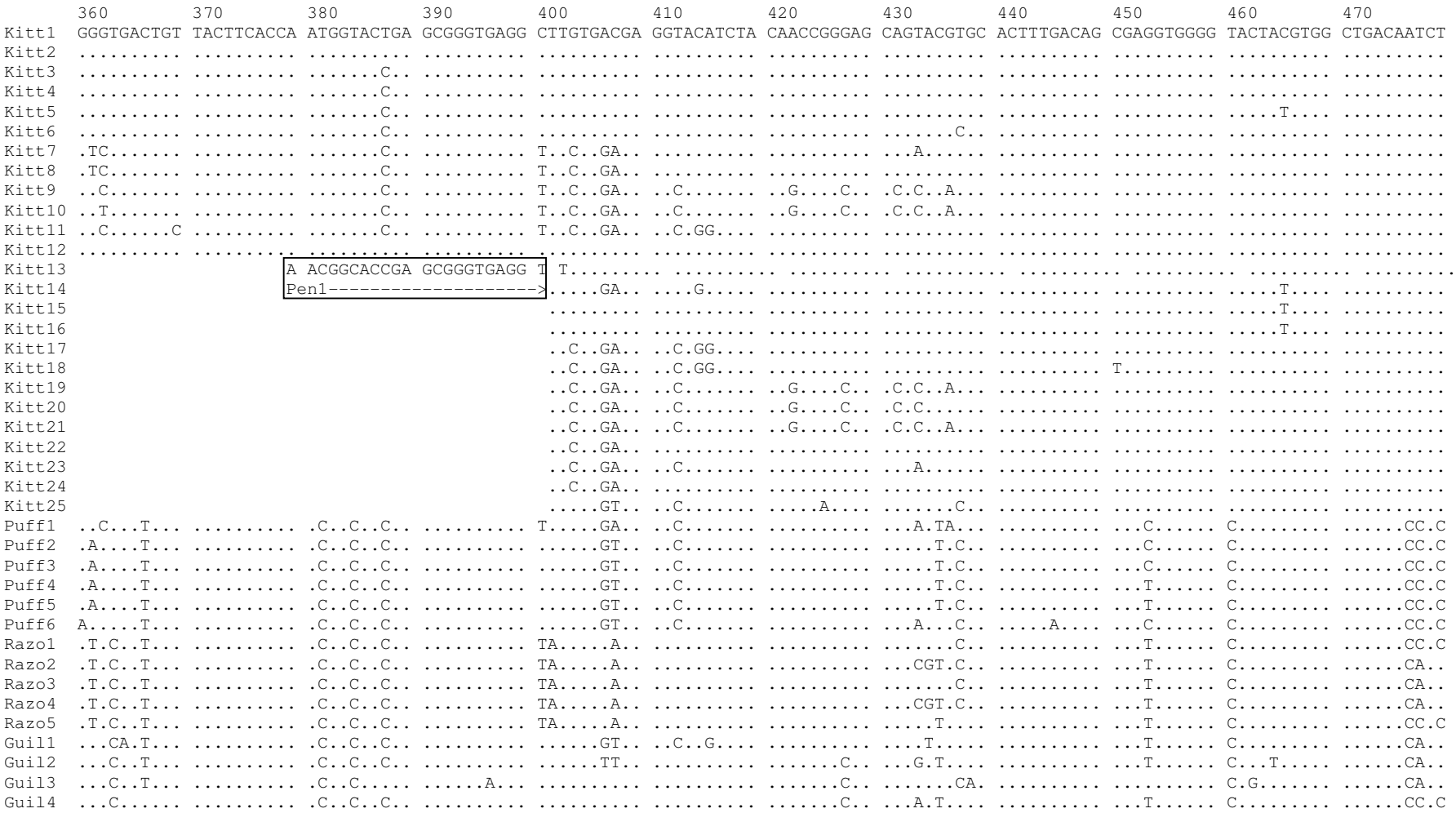
Figure 2: Alignment of the two 1500 bp sequences (RazoBis1 and GuilBis1). Primer positions (LP1/2, Pen1/4), and the limits of exons and introns are given on the figure.

Supplemental Material Figure 1


Intron 1
10 20 30 40 50 60 70 80 90 100 110
Kitt1 TGGGGCCCTA CTGGTGGCAC TGGTGGTGCT GGGAGCCCAC CCGTCTGGTG GCAAGGAGAC GTCAGGTGAG CTCTGAGCCG TGGTGTGGGG AGGTGCTGGG TGTGGGAGGG GG---CTCAG
Kitt2 LP1----------> ..---.....
Kitt3 ..A..... ...G..... ...C..... C..... ..CA..... ..---.....
Kitt4 ..---.....
Kitt5 ..CA.TT... ..---.....
Kitt6 ...C.....A C..... ..C..... ..---A....
Kitt7 ...C.....A C..... ..CA..... ..---A....
Kitt8 ...C.....A C..... ..C..... ..---A....
Kitt9 ...C....T. ..C..... ..---.....
Kitt10 ..C..... ..---.....
Kitt11 ...C.....A C..... ..C..... ..---A....
Kitt12 ..---.....
Puff1 C.....T. C.....---- ---..... ..---A....
Puff2 C.....T. C.....---- ---..... ..---A....
Puff3 C.....T. .....---- ---..... ..---A....
Puff4 C.....T. C.....---- ---..... ..---A....
Puff5 C.....T. C.....---- ---..... ..---A....
Puff6 C.....T. C....C---- ---..... ..---A....
Razo1 C.....T. C..... ..C.C..... ..---A....
Razo2 C.....T. C..... ..C.C..... ..---A....
Razo3 C.....T. C..... ..C.C..... ..---A....
Razo4 C.....T. C..... ..C.C..... ..---A....
Razo5 C.....T. C..... ..C.C..... ..---A....
Guil1 ..G....AG. C.....T. C..... ..C.C..... ..GGGA.T..
Guil2 C.....T. C..... ..C.C..... ..GGGA....
Guil3 C.....T. C..... ..C.C....A ..GG-A....
Guil4 ..A....T.. C.....T. C..... ..A..... ..C.C..... ..GGGA....

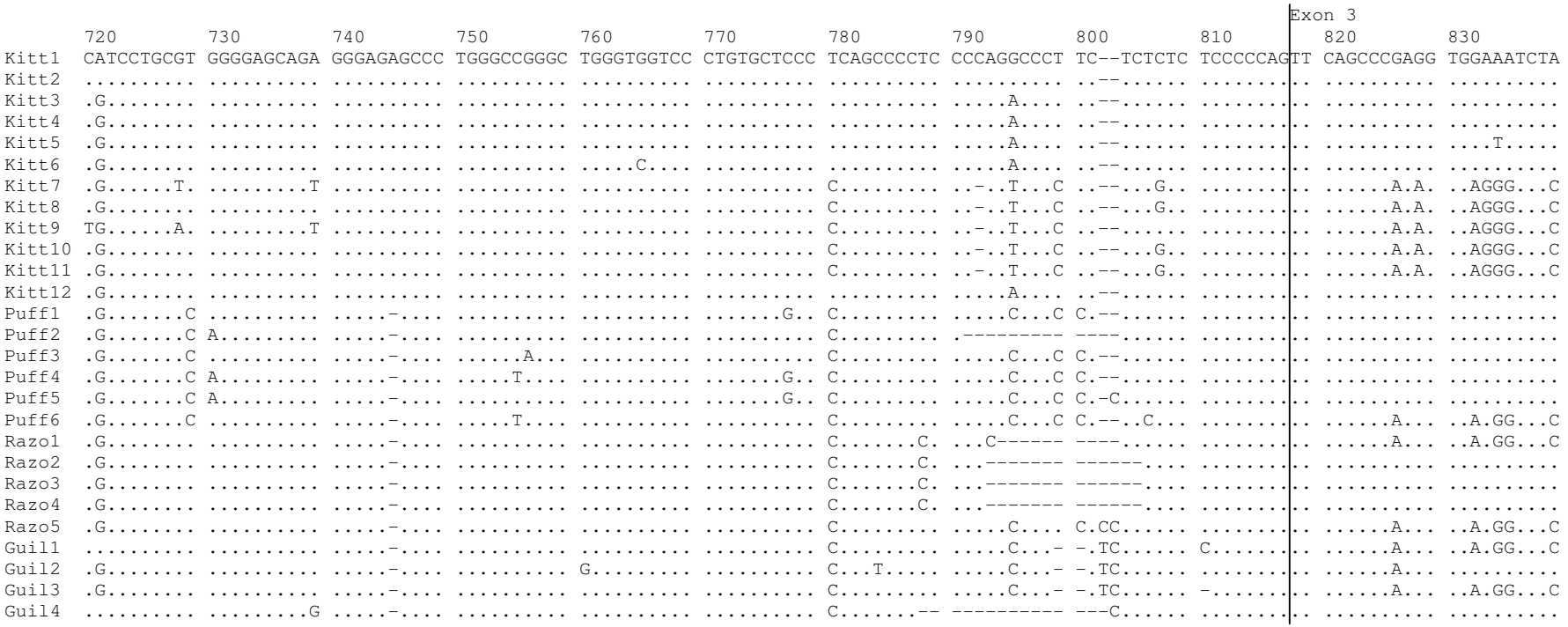
```
       120        130        140        150        160        170        180        190        200        210        220        230
Kitt1  ACCCCTTAGG GTGGATCGGG GTGGAACGGG GTGCAGGAGG ATGCTGTGAA GGAAGGAGAT GGGTGGAAAA GGGGGAGCGG GACAGTGTAG AGGGGGGTGA CCCATGGCAT CGTCTAGCCT
Kitt2  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Kitt3  .......... .......... ......A... .......... .......... ..G..---.. .......... .......T.. ...G...... ..A....... .......... .C..C.....
Kitt4  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Kitt5  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..A....... .......... ..........
Kitt6  .......... .......... .......... .......... .......... ..G....... .......... .......T.. ..TG...... ..-......T .......... .T..C.....
Kitt7  .........T .CA...T... .......... .......... .......... ..G....... .......... .......T.. ...G...... ..-......T .......... .C..C.....
Kitt8  .........T .CA...T... .......... .......... .......... ..G....... .......... .......T.. ..TG...... ..-......T .......... .C..C.....
Kitt9  .......... ......T... .......A.. .......... .......... ..G....... ...C...... .......... .......... ..A......T .......... .C..C.....
Kitt10 .......... ......T... .......A.. .......... .......... ..G....... .......... .......... .......... ..A......T .......... .C..C.....
Kitt11 .........T .CA...T... .......... .......... .......... ..G....... .......... .......T.. ...G...... ..-......T .......... .C..C.....
Kitt12 ...------- ---------- ---....... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Puff1  .......... .....A.... ......T... .......... .......... ..G....... .......... ........A. ...G...... G.A.....-- .......... .C..CG....
Puff2  .......... ...T.A.... ......T... .......... .......... ..G....... .......... ........A. .......... G.A.....-- .......... .C..CG....
Puff3  .........-- --------.. ......T... .......... .......... ..G....... .......... ........A. ...G...... G.A.....-- .......... .C..CG....
Puff4  .........-- --------.. ......T... .......... .......... ..G....... .......... ........A. ...G...... G.A.....-- .......... .C..CG....
Puff5  .......... .....A.... ......T... .......... .......... ..G....... .......... ........A. ...G...... G.A.....-- .......... .C..CG....
Puff6  .......... .....A.... ......T... .......... .......... ..G....... .......... ........A. ...G...... G.A.....-- .......... .C..CG....
Razo1  .......... ....C..... ......G... .......... ........C. ..G....... .......... .......GA. ...G...... G.A.....-- .......... .C..C.....
Razo2  .......... ....C..... ......G... .......... ........C. ..G....... .......... .......GA. ...G...... G.A.....-- .......... .C..C.....
Razo3  .......... .......... ......G... .......... ........C. ..G....... .......... .......GA. ...G...... G.A.....-- .......... .C..C.....
Razo4  .......... .......... ......G... .......... ........C. ..G....... ......G... .......GA. ...G...... G.A.....-- .......... .C..C.....
Razo5  .......... ....C..... ......G... .......... ........C. ..G....... .......... .......GA. ...G...... G.A.....-- .......... .C..C.....
Guil1  .......... ...T...... .......... .......... ........C. ..G....... .......... .......GA. ...G...... G.A.....-- ....G..... .C..C.....
Guil2  .......... ...T...... .......... .......... ........C. ..G....... .......... .......GA. ...G...... G.A.....-- ....C..... .C..C.....
Guil3  .......... .C.T...... .......... .......... ........C. ..G....... .......... .......GA. ...G...... G.A.....-- .......... .C..C.....
Guil4  .......... ...T...... .......... .......... ........C. ..G....... .......... .......GA. ...G...... G.A.....-- ....C..... .C..C.....
```

```
                                                                                                                  Exon 2
       240        250        260        270        280        290        300        310        320        330        340        350
Kitt1  CCCCC-AGCC CTTGGG---- ---------- -------GGC AGCTGGGGAG GGGGAGTCAC GGAAGCAGCC CTCACCTCCC CCATGTCTGC ATGAGCAGGG TATTTCCAGT TCCAGTTTAA
Kitt2  .....-.... ......---- ---------- -------... .......... .......... .......... .......... .......... .......... .......... ..........
Kitt3  .....-.... ......CACT GGTGCAGATC CCCTGGG... .......... .......... .......... ......C... .......... .......... .......... ..........
Kitt4  .....-.... ......---- ---------- -------... .......... .......... .......... .......... .......... .......... .......... ..........
Kitt5  .....-.... ......---- ---------- -------... .......... .......... .......... .......... .......... .C........ .G........ ..........
Kitt6  .....-.... ......CACT GGTGCAGATG CCCTGGG... .......... .......... .......... ..G....... T......... .C........ .........C ..........
Kitt7  .....-.... ......CACT GGTACAGAAC CCCTGGG... .......... .......... .......... ..G....... .......... .C........ .......... A....CA...
Kitt8  .....-.... ......CACT GGTGCAGAAC CCCTGGG... .......... .......... .......... ..G....... .......... .C........ .........G A....CA...
Kitt9  .....-.... ......CACT GGTGCAGAAC CCCTGGG... .......... .......... .......... .......... .......... .......... .......... ..........
Kitt10 .....-.... ......CACT GGTGCAGAAC CCCTGGG... .......... .......... .......... T.G....... .......... .C........ .......... A.........
Kitt11 .....-.... ......CACT GGTGCAGAAC CCCTGGG... .......... .......... .......... ...G...... .......... .C........ .........C G.........
Kitt12 .....-.... ......---- ---------- -------... .......... .......... .......... .......... .......... .......... .......... ..........
Puff1  .....-.... ......CACT GGTGCAGATT CCCTGGG... .......... .......... A.G..G.... ..G....... .-........ .C........ .........C .GAT......
Puff2  .....-.... ......CACT GGTGCAGATT CCCTGGG... .......... .......... A.G..G.... ..G....... .-........ .C........ .........C .GAT......
Puff3  .....-.... ......CACT GGTGCAGATT CCCTGGG... .......... .......... A.G..G.... ..G....... .-........ .C........ .........C .GAT......
Puff4  .....-.... ......CACT GGTGCAGATT CCCTGGG... .......... .......... A.G..G.... ..G....... .-........ .C........ .........C .GAT......
Puff5  .....-.... ......CACT GGTGCAGATT CCCTGGG... .......... A......... A.G..G.... ..G....... .-........ .C........ .........C .GAT......
Puff6  .....-.... ......CACT GGTGCAGATT CCCTGGG... .......... A......... A.G..G.... ..G....... .-........ .C........ .........G AAAT.C....
Razo1  .....C.... ......CACT GGTGCAGATC CCCTGGG... .......... ......G... ..G..G.... ..G....... .-........ CC........ .........G AGAT......
Razo2  .....C.... ......CACT GGTGCAGATC CCCTGGG... .......... ......G... ..G..G.... ..G....... .-........ CC........ .........G AGAT......
Razo3  .....C.... ......CACT GGTGCAGATG CCCTGGG... .......... A.....G... ..G..G.... ..G....... .-........ CC........ .........G AGAT......
Razo4  .....C.... ......CACT GGTGCAGATG CCCTGGG... .......... A.....G... ..G..G.... ..G....... .-........ CC........ .........G AGAT......
Razo5  .....C.... ......CACT GGTGCAGATC CCCTGGG... .......... ......G... ..G..G.... ..G....... .-........ CC........ .........G AGAT......
Guil1  .....-.... ......CACT GGTGCAGATC CCCTGGG... .......... ......G... ..G..G.... ..G....... .-........ .C........ .......... A.AT.C....
Guil2  .....-.... ......CACT GGTGCAGATC CCCTGGG... .......... ......G... ..G..G.... ..G....... .-........ .C........ .......... A.AT..A...
Guil3  .....-.... ......CACT GGTGCAGATC CCCTGGG... .......... ......G... ..G..G.... ..G....... .-........ .C........ .........G AGAT......
Guil4  .....-.... ......CACT GGTGCAGATC CCCTGGG... .......... ......G... ..G..G.... ..G....... .-........ .C........ .........G A.AT...C..
```


360 370 380 390 400 410 420 430 440 450 460 470
Kitt1 GGGTGACTGT TACTTCACCA ATGGTACTGA GCGGGTGAGG CTTGTGACGA GGTACATCTA CAACCGGGAG CAGTACGTGC ACTTTGACAG CGAGGTGGGG TACTACGTGG CTGACAATCT
Kitt2 ....
Kitt3 ....C.. ....
Kitt4 ....C.. ....
Kitt5 ....C.. ....T.... .....
Kitt6 ....C.. ....C.. ....
Kitt7 .TC..... ....C.. ..... T..C..GA.. ....A..... ....
Kitt8 .TC..... ....C.. ..... T..C..GA.. ....
Kitt9 ..C..... ....C.. ..... T..C..GA.. ..C..... ..G....C.. .C.C..A... ....
Kitt10 ..T..... ....C.. ..... T..C..GA.. ..C..... ..G....C.. .C.C..A... ....
Kitt11 ..C......C ....C.. ..... T..C..GA.. ..C.GG.... ....
Kitt12 ....
Kitt13 A ACGGCACCGA GCGGGTGAGG T T..... ....
Kitt14 Pen1----------> .....GA.. ....G..... ....T.... .....
Kitt15 ....T.... .....
Kitt16 ....T.... .....
Kitt17 ..C..GA.. ..C.GG.... ....
Kitt18 ..C..GA.. ..C.GG.... .... T..... ....
Kitt19 ..C..GA.. ..C..... ..G....C.. .C.C...A... ....
Kitt20 ..C..GA.. ..C..... ..G....C.. .C.C..... ....
Kitt21 ..C..GA.. ..C..... ..G....C.. .C.C..A... ....
Kitt22 ..C..GA.. ....
Kitt23 ..C..GA.. ..C..... ....A..... ....
Kitt24 ..C..GA.. ....
Kitt25 .....GT.. ..C..... .....A.... .....C.. ....
Puff1 ..C...T... ..... .C..C..C.. ..... T.....GA.. ..C..... ....A.TA... ....C..... C..... ......CC.C
Puff2 .A....T... ..... .C..C..C.. ....GT.. ..C..... ....T.C.. ....C..... C..... ......CC.C
Puff3 .A....T... ..... .C..C..C.. ....GT.. ..C..... ....T.C.. ....C..... C..... ......CC.C
Puff4 .A....T... ..... .C..C..C.. ....GT.. ..C..... ....T.C.. ....T..... C..... ......CC.C
Puff5 .A....T... ..... .C..C..C.. ....GT.. ..C..... ....T.C.. ....T..... C..... ......CC.C
Puff6 A.....T... ..... .C..C..C.. ....GT.. ..C..... ....A...C.. .....A.... ...C..... C..... ......CC.C
Razo1 .T.C..T... ..... .C..C..C.. ..... TA.....A.. ....C.. ....T..... C..... ......CC.C
Razo2 .T.C..T... ..... .C..C..C.. ..... TA.....A.. ....CGT.C.. ....T..... C..... ......CA..
Razo3 .T.C..T... ..... .C..C..C.. ..... TA.....A.. ....C.. ....T..... C..... ......CA..
Razo4 .T.C..T... ..... .C..C..C.. ..... TA.....A.. ....CGT.C.. ....T..... C..... ......CA..
Razo5 .T.C..T... ..... .C..C..C.. ..... TA.....A.. ....T.... ....T..... C..... ......CC.C
Guil1 ...CA.T... ..... .C..C..C.. ....GT.. ..C..G.... ....T..... ....T..... C..... ......CA..
Guil2 ...C..T... ..... .C..C..C.. ....TT.. ....C.. ...G.T.... ....T..... C...T..... ......CA..
Guil3 ...C..T... ..... .C..C..... ......A... ....C.. .....CA. .... C.G..... ......CA..
Guil4 ...C..... ..... .C..C..C.. ....C.. ...A.T.... ....T..... C..... ......CC.C

```
       480        490        500        510        520        530        540        550        560        570        580        590
Kitt1  TCTGGGCGAG TCTACTGCCA AATACTTCAA CAGCCAGCCC GACTTCCTGG AGCAGACACG GGCTGAGGTG GACACAGTCT GCCGAAACAA CTACGGGTTG GCGACCCCTT TCGCCGTGGA
Kitt2  .......... .......... .......... .......... .......... .......... .......... .....G.... .......... .......... .......... ..........
Kitt3  .......... .......... .......... .......... .......... .......... .......... .....G.... .......... .......... .......... ..........
Kitt4  .......... .......... .......... .......... .......... .......... .......... .....G.... .......... .......... .......... ....T.....
Kitt5  CT....GA.. C..T...... .......... .......... .......... .......... .......... .....GTA.. ....GC.... .......G.. .......... ..........
Kitt6  C.....GA.. C..GA...TG .C....GG.. .......... ...A...... .........A .......... .....G.... .......... ....AACG.. TG...T.... ....T.....
Kitt7  C.....GA.. C..GA...TG .T....GG.. .......... .....A.... ......A... .....CT... ....GGT... ....GT.... .......G.. T......... A.........
Kitt8  C.....GA.. C..GA...TG .T....GG.. .......... .....A.... ......A... .....CT... .....GT... ....GC.... .......G.. .......... ..AT.....T
Kitt9  C......... .......... .......... .......... A..G...... ..G.TG.... .....CT... .....GTA.. ....GC.... .......G.. .......... ..........
Kitt10 C......... C...G..... .......... .........A ...A...... ......A... .....CT... ....GGT... ....GC.... .......G.. T......... ..AT......
Kitt11 C......... C...G..... .......... .........A ...A...... ..G.TG.... .....CT... .....GT... ....GC.... .......G.. TTT....... ....T.....
Kitt12 .......... .......... .......... .......... .......... .......... .......... .....G.... .......... .......... ..........
Kitt13 .......... .......... .......... .......... .......... .......... .......... .....G..CT GCCAACACAA CTACGGG
Kitt14 CT....GA.. C..T...... .......... .......... .......... .......... ......T... .....GTA<----------------Pen4
Kitt15 CT....GA.. C..T...... .......... .......... .......... .......... .......... .....GTA
Kitt16 CT....GA.. C..T...... .......... .......... .......... ...G...... .......... ..T..GTA
Kitt17 C......... C...G..... .......... .........A ...A...... ......A... .....CT... .T...GT.
Kitt18 C......... C..GT..... .......... .........A ...A...... ......A... .....CT... .....GT.
Kitt19 C......A.. .......... .......... .......... A..G...... ..G.TG.... .....CT... .....GT.
Kitt20 C......... .......... .......G.. .........A ...A...... ...G..A..A .....CT... .....GT.
Kitt21 C......... .......... .......... .........A ...A...... ...G..A... .....CT... .....GT.
Kitt22 C.....GA.. C..T.....G .G....GG.. .......... .....A.... ......A... .....CT... .....GT.
Kitt23 C.....G... C..GA...TG ......GG.. .......... ...A.A.... ......A..A .....CT... .....GT.
Kitt24 C.....GA.. C..GA...TG .T....GG.. .......... .....A.... ......A... .....TT... .....GT.
Kitt25 C.....GA.. C..T.....G .G....GG.. .......... .....A.... ......G... .......... .....GTA
Puff1  C.....GA.. C..GAG.T.G .G....GG.. .........G .....A.... ......G... .......... ....GGTA.. ....GC.... ..T.AATG.. TG..TT.... ..A.......
Puff2  C.....GA.. C..GA..T.G .G.C..GG.. ........TG ...A...... ...G..A... .......... .....G.... .......... ..T.AATG.. TG..TT.... ..........
Puff3  C.....GA.. C..GA..T.G .G.C..GG.. ........TG .G.A...... ...G..A... .......... .....G.... .......... ..T.AATG.. TG..TT.... ..........
Puff4  C.....GA.. C..GA..T.G .G.C..GG.. ........TG ...A...... ...G..A... .......... .....G.... .......... ..T.AATG.. TG..TT.... ..A.......
Puff5  C.....GA.. C..GA..T.G .G.C..GG.. ........TG ...A...... ...G..A... .......... .....G.... .......... ..T.AATG.. TG..TT.... ..........
Puff6  C.....GA.. C..GA..T.G .G....GG.. .........G .....A.... .C.G..G... .......... .....G.... .......... ....A..G.. TTT....... ..A.......
Razo1  C.....GA.. C..G.....G .G....GG.. .........G ...G...... ...G.G.... .......... .....GT... .......... ....A..G.. .A........ ....T.....
Razo2  C.....GA.. C..GA....G .G....GG.. .........G ...C...... ...G.GAT.. .......... .....GT... .......... ....A..G.. TG........ ..........
Razo3  C.....GA.. C..GA....G .G....GG.. .........G ...G...... ...G.GAT.. .......... .....GT... .......... ....A..G.. TG........ ..........
Razo4  C.....GA.. C..T.....G .G....GG.. .........G ...C...... ...G.GAT.. .......... .....GT... .......... ....A..G.. TG........ ..........
Razo5  ......GA.. C..G.....G .G.....G.. .........G ...G...... ...G.G.... .......... .....GT... .......... ....A..G.. .A........ ..........
Guil1  C.....GA.. C..GA....G .G....GG.. .........G .....A.... ...G..G... .......... .....GT... ....GC.... ....AA.G.. T......... ..AT......
Guil2  C.....GA.. C...T....G .G....GGG. .........G ...A...... .......... ......T... ....GGTA.. ......T... ....AA.G.. T.A....... ..AT......
Guil3  C.....GA.. C..GA....G .G....GG.. .........G ...A...... ...G...... .......... .....GTA.. .......... ....AA.G.. TTTCT..... ..A.......
Guil4  C.....GA.. C...T....G .G....GG.. .........A ..A....... .......... .......... .....GT... .......... ....AA.G.. .T........ ..A.......
```

```
                 Intron 2
        600        610        620        630        640        650        660        670        680        690        700        710
Kitt1   GAGGAGAGGT GAGTGTGTGG CAGAACATTT CCC-GGGGGG ACAGGCACAA GCCAAGCCCC GGGCAGTCCC TGC-GCCCTT CCATGG-GGA CGTGAGTCGC TTGTAGC--- ---------A
Kitt2   ........... .......... .......... ...-...... .......... .......... .......... ...-...... ......-... .......... .......--- ---------.
Kitt3   ........... .......... .......... ...-...... .......... .......... .......... ...-...... ......-... .......... .......--- ---------.
Kitt4   ........... .......... .......... ...-...... .......... .......... .......... ...-...... ......-... .......... .......--- ---------.
Kitt5   ........... .......... .......... ..T-...... .......... .......... .......... ...-...... .....T-.A. ..C......T G......--- ---------.
Kitt6   ........... .......... .......... ...-.....- .......... .......... .......... ...-...... .....T-.A. ..C....... .......--- ---------.
Kitt7   ........... .......... ......G... ...C...... .TG....... .......... .......... ...-...... ......-... .......T.. .......--- ---------.
Kitt8   ........... .......... .......... ..T-.T.... .TG....... .......... .......... ...-...... ...C.A-.A. ..C......T G......--- ---------.
Kitt9   ........... .......... .......... ...-...... .......... .......... A......... ...-...... ..TCAA-.A. .AC....... C......--- ---------.
Kitt10  ........... .......... .......... ..T-.T.... .TG....... .......... .......... ...-...... ...C.A-.A. ..C......T G......--- ---------.
Kitt11  ........... .......... .......... ..T-.T.... .TG....... .......... .......... ...-...... ...C.A-.A. ..C...C..T G......--- ---------.
Kitt12  ........... .......... .......... ...-...... .......... .......... .......... ...-...... ......-... .......... .......--- ---------.
Puff1   ........... ...C...... ......G... ...-C..CA. G......... .........T .......... ...-A..... ...C..-... ..CA...T.. ...C...--- ---------.
Puff2   ........... ...CA..... ......G... ...-T....A .TG.A..... .........T .......... ...-...... ...C..-... ..C....T.. ..CC...--- ---------.
Puff3   ........... ...C...... ......G... ...-T....A .TG.A..... .........T .......... ...-...... ...C.A-... ..CA...T.. ..CC...--- ---------.
Puff4   ........... ...C...... ......G... ...-C..CA. G......... .........T .......... ...-...... ...C..-... ..CA...T.. ..CC...--- ---------.
Puff5   ........... ...C...... ......G... ...-T....A .TG.A..... .........T .......... ...-...... ...C..-... ..CA...T.. ..CC...--- ---------.
Puff6   ........... .......... .......... ...-C..CA. G......... .........T ......C... ...C...... ...CAA-.A. .........C. ...C...CTC CCC--AGGC.
Razo1   ........... .....C.... ......G... ...-T..... .......... .......... ......C... ...-A....C ...CAA-.A. G.C.....C. ...C...C-C CCCCGAGGC.
Razo2   ........... .....C.... ......G... ...-T..... .......... .......... ......C... ...-.....C ...CA--.A. --C.....C. ...C...C-C CCCCGAGGC.
Razo3   ........... .....C.... ......G... ...-T..... .......... .......... ......C... ...-.....C ...CA--.A. --C.....C. ...C...C-C CCCCGAGGC.
Razo4   ........... .....C.... ......G... ...-T..... .......... .......... ......C... ...-.....C ...CA--.A. --C.....C. ...C...C-C CCCCGAGGC.
Razo5   ........... .....C.... ......G... ...-T..... .......... .......... ......C... ...-A....C ...CAA-.A. G.C.....C. ...C...C-C CCCCGAGGC.
Guil1   ........... .......... .......... ...-T..... .......... .......... ......C... ...-.....C ...CAA-.A. ..C.....C. ...C...CTC CCCCGAGGC.
Guil2   ........... ...C...... .......... ...-T..... .......... .......... ......C... ...-.....C ...CAA-.A. ..C.....C. ...C...CTC CCCCGAGGC.
Guil3   ........... .......C.. .......... ...-T..... .......... .......... ......C... ...-.....C ...CAA-.A. G.C.....C. ...C...CTC CCCCGAGGC.
Guil4   ........... .......... .......... ...-T..... .......... .......... ......C... ..T-...... ...C..-... ..CA...T.. ...C...--- ---------.
```


Exon 3
720 730 740 750 760 770 780 790 800 810 820 830
Kitt1 CATCCTGCGT GGGGAGCAGA GGGAGAGCCC TGGGCCGGGC TGGGTGGTCC CTGTGCTCCC TCAGCCCCTC CCCAGGCCCT TC--TCTCTC TCCCCCAGTT CAGCCCGAGG TGGAAATCTA
Kitt2 ....--..... ....
Kitt3 .G..... ....A.... ..--..... ....
Kitt4 .G..... ....A.... ..--..... ....
Kitt5 .G..... ....A.... ..--..... ....T.....
Kitt6 .G..... ....C.... ....A.... ..--..... ....
Kitt7 .G.....T. .....T .... C..... ..-..T...C ..--...G.. ....A.A. ..AGGG...C
Kitt8 .G..... .... C..... ..-..T...C ..--...G.. ....A.A. ..AGGG...C
Kitt9 TG.....A. .....T .... C..... ..-..T...C ..--..... ....A.A. ..AGGG...C
Kitt10 .G..... .... C..... ..-..T...C ..--...G.. ....A.A. ..AGGG...C
Kitt11 .G..... .... C..... ..-..T...C ..--...G.. ....A.A. ..AGGG...C
Kitt12 .G..... ....A.... ..--..... ....
Puff1 .G.......C ....-.... ....G.. C..... .....C...C C.--..... ....
Puff2 .G.......C A..... .....-.... .... C..... .--------- ----..... ....
Puff3 .G.......C ....-.... ......A... .... C..... .....C...C C.--..... ....
Puff4 .G.......C A..... .....-.... .....T.... ....G.. C..... .....C...C C.--..... ....
Puff5 .G.......C A..... .....-.... ....G.. C..... .....C...C C.-C..... ....
Puff6 .G.......C ....-.... .....T.... .... C..... .....C...C C.--..C... ....A... ..A.GG...C
Razo1 .G..... ....-.... .... C.....C. ...C------ ----..... ....A... ..A.GG...C
Razo2 .G..... ....-.... .... C.....C. ...------- ------.... ....
Razo3 .G..... ....-.... .... C.....C. ...------- ------.... ....
Razo4 .G..... ....-.... .... C.....C. ...------- ------.... ....
Razo5 .G..... ....-.... .... C..... .....C.... C.CC..... ....A... ..A.GG...C
Guil1 ....-.... .... C..... .....C...- -.TC..... C..... ....A... ..A.GG...C
Guil2 .G..... ....-.... ..... G..... .... C...T..... .....C...- -.TC..... ....A... .....
Guil3 .G..... ....-.... .... C..... .....C...- -.TC..... -..... ....A... ..A.GG...C
Guil4 ....G .....-.... .... C.....-- ---------- ---C..... ....

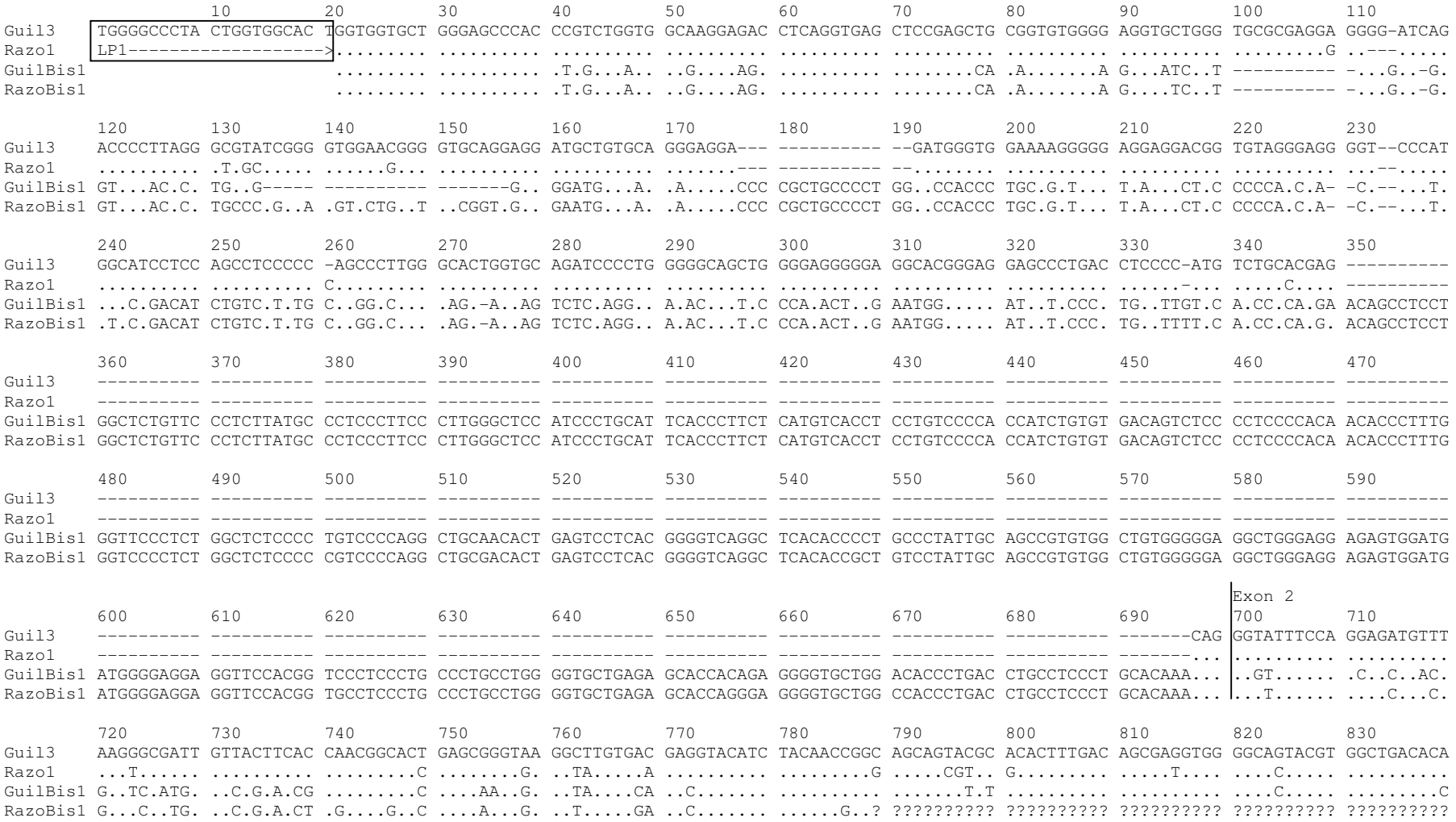
```
       840        850        860        870        880        890        900        910        920        930        940        950
Kitt1  CCCAGTGCAG TCGAGCTCCC TGCCCCAGAC CGACAGGCTG GTTTGCGCCG TGATGGATTT CTACCCCCCG GAGATTGAGG TGAAGTGGTT GAAGAACGGG CAGGAGGAGA CGGAGCACGT
Kitt2  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Kitt3  .......... .......... .......... .......... .......... .......... .......G.. .......... .......... .......... .......... ..........
Kitt4  .......... .......... .......... .......... .......... .......... .......G.. .......... .......... .......... .......... ..........
Kitt5  .......... .......... .......... .......... .......... .......... .......G.. .......... .......... .......... .......... ..........
Kitt6  .......... .......... .......... .......... .......... .......... .......G.. .......... .......... .......... .......... ..........
Kitt7  T..CA..... .......... .......... .......... .......... ...C..GG.. T.....TG.. .......... .......... .......... .......... ..........
Kitt8  ...CA..... .......... .......... .......... .......... ...C..GG.. T.....TG.. .......... .......... .......... .......... ..........
Kitt9  ...CA..... .......... .......... .......... .......... ...C..GGC. T.....TG.. .......... .......... .......... .......... ..........
Kitt10 ...CA..... .......... .......... .......... .......... ...C..GG.. T.....TG.. .......... .......... .......... .......... ..........
Kitt11 T..CA..... .......... .......... .......... .......... ...C..GG.. T.....TG.. .......... .......... .......... .......... ..........
Kitt12 .......... .......... .......... .......... .......... .......... .......G.. .......... .......... .......... .......... ..........
Puff1  ...GC..... .......... .......... .A........ .......... .......... ......TG.. .......... ........C. .......... .......... ..........
Puff2  ...GC..... .......... .......... .A........ .......... .......... ......TG.. .......... ........C. .......... .......... ..........
Puff3  ...GC..... .......... .......... .A........ .......... .......... ......TG.. .......... ........C. .......... .......... ..........
Puff4  ...GC..... .......... .......... .A........ .......... .......... ......TG.. .......... ........C. .......... .......... ..........
Puff5  ...GC..... .......... .......... .A........ .......... .......... ......TG.. .......... ........C. .......... .......... T.........
Puff6  .......... .......... .......... .A........ ........T. ...C..GG.. T......G.. .......... ........C. .......... .......... ..........
Razo1  .......... .......... .......... .......... .......... ...C..GG.. T......G.. .......... ........C. .......... .......... ..........
Razo2  ...G...... .......... .......... .......... .......... .......... .......G.. .......... ........C. .......... .......... ..A.......
Razo3  ...G...... .......... .......... .......... .......... .......... .......G.. .......... ........C. .......... .......... ..........
Razo4  ...G...... .......... .......... .......... .......... .......... .......G.. .......... ........C. .......... .......... ..........
Razo5  .......... .......... .......... .......... .......... ...C..GG.. T......G.. .......... ........C. .......... .......... ..........
Guil1  .......... .......... .......... .......... .......... ...C..GG.. T......G.. .......... .......... .......... .......... ..........
Guil2  ...G...... .......... .......... .......... .......... .......... ......TG.. .......... .......... .......... .......... ..........
Guil3  ...G...... .......... .......... .......... .......... ...C..GG.. T......G.. .......... .......... .......... .......... ..........
Guil4  ...G...... .......... .......... .......... .......... .......... .......G.. .......... .......... .......... .......... ..........
```

```
       960        970        980        990        1000       1010       1020       1030       1040       1050       1060       1070
Kitt1  GGTGTCCACG GAGGTGATGC AGAACGGAGA CTGGACCTAC CAGGTGCTGG TGATGCTGGA AACCACCCCG CAGCGCGGGG ACACCTACAT GTGCCAGGTG GAGCACGTCA GCCTGCAGCA
Kitt2  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Kitt3  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .........G
Kitt4  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .........G
Kitt5  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .........G
Kitt6  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .........G
Kitt7  .......... .......... .......... .......... .......... .......... .......... .......... .........C .......... .......... ..........
Kitt8  .......... .......... .......... .......... .......... .......... .G........ .......... .........C .......... .........G ..........
Kitt9  .......... .......... .......... .......... .......... .......... .......... .......... .........C .......... .......... ..........
Kitt10 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Kitt11 .......... .......... .......... .......... .......... .......... .......... .......... .........C .......... .......... ..........
Kitt12 .........A .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Puff1  .......... .......... .......... .......... .......... .......... .......... .......... .........C .......... .......... ..........
Puff2  .......... .......... .......... .......... .......... .......... .......... .......... .........C .......... .......... ..........
Puff3  .......... .......... .......... .......... .......... .......... .......... .......... .........C .......... .......... ......G...
Puff4  .......... .......... .......... .......... .......... .......... .......... .......... .........C .......... .......... ..........
Puff5  .......... .......... .......... .......... .......... .......... .......... ......A... .........C .......... .......... ..........
Puff6  .......... .......... .......... .......... .......... .......... .......... .......... .........C .......... .......... ..........
Razo1  .......... .......... .......... .......... .......... .......... ....G..... .......... .........C .......... .......... ..........
Razo2  .......... .......... .......... .......... .......... .......... ....G..... .......... .........C .......... .......... ..........
Razo3  .......... .......... .......... .......... .......... .......... ....G..... .......... .........C .......... .......... ..........
Razo4  .......... .......... .......... .......... .......... .......... ....G..... .......... .........C .......... .......... ..........
Razo5  .......... .......... .......... .......... .......... .......... ....G..... .......... .........C .......... .....T.... ..........
Guil1  .......... .......... .......... .......... .......... .......... .......... .......... .........C .......... .......... ......G...
Guil2  ......G... .......... .......... .......... .......... .......... .......... .......... .........C .......... .......... ......G...
Guil3  .......... .......... .......... .......... .......... .......... .......... .......... .........C .......... .......... ......G...
Guil4  .......... .......... .......... .......... .......... .......... .......... .......... .........C .......... .......... ..T...G...
```

```
                                 |Intron 3
       1080       1090       |1100       1110       1120       1130       1140       1150       1160       1170       1180       1190
Kitt1  CCCCATCACC CAGCACTGGG |GTAAGGCCCT GCCCTGTGCA GCCCC----- ---------- ---------T GGTGTGGGTG GGGAGGGGGC CGGGGCCCCC C-AGCCCTGA CCCCATGCTC
Kitt2  .......... .......... |.......... .......... .....----- ---------- ---------. .......... .......... .......... .-........ ....G.....
Kitt3  ....G..... .......... |.......... .........G .....----- ---------- ---------. .......... .......... .......... .-........ ....GC....
Kitt4  ....G..... .......... |.......... .......... .....----- ---------- ---------. .......... .......... .......... .-........ ....G.....
Kitt5  ....G..... .......... |.......... .........G .....----- ---------- ---------. .......... .......... .......... .-........ ....GC....
Kitt6  ....G..... .......... |.......... ........TG .....----- ---------- ---------. .......... .......... .......... .-........ ....GC....
Kitt7  .......... .......... |.......... .........G .....TGGGG GGTGGGGGAG GAGAGCCCC. ....C..... .......... .......... .-........ ....GC....
Kitt8  ....G..... .......... |.......... .........G .....TGGGG GGTGGGGGAG GAGAGCCCC. .......... .......... .......... .-........ ....GC....
Kitt9  .......... .......... |.......... .......... .....TGGGG GGTGGGGGAG GAGAGCCCC. .......... .......... .......... .-......C. ....GC....
Kitt10 ....G..... .......... |.......... .........G .....TGGGG GGTGGGGGAG GAGAGCCCC. .......... .......... .......... .-......C. ....GC....
Kitt11 .......... .......... |.......... .........G .....TGGGG GGTGGGGGAG GAGAGCCCC. ....C..... .......... .......... .-........ ....GC....
Kitt12 .......... .......... |.......... .........G .....----- ---------- ---------. .......... .......... .......... .-........ ....GC....
Puff1  .......... .......... |.......... .........G .....CGGGG G-TGGGGAGG --GAGCCCC. ....C..... ......T..A .......... .-........ ....CC....
Puff2  .......... .......... |.......... .........G .....CGGGG G-TGGGGAGG --GAGCCCC. ....C..... ......T..A .......... .-........ ....CC....
Puff3  .......... .......... |.......... .........G .....CGGGG G-TGGGGAGG --GAGCCCC. ....G..... ......T..A .......... .-........ ....CC....
Puff4  .......... .......... |.......... .........G .....CGGGG G-TGGGGAGG --GAGCCCC. ....C..... ......T..A .......... .-........ ....CC....
Puff5  .......... .......... |.......... .........G .....CGGGG G-TGGGGAGG --GAGCCCC. ....G..... ......T..A .......... .-........ ....CC....
Puff6  .......... .......... |.......... .........G .....CGGGG G-TGGGGAGG --GAGCCCC. ....C..... ......T..A .......... .-........ ....CC....
Razo1  ....G..... .......... |.......... .........G .....CGGGG G-TGGGGAG- -AGAGCCCC. ....C..... ...C..T..A ......A... .-........ ....CC....
Razo2  ....G..... .......... |.......... .........G .....CGGGG G-TGGGGAG- -AGAGCCCC. ....C..... ...C..T..A .......... .C........ ....CC....
Razo3  ....G..... .......... |.......... .........G .....CGGGG G-TGGGGAG- -AGAGCCCC. ....C..... ...C..T..A ....A..... .-........ ....CC....
Razo4  ....G..... .......... |.......... .........G .....CGGGG G-TGGGGAG- -AGAGCCCC. ....C..... ...C..T..A .......... .-........ ....CC....
Razo5  ....G..... .......... |.......... .........G .....CGGGG G-TGGGGAG- -AGAGCCCC. ....C..... ...C..T..A .......... .C........ ....CC....
Guil1  ....G..... .......... |.......... .........G .....CGGGG G-TGGGGAG- -AGAGCCCC. .-..C..... ......T..A .......... .-........ ....CC....
Guil2  ....G..... .......... |.......... .........G .....CGGGG G-TGGGAGG- -AGAGCCCC. ....C..... ...T..T..A .......... .-........ ....CC....
Guil3  ....G..... .......... |.......... .........G .....CGGGG G-TGGGGAG- -AGAGCCCC. .-..C..... ......T..A .......... .-........ ....CC....
Guil4  ....G..... ....T..... |.......... T..T.....G .....CGGGG G-TGGGGAG- -AGAGCCCC. .-..CT.... ......T..A ....C..... .-........ ....CC....
```

```
         1200       1210       1220       1230       1240       1250
Kitt1  TCGTCCCCGC AGAGCTGCGG TCGGACGGCG GCAGGAGCAA CATGCTGACG GGGATCG
Kitt2  .......... .......... .......... ......<------------------LP2
Kitt3  .......... ........A. .......... ......
Kitt4  .......... ........A. ..A....... ......
Kitt5  .......... ........A. .......... ......
Kitt6  .......... ........A. .......... ......
Kitt7  .......... ..G.....A. .......... ......
Kitt8  .......... ........A. .......... ......
Kitt9  ..A....... ..G.....A. .......... ......
Kitt10 .......... ..G.....A. .......... ......
Kitt11 .......... ..G.....A. .......... ......
Kitt12 .......... ........A. ......A... ......
Puff1  .......... ........A. .......C.. .G....
Puff2  .......... ........A. .......C.. ......
Puff3  .......... ........A. .......C.. ......
Puff4  .......... ........A. .......C.. ......
Puff5  .......... ........A. .......C.. ......
Puff6  .......... ........A. .......C.. ......
Razo1  .......... ........A. .......C.. ......
Razo2  .......... ........A. .......C.. ......
Razo3  .......... ........A. .......C.. ......
Razo4  .......... ........A. .......C.. ......
Razo5  .......... ........A. .......C.. ......
Guil1  .......... ........A. .......C.. ......
Guil2  .......... ........A. .......C.. ......
Guil3  .......... ........A. .......C.. .....T
Guil4  .T..G.G... ....T...A. .......C.. ......
```

Supplemental Material Figure 2



```
         840        850        860        870        880        890        900        910        920        930        940        950
Guil3    CTCCTGGGGA AGCCTGATGC CGAGTACTGG AACAGCCAGC CGGACATCCT GGAGCGGACA CGGGCTGAGG TGGACACGTA CTGCCGAAAC AACTACAAGG TGTTTCTCCC TTTCACCGTG
Razo1    .......... .......... .......... .......... .....C.... .......GAT .......... .........T .......... .......G.. ...GGAC... ....G.....
GuilBis1 .C........ .......... .A........ .......... .A..AG.... .......G.. .......C.. .........T ......GC.. ......G... ..GCGAC... .....T....
RazoBis1 ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????

                   Intron 2
         960        970        980        990        1000       1010       1020       1030       1040       1050       1060       1070
Guil3    GAGAGGAGAG GTGAGTGTGC GGCAGAACAT TTCCC-TGGG GGACAGGCAC AAGCCAAGCC CCGGG----- ---------- ---------- ---------- ---------- ------CAGC
Razo1    .......... .......C.T ........G. .....-.... .......... .......... .....----- ---------- ---------- ---------- ---------- ------....
GuilBis1 .......... .........T .......... .....-...C A.G....... .......... G....----- ---------- ---------- ---------- ---------- ------....
RazoBis1 ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????

         1080       1090       1100       1110       1120       1130       1140       1150       1160       1170       1180       1190
Guil3    CCCTGC-GCC CTCCCACAA- GAAGGCGAGT CCCTTGCAGC CTCCCCCGAG GCACGTCCTG CGTGGGGAGC AGAGGGAG-G CCCTGGGCCG GGCTGGGTGG TCCCTGTGCT CCCCCAGCCC
Razo1    ......-... .........-- ...--..... .......... .-........ .......... .......... ........-. .......... .......... .......... ..........
GuilBis1 ......-... .........A .......... .......... .......... .......... .......... ........-. .......... .......... .......... ..........
RazoBis1 ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????

                                        Exon 3
         1200       1210       1220       1230       1240       1250       1260       1270       1280       1290       1300       1310
Guil3    CTCCCCAGCC CC--CTCTCT CTC-CCCCCA GTTCAGCCCA AGGTGAAGGT CTCCCCGGTG CAGTCGAGCT CCCTGCCCCA GACCGACAGG CTGGTTTGCG CCGTGACGGG GTTTTACCCC
Razo1    .C....---- ---------. ...T...... .........G .....G.AA. ..A....... .......... .......... .......... .......... ......T..A T..C......
GuilBis1 .......... ..CT.--... ...T...... .......... .....G.AA. ..A....... .......... .......... .......... .......... ......T..A T..C.....T
RazoBis1 ????????.. ..CT.----- -..T...... .......... .......... ......A... .......... .......... .......... .......... .......... .........T

         1320       1330       1340       1350       1360       1370       1380       1390       1400       1410       1420       1430
Guil3    GCGGAGATTG AGGTGAAGTG GTTGAAGAAC GGGCAGGAGG AGACGGAGCA CGTGGTGTCC ACGGAGGTGA TGCAGAACGG AGACTGGACC TACCAGGTGC TGGTGATGCT GGAAACCACC
Razo1    .......... .......... .C........ .......... .....A.... .......... .......... .......... .......... .......... .......... .......G..
GuilBis1 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
RazoBis1 .......... .......... .C........ .......... .......... .......... .......... .......... .......... .......... .........C .......G..

                                                                                  Intron 3
         1440       1450       1460       1470       1480       1490       1500       1510       1520       1530       1540       1550
Guil3    CCGCAGCGCG GGGACACCTA CACGTGCCAG GTGGAGCACG TCAGCCTGCG GCACCCCGTC ACCCAGCACT GGGGTAAGGC CCTGCCCTGT GCGGCCCCCG GGGG-TGGGG AG--AGAGCC
Razo1    .......... .......... .......... .......... .........A .......... .......... ..........|.......... .......... ....-..... ..--......
GuilBis1 .......... .......... .......... .......... .......... .......... .......... ..........|.......... .......... ....-..... ..AG..--..
RazoBis1 .......... .......... .......... .......... .........A .......... .......... ..........|.......... .......... ....-..... ..AG..--..

         1560       1570       1580       1590       1600       1610       1620       1630       1640       1650       1660
Guil3    CCTG-TGCGG GTGGGGAGGT GGACGGGGCC CCCC-AGCCC TGACCCCCCG CTCTCGTCCC CGCAGAGCTG CAGTCGGACG CCGGCAGGTG CAACATGCTG ACGGGGATCG
Razo1    ....G..... ......C... .......... ....C..... .......... .......... .......... .......... ........A<--------------------LP2
GuilBis1 ....G..... .......... .......... ....-..... .......... .......... .......... .......... ........A
RazoBis1 ....G..... ......C... .......... ....C..... .......... .......... .......... .......... ........A
```

Références utilisées dans l'ensemble de la these (n = 233)

Aguilar, A., Roemer, G., Debenham, S., Binns, M., Garcelon, D. & Wayne, R. K. 2004. High MHC diversity maintained by balancing selection in an otherwise genetically monomorphic mammal. *Proceedings of the National Academy of Sciences USA*, **101,** 3490-3494.

Amos, W., Worthington Wilmer, J., Fullard, K., Burg, T. M., Croxall, J. P., Bloch, D. & Coulson, T. 2001. The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society of London B*, **268,** 2021-2027.

Anders, A. D., Dearborn, D. C., Faaborg, J. & Thompson III, F. R. 1997. Juvenile survival in a population of Neotropical migrant birds. *Conservation Biology*, **11,** 698-707.

Andersson, M. 1994. *Sexual selection*. Princeton: Princeton University Press.

Aubin, T. 2004. Penguins and their noisy world. *Anais da Academia Brasileira Ciências*, **76,** 279-283.

Aubin, T. & Jouventin, P. 1998. Cocktail-party effect in king penguin colonies. *Proceedings of the Royal Society of London B*, **265,** 1665-1673.

Aubin, T. & Jouventin, P. 2002. Localisation of an acoustic signal in a noisy environment: the display call of the king penguin *Aptenodytes patagonicus*. *Journal of Experimental Biology*, **205,** 3793-3798.

Aubin, T., Mathevon, N., Staszewski, V. & Boulinier, T. 2007. Acoustic communication in the kittiwake *Rissa tridactyla*: potential cues for sexual and individual signatures in long calls. *Polar Biology*.

Avital, E., Jablonka, E. & Lachmann, M. 1998. Adopting adoption. *Animal Behaviour*, **55,** 1451-1459.

Baird, P. H. 1994. Black-legged kittiwake. In: *The Birds of North America*, pp. 1-24: A. Poole and F. Gill, Editors.

Baker, R. H., Ashwell, R. I. S., Richards, T. A., Fowler, K., Chapman, T. & Pomiankowski, A. 2001. Effects of multiple mating and male eye span on female reproductive output in the stalk-eyed fly *Cytodiopsis dalmanni*. *Behavioral Ecology*, **12,** 732-739.

Bang, B. G. & Cobb, S. 1968. The size of the olfactory bulb in 108 species of bird. *Auk*, **85,** 55-61.

Beer, C. G. 1969. Laughing gull chicks: recognition of their parents' voices. *Science*, **166,** 1030-1032.

Beer, C. G. 1970a. On the responses of Laughing gull chicks (*Larus atricilla*) to the calls of adults I: Recognition of the voices of the parents. *Animal Behaviour*, **18,** 652-660.

Beer, C. G. 1970b. On the responses of Laughing gull chicks (*Larus atricilla*) to the calls of adults II: Age changes and responses to different types of calls. *Animal Behaviour*, **18,** 661-677.

Beer, C. G. 1979. Vocal communication between Laughing gull parents and chicks. *Behaviour*, **70,** 118-146.

Belkhir, K., Castric, V. & Bonhomme, F. 2002. IDENTIX, a software to test for relatedness in a population using permutation methods. *Molecular Ecology Notes*, **2,** 611-614.

Bensch, S., Hasselquist, D. & Von Schantz, T. 1994. Genetic similarity between parents predicts hatching failure nonincestuous inbreeding in the Great reed warbler? *Evolution*, **48,** 317-326.

Bergelson, J., Kreitman, M., Stahl, E. A. & Tian, D. 2001. Evolutionary dynamics of plant R-genes. *Science*, **292,** 2281-2285.

Bergstrom, C. T. & Real, L. A. 2000. Towards a theory of mutual mate choice: Lessons from two-sided matching. *Evolutionary Ecology Research*, **2,** 493-508.

Blomqvist, D., Andersson, M., Küpper, C., Cuthill, I. C., Kis, J., Lanctot, R. B., Sandercock, B. K., Székely, T., Wallander, J. & Kempenaers, B. 2002. Genetic simimarity between mates and extra-pair parentage in three species of shorebirds. *Nature*, **419,** 613-615.

Boehm, T. & Zufall, F. 2006. MHC peptides and the sensory evaluation of genotype. *Trends in Neurosciences*, **29,** 100-107.

Bohnet, S., Rogers, L., Sasaki, G. & Kolattukudy, P. E. 1991. Estradiol induces proliferation of peroxisome-like microbodies and the production of 3-hydroxy fatty acid diesters, the female pheromones, in the uropygial glands of male and female mallards. *Journal of Biological Chemistry*, **266,** 9795-9804.

Bonadonna, F., Cunningham, G. B., Jouventin, P., Hesters, F. & Nevitt, G. A. 2003. Evidence for nest-odour recognition in two species of diving petrel. *Journal of Experimental Biology*, **206,** 3719-3722.

Bonadonna, F. & Nevitt, G. A. 2004. Partner-specific odor recognition in an Antarctic seabird. *Science*, **306,** 835.

Bonadonna, F., Villafane, M., Bajzak, C. & Jouventin, P. 2004. Recognition of burrow's olfactory signature in Blue petrels, *Halobaena caerulea*: an efficient discrimination mechanism in the dark. *Animal Behaviour*, **67,** 893-898.

Bonneaud, C., Chastel, O., Federici, P., Westerdahl, H. & Sorci, G. 2006. Complex MHC-based mate choice in a wild passerine. *Proceedings of the Royal Society of London B*, **273,** 1111-1116.

Bonneaud, C., Mazuc, J., Chastel, O., Westerdahl, H. & Sorci, G. 2004a. Terminal investment induced by immune challenge and fitness traits associated with major histocompatibility complex in the House sparrow. *Evolution*, **12,** 2823-2830.

Bonneaud, C., Sorci, G., Morin, V., Westerdahl, H., Zoorob, R. & Wittzell, H. 2004b. Diversity of MHC class I and IIB genes in House sparrows (*Passer domesticus*). *Immunogenetics*, **55,** 855-865.

Brown, J. H., Jardetzky, T. S., Gorga, J. C., Stern, L. J., Urban, R. G., Strominger, J. L. & Wiley, D. C. 1993. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature*, **364,** 33-39.

Brown, J. L. 1996. A theory of mate choice based on heterozygosity. *Behavioral Ecology*, **8,** 60-65.

Buckley, F. G. & Buckley, P. A. 1972. The breeding ecology of Royal terns *Sterna (Thalasseus) maxima maxima*. *Ibis*, **114,** 344-359.

Burley, N. T. & Foster, V. 2006. Variation in female choice of mates: condition influences selectivity. *Animal Behaviour*, **72,** 713-719.

Cadiou, B., Monnat, J.-Y. & Danchin, E. 1994. Prospecting in the kittiwake, *Rissa tridactyla*: different behavioural patterns and the role of squatting in recruitment. *Animal Behaviour*, **47,** 847-856.

Cam, E., Link, W. A., Cooch, E. G., Monnat, J.-Y. & Danchin, E. 2002. Individual covariation in life-history traits: seeing the trees despite the forest. *American Naturalist*, **159,** 96-105.

Cam, E. & Monnat, J.-Y. 2000a. Apparent inferiority of first-time breeders in the kittiwake: the role of heterogeneity among age classes. *Journal of Animal Ecology*, **69,** 380-394.

Cam, E. & Monnat, J.-Y. 2000b. Stratification based on reproductive state reveals contrasting patterns of age-related variation in demographic parameters in the kittiwake. *OIKOS*, **90,** 560-574.

Cam, E., Monnat, J.-Y. & Hines, J. E. 2003. Long-term fitness consequences of early conditions in the kittiwake. *Journal of Animal Ecology*, **72,** 411-424.

Carter, L. R. & Spear, L. B. 1986. Costs of adoption in western gulls. *Condor*, **88,** 253-256.

Cézilly, F., Dubois, F. & Pagel, M. 2000. Is mate fidelity related to site fidelity? A comparative analysis in Ciconiiforms. *Animal Behaviour*, **59,** 1143-1152.

Chardine, J. W. 2002. Geographic variation in the wingtip patterns of black-legged kittiwakes. *Condor*, **104,** 687-693.

Charrier, I., Mathevon, N., Jouventin, P. & Aubin, T. 2001. Acoustic communication in a black-headed gull colony: how do chicks identify their parents ? *Ethology*, **107,** 961-974.

Chilton, G. & Lein, M. R. 1996. Long-term changes in songs and song dialect boundaries of puget soung white-crowned sparrows. *Condor*, **98,** 567-580.

Cohen, L. B. & Dearborn, D. C. 2004. Great frigatebirds, *Fregata minor*, choose mates that are genetically similar. *Animal Behaviour*, **68,** 1229-1236.

Coltman, D. W. & Slate, J. 2003. Microsatellite measures of inbreeding: a meta-analysis. *Evolution*, **57,** 971-983.

Cooper, G., Amos, W., Bellamy, R., Ruby Siddiqui, M., Frodsham, A., Hill, A. V. S. & Rubinsztein, D. C. 1999. An empirical exploration of the $(\delta\mu)^2$ genetic distance for 213 human microsatellite markers. *American Journal of Human Genetics*, **65,** 1125-1133.

Coulson, J. C. 1966. The influence of the pair-bond and age on the breeding biology of the kittiwake gull *Rissa tridactyla*. *Journal of Animal Ecology*, **35,** 269-279.

Coulson, J. C. 1983. The changing status of the Kittiwake *Rissa tridactyla* in the British Isles, 1969-1979. *Bird Study*, **30,** 9-16.

Coulson, J. C. & Thomas, C. S. 1980. A study of the factors influencing the pair-bond in the kittiwake gull. *Proceedings of the International Ornithological Congress*, **17,** 823-833.

Coulson, J. C. & Thomas, C. S. 1985. Changes in the biology of the kittiwake Rissa tridactyla: a 31-year study of a breeding colony. *Journal of Animal Ecology*, **54,** 9-26.

Coulson, T. N., Albon, S. D., Slate, J. & Pemberton, J. M. 1999. Microsatellite loci reveal sex-dependent responses to inbreeding and outbreeding in red deer calves. *Evolution*, **53,** 1951-1960.

Cullen, E. 1956. Adaptations in the kittiwake to cliff-nesting. *Ibis*, **99,** 275-302.

Cunningham, G. B., Van Buskirk, R. W., Bonadonna, F., Weimerskirch, H. & Nevitt, G. A. 2003. A comparison of the olfactory abilities of three species of procellariiform chicks. *Journal of Experimental Biology*, **206,** 1615-1620.

Danchin, E. 1983. Les comportements liés à l'occupation du site de reproduction chez la mouette tridactyle (*Rissa tridactyla*) : les comportements de pré-atterrissage. *CNRS-CRBPO***,** 226-246.

Danchin, E. 1987. The behaviour associated with the occupation of breeding site in the kittiwake gull *Rissa tridactyla*: the social status of landing birds. *Animal Behaviour*, **35,** 81-93.

Danchin, E. (1987). Description and functional signification of the behaviour and the displays of the kittiwake (*Rissa tridactyla*). *(Unpublished).*

Danchin, E. 1988a. Rôle des facteurs comportementaux dans les mécanismes de régulation des populations d'oiseaux coloniaux. Cas de la mouette tridactyle (*Rissa tridactyla*). Paris: Université Pierre et Marie Curie.

Danchin, E. 1988b. Social interactions in Kittiwakes colonies: social facilitation and/or favourable social environment. *Animal Behaviour*, **36,** 443-451.

Danchin, E. & Nelson, J. B. 1991. Behavioral adaptations to cliff nesting in the kittiwake (*Rissa tridactyla*): convergences with the gannet (*Sula bassana*) and the black noddy (*Anous tenuirostris*). *Colonial Waterbirds*, **14,** 103-107.

Danchin, E., Boulinier, T. & Massot, M. 1998. Conspecific reproductive success and breeding habitat selection: implications for the study of coloniality. *Ecology*, **79,** 2415-2428.

Danchin, E., Cadiou, B., Monnat, J.-Y. & Estrella, R. R. 1991. Recruitment in long-lived birds: conceptual framework and behavioural mechanisms. In: *Acta XXth Congressus Internationalis Ornithologicus*, pp. 1641-1656. Wellington: Hutcheson, Bowman and Stewart Ltd.

Danchin, E., Vitiello, V., Vienne, A., Richard, O., Gouret, P., McDermott, M. F. & Pontarotti, P. 2004. The major histocompatibility complex origin. *Immunological Reviews*, **198,** 216-232.

Darwin, C. 1871. *The descent of man and selection in relation to sex*. London: John Murray.

Doherty, P. F., Sorci, G., Royle, J. A., Hines, J. E., Nichols, J. D. & Boulinier, T. 2003. Sexual selection affects local extinction and turnover in bird communities. *Proceedings of the National Academy of Sciences USA*, **100,** 5858-5862.

Draganoiu, T. I., Nagle, L., Musseau, R. & Kreutzer, M. 2005. Parental care and brood division in a songbird, the black redstart. *Behaviour*, **142,** 1495-1514.

Drickamer, L. C., Gowaty, P. A. & Holmes, C. M. 2000. Free female mate choice in house mice affects reproductive success and offspring viability and performance. *Animal Behaviour*, **59,** 371-378.

Edward, P. J. 1985. Brood division and transition to independance in Blackbirds, *Turdus merula*. *Ibis*, **127,** 42-59.

Edwards, S. V., Kingan, S. B., Calkins, J. D., Balakrishnan, C. N., Jennings, W. B., Swanson, W. J. & Sorenson, M. D. 2005. Speciation in birds: genes, geography, and sexual selection. *Proceedings of the National Academy of Sciences USA*, **102,** 6550-6557.

Edwards, S. V., Wakeland, E. K. & Potts, W. K. 1995. Contrasting histories of avian and mammalian MHC genes revealed by class II ß sequences from songbirds. *Proceedings of the National Academy of Sciences USA*, **92,** 12200-12204.

Ekblom, R., Grahn, M. & Höglund, J. 2003. Patterns of polymorphism in the MHC Class II of a non-passerine bird, the Great snipe (*Gallinago media*). *Immunogenetics*, **54,** 734-741.

Ekblom, R., Saether, S. A., Grahn, M., Fiske, P., Kålås, J. A. & Höglund, J. 2004. Major histocompatibility complex variation and mate choice in a lekking bird, the Great snipe (*Gallinago media*). *Molecular Ecology*, **13,** 3821-3828.

Ellers, J. & Slabbekoorn, H. 2003. Song divergence and male dispersal among bird populations: a spatially explicit model testing the role of vocal learning. *Animal Behaviour*, **65,** 671-681.

Evans, R. M. 1970. Parental recognition and the 'mew call' in Black-billed gulls (*Larus bulleri*). *Auk*, **87,** 503.

Fairweather, J. A. & Coulson, J. C. 1995. Mate retention in the kittiwake, *Rissa tridactyla*, and the significance of nest site tenacity. *Animal Behaviour*, **50,** 455-464.

Falls, J. B. 1982. Individual recognition by sounds in birds. In: *Acoustic communication in birds* (Ed. by Kroodsma, D. E. & Miller, E. H.), pp. 235-278. New York: Academic Press.

Fisher, R. A. 1915. The evolution of sexual preferences. *Eugenics Review*, **7,** 184-192.

Fisher, R. A. 1930. *The genetical theory of natural selection*. Oxford: Clarendon Press.

Foerster, K., Delhey, K., Johnsen, A., Lifjeld, J. T. & Kempenaers, B. 2003. Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature*, **425,** 714-717.

Freeman-Gallant, C. R., Meguerdichian, M., Wheelwright, N. T. & Sollecito, S. V. 2003. Social pairing and female mating fidelity predicted by restriction fragment length polymorphism similarity at the major histocompatibility complex in a songbird. *Molecular Ecology*, **12,** 3077-3083.

Friesen, V. L., Burg, T. M. & McCoy, K. D. 2007. Mechanisms of population differenciation in seabirds. *Molecular Ecology*, **16,** 1765-1785.

Frings, H. & Boyd, W. A. 1952. Evidence of olfactory discrimination by the Bobwhite quail. *American Midland Naturalist*, **48,** 181-184.

Garamszegi, L. Z., Møller, A. P., Török, J., Michl, G., Péczely, P. & Richard, M. 2004. Immune challenge mediates vocal communication in a passerine bird: an experiment. *Behavioral Ecology*, **15,** 148-158.

Gentner, T. Q. & Hulse, S. H. 1998. Perceptual mechanisms for individual vocal recognition in European starlings, *Sturnus vulgaris*. *Animal Behaviour*, **56,** 579-594.

Gill, V. A. & Hatch, S. A. 2002. Components of productivity in black-legged kittiwakes *Rissa tridactyla*: response to supplemental feeding. *Journal of Avian Biology*, **33,** 113-126.

Gill, V. A., Hatch, S. A. & Lanctot, R. B. 2002. Sensitivity of breeding parameters to food supply in Black-legged kittiwakes *Rissa tridactyla*. *Ibis*, **144,** 268-283.

Given, A. D., Mills, A. & Baker, J. 2002. Isolation of polymorphic microsatellite loci from the red-billed gull (*Larus novaehollandiae scopulinus*) and amplification in related species. *Molecular Ecology Notes*, **2,** 416-418.

Grant, P. R. & Grant, B. R. 1997. Genetics and the origin of bird species. *Proceedings of the National Academy of Sciences USA*, **94,** 7768-7775.

Green, D. J. & Cockburn, A. 2001. Post-fledging care, philopatry and recruitment in brown thornbills. *Journal of Animal Ecology*, **70,** 505-514.

Grenfell, B. T., Pybus, O. G., Gog, J. R., Wood, J. L. N., Daly, J. M., Mumford, J. A. & Holmes, E. C. 2004. Unifying the epidemiological and evolutionary dynamics of pathogens. *Science*, **303,** 327-332.

Griffith, S. C., Owens, I. P. F. & Thuman, K. A. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology*, **11,** 2195-2212.

Griswold, D. A., Harrer, M. F., Sladkin, C., Alessandro, D. A. & Gould, J. L. 1995. Intraspecific recognition by laughing gull chicks. *Animal Behaviour*, **50,** 1341-1348.

Hagelin, J. C., Jones, I. L. & Rasmussen, L. E. L. 2003. A tangerine-scented social odour in a monogamous seabird. *Proceedings of the Royal Society of London B*, **270,** 1323-1329.

Hansson, B. 2004. Marker-based relatedness predicts egg-hatching failure in great reed warblers. *Conservation Genetics*, **5,** 339-348.

Hansson, B. & Westerberg, L. 2002. On the correlation between heterozygosity and fitness in natural populations. *Molecular Ecology*, **11,** 2467-2474.

Hasegawa, H. & DeGange, A. R. 1982. The Short-tailed albatross *Diomedea albatrus*, its status, distribution and natural history. *American Birds*, **36,** 806-814.

Hatch, S. A., Roberts, B. D. & Fadely, B. S. 1993. Adult survival of Black-legged kittiwakes *Rissa tridactyla* in a Pacific colony. *Ibis*, **135,** 247-254.

Healy, S. & Guilford, T. 1990. Olfactory-bulb size and nocturnality in birds. *Evolution*, **44,** 339-346.

Heath, J., Slater, A., Daniels, D. & Merrifield, S. 1982. Duration of Vocal Behavior During the Greeting Ceremony of the Kittiwake (Rissa-Tridactyla). *Behaviour Analysis Letters*, **2,** 221-225.

Helfenstein, F. 2002. Stratégies de reproduction et conflits sexuels. In: *Ecologie*, pp. 137. Paris: Université Pierre et Marie Curie.

Helfenstein, F., Danchin, E. & Wagner, R. H. 2004a. Assortative mating and sexual size dimorphism in black-legged kittiwakes. *Waterbirds*, **27,** 350-354.

Helfenstein, F., Danchin, E. & Wagner, R. H. 2004b. Is male unpredictability a paternity assurance strategy? *Behaviour*, **141,** 675-690.

Helfenstein, F., Tirard, C., Danchin, E. & Wagner, R. H. 2004c. Low frequency of extra-pair paternity and high frequency of adoption in Black-legged kittiwakes. *Condor*, **106,** 149-155.

Helfenstein, F., Wagner, R. H., Danchin, E. & Rossi, J.-M. 2003. Fonctions of courtship feeding in black-legged kittiwakes: natural and sexual selection. *Animal Behaviour*, **65,** 1027-1033.

Hess, C. M. & Edwards, S. V. 2002. The evolution of the major histocompatibility complex in birds. *BioScience*, **52,** 423-431.

Hoffman, J. I., Fordaca, J., Trathan, P. N. & Amos, W. 2007. Female fur seals show active choice for males that are heterozygous and unrelated. *Nature*, **445,** 912-914.

Höglund, J., Piertney, S. B., Alatalo, R. V., Lindell, J., Lundberg, A. & Rintamäki, P. T. 2002. Inbreeding depression and male fitness in black grouse. *Proceedings of the Royal Society of London B*, **269,** 711-715.

Holley, A. J. F. 2000. Ring-billed gull adoption: what arms race? *Animal Behaviour*, **60,** F15-F16.

Hutchinson, R. E., Stevenson, J. G. & Thorpe, W. H. 1968. The basis for individual recognition by voice in the Sandwich tern (*Sterna sandvicensis*). *Behaviour*, **32,** 150-157.

Insley, S. J., Paredes, R. & Jones, I. L. 2003. Sex differences in razorbill *Alca torda* parent-offspring vocal recognition. *Journal of Experimental Biology*, **206,** 25-31.

Irwin, D. E. 2000. Song variation in an avian ring species. *Evolution*, **54,** 998-1010.

Jodice, P. G. R., Lanctot, R. B., Gill, V. A., Roby, D. D. & Hatch, S. 2000. Sexing adult black-legged kittiwakes by DNA, behavior, and morphology. *Waterbirds*, **23,** 405-415.

Johnsen, A., Andersen, V., Sundling, C. & Lifjeld, J. T. 2000. Female bluethroats enhance offspring immunocompetence through extra-pair copulations. *Nature*, **406,** 296-299.

Johnsen, A., Delhey, K., Schlicht, E., Peters, A. & Kempenaers, B. 2005. Male sexual attractiveness and parental effort in Blue tits: a test of the differential allocation hypothesis. *Animal Behaviour*, **70,** 877-888.

Johnson, L. S., Rauch, R. L. & Dellone, S. N. 2004. The process and causes of fledging in a cavity-nesting passerine bird, the house wren (*Troglodytes aedon*). *Ethology*, **110,** 693-705.

Jones, I. L. & Hunter, F. M. 1993. Mutual sexual selection in a monogamous seabird. *Nature*, **362,** 238-239.

Kempenaers, B., Adriaensen, F., Van Noordwijk, A. J. & Dhondt, A. A. 1996. Genetic similarity, inbreeding and hatching failure in blue tits: are unhatched eggs infertile? *Proceedings of the Royal Society of London B*, **263,** 179-185.

Kempenaers, B., Lanctot, R. B., Gill, V. A. & Hatch, S. A. 2006. Do females trade copulations for food? An experimental study in Black-legged Kittiwakes. *Behavioral Ecology*, **147,** 192-193.

Kikkawa, E. F., Tsuda, T. T., Naruse, T., Sumiyama, D., Fukuda, M., Kurita, M., Murata, K., Wilson, R. P., Le Maho, Y., Tsuda, M., Kulski, J. K. & Inoko, H. 2005. Analysis

of the sequence variations in the *MHC DRB1*-like gene of the endangered Humboldt penguin (*Spheniscus humbodlti*). *Immunogenetics*, **57,** 99-107.

Kleven, O. & Lifjeld, J. T. 2004. Extrapair paternity and offspring immunocompetence in the reed bunting, *Emberiza schoeniclus*. *Animal Behaviour*, **68,** 283-289.

Knapp, L. A., Robson, J. & Waterhouse, J. S. 2006. Olfactory signals and the MHC: a review and a case study in *Lemur catta*. *American Journal of Primatology*, **68,** 568-584.

Kokko, H. & Ots, I. 2006. When not to avoid inbreeding. *Evolution*, **60,** 467-475.

Krokene, C. & Lifjeld, J. T. 2000. Variation in the frequency of extra-pair paternity in birds; a comparison of an island and a mainland population of Blue tits. *Behaviour*, **137,** 1317-1330.

Kroodsma, D. E. 2004. The diversity and plasticity of birdsong. In: *Nature's music* (Ed. by Marler, P. & Slabbekoorn, H.): Elsevier Academic Press.

Kroodsma, D. E. & Miller, E. H. 1996. *Ecology and evolution of acoustic communication in birds*. Ithaca, New York: Cornell University Press.

Lambrechts, M. M. & Hossaert-McKey, M. 2006. Olfaction, volatile compounds and reproduction in birds. *Acta Zoologica Sinica*, **53S,** 284-287.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. *Proceedings of the National Academy of Sciences USA*, **78,** 3721-3725.

Landry, C., Garant, D., Duchesne, P. & Bernatchez, L. 2001. 'Good genes as heterozygosity': the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). *Proceedings of the Royal Society of London B*, **268,** 1279-1285.

Leedman, A. W. & Magrath, R. D. 2003. Long-term brood division and exclusive parental care in a cooperatively breeding passerine. *Animal Behaviour*, **65,** 1093-1108.

Leibovici, M., Lapointe, F., Aletta, P. & Ayer-Le Lièvre, C. 1996. Avian olfactory receptors: differenciation of olfactory neurons under normal an experimental conditions. *Developmental Biology*, **175,** 118-131.

Lessells, C. M. 2002. Parentally biased favouritism: why should parents specialize in caring for different offspring? *Philosophical Transactions of the Royal Society of London B*, **357,** 381-403.

Lieutenant-Gosselin, M. & Bernatchez, L. 2006. Local heterozygosity-fitness correlations with global positive effects of fitness in threespine stickleback. *Evolution*, **60,** 1658-1668.

Lloyd, C., Tasker, M. L. & Partridge, K. 1991. Kittiwake *Rissa tridactyla*. In: *The status of seabirds in Great Britain and Ireland*, pp. 182-193.

Longmire, J. L., Lewis, A. K., Brown, N. C., Buckingham, J. M., Clark, L. M., Jones, M. D., Meincke, L. J., Meyne, J., Ratliff, R. L., Ray, F. A., Wagner, R. P. & Moyzis, R. K. 1988. Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family Falconidae. *Genomics*, **2,** 14-24.

Lynch, M. & Ritland, K. 1999. Estimation of pairwise relatedness with molecular markers. *Genetics*, **152,** 1753-1766.

Mäntylä, E., Klemola, T. & Haukioja, E. 2004. Attraction of willow warblers to sawfty-damaged mountain birches: novel function of inducible plant defences? *Ecology Letters*, **7,** 915-918.

Mathevon, N., Charrier, I. & Jouventin, P. 2003. Potential for individual recognition in acoustic signals: a comparative study of two gulls with different nesting patterns. *Comptes-Rendus Biologies*, **326,** 329-337.

Mathieu, E., Autem, M., Roux, M. & Bonhomme, F. 1990. Epreuves de validation dans l'analyse de structures génétiques multivariées: comment tester l'équilibre panmictique? *Revue de Statistique Appliquée*, **38,** 47-66.

Mays, H. L. & Hill, G. E. 2004. Choosing mates: good genes versus genes that are a good fit. *Trends in Ecology & Evolution*, **19,** 554-559.

McClelland, E. E., Penn, D. J. & Potts, W. K. 2003. Major histocompatibility complex heterozygote superiority during coinfection. *Infection & Immunity*, **71,** 2079-2086.

McCoy, K. D., Boulinier, T. & Tirard, C. 2005. Comparative host-parasite population structures: disentangling prospecting and dispersal in the Black-legged kittiwake *Rissa tridactyla*. *Molecular Ecology*, **14,** 2825-2838.

Medrano, J. F., Aasen, E. & Sharrow, L. 1990. DNA extraction from nucleated red blood cells. *Biotechniques*, **8,** 43.

Mennerat, A., Bonadonna, F., Perret, P. & Lambrechts, M. M. 2005. Olfactory conditioning experiments in a food-searching passerine bird in semi-natural conditions. *Behavioural Processes*, **70,** 264-270.

Middleton, H. A., Green, D. J. & Krebs, E. A. 2007. Fledgling begging and parental responsiveness in American dippers (Cinclus mexicanus). *Behaviour*, **144,** 485-501.

Milinski, M. & Wedekind, C. 2001. Evidence for MHC-correlated perfume preferences in humans. *Behavioral Ecology*, **12,** 140-149.

Miller, D. E. & Emlen, J. T. J. 1975. Individual chick recognition and family integrity in the Ring-billed gull. *Behaviour*, **52,** 124-143.

Møller, A. P., Merino, S., Brown, C. R. & Robertson, R. J. 2001. Immune defense and host sociality: a comparative study of swallows and martins. *American Naturalist*, **158,** 136-145.

Monnat, J.-Y., Danchin, E. & Estrella, R. R. 1990. Assessment of environmental quality within the framework of prospecting and recruitment: the squatterism in the Kittiwake. *Comptes-Rendus de l'Académie des Sciences de Paris*, **311,** 391-396.

Morris, R. D., Woulfe, M. & Wichert, G. D. 1991. Hatching synchrony, chick care and adoption in the common tern: can disadvantaged chicks win? *Canadian Journal of Zoology*, **68,** 661-668.

Morton, E. S., Forman, L. & Braun, M. 1990. Extrapair fertilizations and the evolution of colonial breeding in purple martins. *Auk*, **107,** 275-283.

Moseley, L. J. 1979. Individual auditory recognitino in the least tern (Sterna albifrons). *Auk*, **96,** 31-39.

Moum, T., Johansen, S., Enkstad, K. E. & Piatt, J. F. 1994. Phylogeny and evolution of the auks (subfamily Alcinae) based on mitochondrial DNA sequences. *Proceedings of the National Academy of Sciences USA*, **91,** 7912-7916.

Naves, L. C., Monnat, J.-Y. & Cam, E. 2006. Breeding performance, mate fidelity, and nest site fidelity in a long-lived seabird: behaving against the current? *OIKOS*, **115,** 263-276.

Nef, S., Allaman, I., Fiumelli, H., De Castro, E. & Nef, P. 1996. Olfaction in birds: differential embryonic expression of nine putative odorant receptor genes in the avian olfactory system. *Mechanisms of Development*, **55,** 65-77.

Neff, B. D. & Pitcher, T. E. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Molecular Ecology*, **14,** 19-38.

Nei, M. & Gojobori, T. 1986. Simple method for estimating the number of synonymous and nonsynonymous nucleotide substitutions. *Molecular Biology and Evolution*, **3,** 418-426.

Nelson, D. A. & Marler, P. 1994. Selection-based learning in bird song development. *Proceedings of the National Academy of Sciences USA*, **91,** 10498-10501.

Nevitt, G. A., Reid, J. M. & Trathan, P. 2004. Testing olfactory foraging strategies in an Antarctic seabird assemblage. *Journal of Experimental Biology*, **207,** 3537-3544.

Oetting, W. S., Lee, H. K., Flanders, D. J., Wiesner, G. L., Sellers, T. A. & King, R. A. 1995. Linkage analysis with multiplexed short tandem repeat polymorphisms using infrared fluorescence and M13 taied primers. *Genomics*, **30,** 450-458.

Olsén, K. H., Grahn, M., Lohm, J. & Langefors, Å. 1998. MHC and kin discrimination in juvenile Arctic charr, *Salvelinus alpinus* (L.). *Animal Behaviour*, **56,** 319-327.

Olsson, M., Madsen, T., Nordby, J., Wapstra, E., Ujvari, B. & Wittsell, H. 2003. Major histocompatibility complex and mate choice in sand lizards. *Proceedings of the Royal Society of London B*, **Supplement,** S1-S3.

Parker, N., Cam, E., Lank, D. B. & Cooke, F. 2003. Post-fledging survival of marbled murrelets *Brachyramphus marmoratus* estimated with radio-marked juveniles in Desolation Sound, British Columbia. *Marine Ornithology*, **31,** 207-212.

Penn, D. J. 2002. The scent of genetic compatibility: sexual selection and the major histocompatibility complex. *Ethology*, **108,** 1-21.

Penn, D. J., Damjanovich, K. & Potts, W. K. 2002. MHC heterozygosity confers a selective advantage against multiple-strain infections. *Proceedings of the National Academy of Sciences USA*, **99,** 11260-11264.

Penn, D. J. & Potts, W. K. 1998a. MHC-disassortative mating preferences reversed by cross-fostering. *Proceedings of the Royal Society of London B*, **265,** 1299-1306.

Penn, D. J. & Potts, W. K. 1998b. Untrained mice discriminate MHC-determined odors. *Physiology & Behavior*, **63,** 235-243.

Petit, C., Hossaert-McKey, M., Perret, P., Blondel, J. & Lambrechts, M. M. 2002. Blue tits use selected plants and olfaction to maintain an aromatic environment for nestlings. *Ecology Letters*, **5,** 585-689.

Piertney, S. B. & Oliver, M. K. 2006. The evolutionary ecology of the major histocompatibility complex. *Heredity*, **96,** 7-21.

Plissner, J. A. & Gowaty, P. A. 1988. Evidence of reproductive error in the adoption of nestling Eastern bluebirds (*Sialis sialis*). *Auk*, **105,** 575-578.

Poesel, A., Kunc, H. P., Foerster, K., Johnsen, A. & Kempenaers, B. 2006. Early birds are sexy: male age, dawn song and extrapair paternity in Blue tits, *Cyanistes* (formerly *Parus*) *caeruleus*. *Animal Behaviour*, **72,** 531-538.

Price, T. D. 1998. Sexual selection and natural selection in bird speciation. *Philosophical Transactions of the Royal Society of London B*, **353,** 251-260.

Prosser, C. L. 1950. *Comparative animal physiology*. Philadelphy: W.S. Saunders Co.

Queller, D. C. & Goodnight, K. F. 1989. Estimating relatedness using genetic markers. *Evolution*, **43,** 258-275.

Rantala, M. J., Eriksson, C. J. P., Vainikka, A. & Kortet, R. 2006. Male steroid hormones and female preference for male body odor. *Evolution and Human Behavior*, **27,** 259-269.

Rätti, O., Hovi, M., Lundberg, A., Tegelström, H. & Alatalo, R. V. 1995. Extra-pair paternity and male characteristics in the pied flycatcher. *Behavioral Ecology and Sociobiology*, **37,** 419-425.

Raymond, M. & Rousset, F. 1995. GENEPOP (ver. 1.2): a population genetics software for exact test and ecumenicism. *Heredity*, **86,** 248-249.

Reid, J. M. 2007. Secondary sexual ornamentation and non-additive genetic benefits of female mate choice. *Proceedings of the Royal Society of London B*, **274,** 1395-1402.

Reid, J. M., Arcese, P., Cassidy, A., Hiebert, S. M., Smith, J. N. M., Stoddard, P. K., Marr, A. B. & Keller, L. F. 2004. Song repertoire size predicts initial mating success in male song sparrows, Melospiza melodia. *Animal Behaviour*, **68,** 1055-1063.

Reid, J. M., Arcese, P., Cassidy, A. L. E. V., Marr, A. B., Smith, J. N. M. & Keller, L. F. 2005. Hamilton and Zuk meet heterozygosity? Song repertoire size indicates inbreeding and immunity in Song sparrows (*Melospiza melodia*). *Proceedings of the Royal Society of London B*, **272,** 481-487.

Reneerkens, J., Piersma, T. & Sinninghe Damsté, J. S. 2005. Switch to diester preen waxes may reduce avian nest predation by mammalian predators using olfactory cues. *Journal of Experimental Biology*, **208,** 4199-4202.

Reusch, T. B. H., Häberli, M. A., Aeschlimann, P. B. & Milinski, M. 2001. Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature*, **414,** 300-302.

Richardson, D. S., Komdeur, J., Burke, T. & Von Schantz, T. 2005. MHC-based patterns of social and extra-pair mate choice in the Seychelles warbler. *Proceedings of the Royal Society of London B*, **272,** 759-767.

Riedman, M. L. 1982. The evolution of alloparental care and adoption in mammals and birds. *Quaterly Review of Biology*, **57,** 405-435.

Riffaut, L., McCoy, K. D., Tirard, C., Friesen, V. L. & Boulinier, T. 2005. Population genetics of the Common guillemot *Uria aalge* in the North Atlantic: geographic impact of oil spills. *Marine Ecology Progress Series*, **291,** 263-273.

Roberts, B. D. & Hatch, S. A. 1993. Behavioral ecology of Black-legged kittiwakes during chick rearing in a failing colony. *Condor*, **95,** 330-342.

Roberts, B. D. & Hatch, S. A. 1994. Chick movements and adoption in a colony of Black-legged kittiwakes. *Wilson Bulletin*, **106,** 289-298.

Roberts, S. C. & Gosling, L. M. 2003. Genetic similarity and quality interact in mate choice decisions by female mice. *Nature Genetics*, **35,** 103-106.

Roper, T. J. 1999. Olfaction in birds. *Advances in the Study of Behavior*, **28,** 247-332.

Rubenstein, D. R. 2007. Female extrapair mate choice in a cooperative breeder: trading sex for help and increasing offspring heterozygosity. *Proceedings of the Royal Society of London B*, **274,** 1895-1903.

Searby, A., Jouventin, P. & Aubin, T. 2004. Acoustic recognition in macaraoni penguins: an original signature system. *Animal Behaviour*, **67,** 615-625.

Searcy, W. A., Nowicki, S., Hughes, M. & Peters, S. 2002. Geographic song discrimination in relation to dispersal distances in song sparrows. *American Naturalist*, **159,** 221-230.

Shiina, T., Shimizu, S., Hosomichi, K., Kohara, S., Watanabe, S., Hanzawa, K., Beck, S., Kulski, J. K. & Inoko, H. 2004. Comparative genomic analysis of two avian (quail and chicken) MHC regions. *Journal of Immunology*, **172,** 6751-6763.

Sibley, C. G. & Ahlquist, J. E. 1991. *Phylogeny and classification of birds: A study in molecular evolution*.: Yale University Press.

Slabbekoorn, H. 2004. Singing in the wild: the ecology of bird song. In: *Nature's music, the science of birdsong* (Ed. by Marler, P. & Slabbekoorn, H.), pp. 178-205.

Slabbekoorn, H. & Smith, T. B. 2000. Does bill size polymorphism affect courtship song characteristics in the African finch *Pyrenestes ostrinus*? *Biological Journal of the Linnean Society*, **71,** 737-753.

Slabbekoorn, H. & Smith, T. B. 2002. Bird song, ecology and speciation. *Proceedings of the Royal Society of London B*, **357,** 493-503.

Slagsvold, T. 1997. Brood division in birds in relation to offspring size: sibling rivalry and parental control. *Animal Behaviour*, **54,** 1357-1368.

Sluys, R. 1982. Geographical variation of the kittiwake. *Rissa tridactyla*. *Le Gerfaut*, **72,** 221-230.

Soini, H. A., Schrock, S. E., Bruce, K. E., Wiesler, D., Ketterson, E. D. & Novotny, M. V. 2006. Seasonal variation in volatile compound profiles from preen gland secretions of the Dark-eyed junco (*Junco hyemalis*). *Journal of Chemical Ecology*.

Storey, A. E., Anderson, R. E., Porter, J. M. & McCharles, A. M. 1992. Absence of parent-young recognition in kittiwakes: a re-examination. *Behaviour*, **120,** 302-323.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. 2007. *MEGA4*: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution***,** 10.1093/molbev/msm092.

The-MHC-Sequencing-Consortium. 1999. Complete sequence and gene map of a human major histocompatibility complex. *Nature*, **401,** 921-923.

Thuman, K. A. & Griffith, S. C. 2005. Genetic similarity and the nonrandom distribution of paternity in a genetically highly polyandrous shorebird. *Animal Behaviour*, **69,** 765-770.

Tiira, K., Laurila, A., Enberg, K., Piironen, J., Aikio, S., Ranta, E. & Primmer, C. R. 2005. Do dominants have higher heterozygosity? Social status and genetic variation in brown trout, Salmo trutta. *Behavioral Ecology and Sociobiology*, **59,** 657-665.

Tinbergen, N. 1953. *The herring gull' world*. London: Collins.

Tinbergen, N. 1959. Comparative studies of the behaviour of gulls (*Laridae*): a progress report. *Behaviour*, **15,** 1-70.

Tirard, C., Helfenstein, F. & Danchin, E. 2002. Polymorphic microsatellites in the Black-legged kittiwake *Rissa tridactyla*. *Molecular Ecology Notes*, **2,** 431-433.

Tregenza, T. & Wedell, N. 2000. Genetic compatibility, mate choice and patterns of parentage: Invited review. *Molecular Ecology*, **9,** 1013-1027.

Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual selection and the descent of man, 1871-1971* (Ed. by Campbell, B.), pp. 136-179. Chicago, IL: Aldine Publishing Company.

Tsuda, T. T., Tsuda, M., Naruse, T., Kawata, H., Ando, A., Shiina, T., Fukuda, M., Kurita, M., Le Maho, Y., Kulski, J. K. & Inoko, H. 2001. Phylogenetic analysis of penguin (*Spheniscidae*) species based on sequence variation in MHC Class II genes. *Immunogenetics*, **53,** 712-716.

Tubaro, P. L. & Segura, E. T. 1995. Geographic, ecological and subspecific variation in the song of the rufous-browed peppershrike (*Cyclarhis gujanensis*). *Condor*, **97,** 792-803.

Van de Casteele, T., Galbusera, P. & Matthysen, E. 2001. A comparison of microsatellite-based pairwise relatedness estimators. *Molecular Ecology*, **10,** 1539-1549.

Van de Casteele, T., Galbusera, P., Schenck, T. & Matthysen, E. 2003. Seasonal and lifetime reproductive consequences of inbreeding in the great tit *Parus major*. *Behavioral Ecology*, **14,** 165-174.

Van Dongen, W. F. D. & Mulder, R. A. 2006. Habitat density, song structure and dialects in the Madagascar paradise flycatcher *Terpsiphone mutata*. *Journal of Avian Biology*, **37,** 349-356.

Wagner, R. H. 1997. Hidden leks: sexual selection and the clumping of avian territories. In: *Extra-pair mating tactics in birds* (Ed. by Parker, P. G. & Burley, N.), pp. 123-145. Washington, D.C.: Ornithological Monographs, American Ornithologists' Union.

Wallraff, H. G. 2004. Avian olfactory navigation: its empirical foundation and conceptual state. *Animal Behaviour*, **67,** 189-204.

Warden, C. J., Jenkins, T. N. & Warner, L. H. 1936. Comparative psychology. In: *Vertebrates*. New York: Ronald Press.

Weatherhead, P. J., Dufour, K. W., Lougheed, S. C. & Eckert, C. G. 1999. A test of the good-genes-as-heterozygosity hypothesis using Red-winged blackbirds. *Behavioral Ecology*, **10,** 619-625.

Wedekind, C. 2002. The MHC and body odors: arbitrary effects caused by shifts of mean pleasantness. *Nature*, **31,** 237.

Wedekind, C. & Füri, S. 1997. Body odour preferences in men and women: do they aim for specific MHC combinations or simply heterozygosity? *Proceedings of the Royal Society of London B*, **264,** 1471-1479.

Wedekind, C. & Penn, D. 2000. MHC genes, body odours, and odour preferences. *Nephrology Dialysis Transplantation*, **15,** 1269-1271.

Wedekind, C., Seebeck, T., Bettens, F. & Paepke, A. J. 1995. MHC-dependent mate preferences in humans. *Proceedings of the Royal Society of London B*, **260,** 245-249.

Wedekind, C., Walker, M., Portmann, J., Cenni, B., Müller, R. & Binz, T. 2004. MHC-linked susceptibility to a bacterial infection, but no MHC-linked cryptic female choice in Whitefish. *Journal of Evolutionary Biology*, **17,** 11-17.

Wegner, K. M., Kalbe, M., Kurtz, J., Reusch, T. B. H. & Milinski, M. 2003. Parasite selection for immunogenetic optimality. *Science*, **301,** 1343.

Westerdahl, H. 2003. Avian MHC: variation and selection in the wild. Lund: Lund University.

Westerdahl, H. 2004. No evidence of an MHC-based female mating preference in great reed warblers. *Molecular Ecology*, **13,** 2465-2470.

Westerdahl, H., Waldenström, J., Hansson, B., Hasselquist, D., Von Schantz, T. & Bensch, S. 2005. Associations between malaria and MHC genes in a migratory songbird. *Proceedings of the Royal Society of London B*, **272,** 1511-1518.

Wittzell, H., Madsen, T., Westerdahl, H., Shine, R. & Von Schantz, T. 1999. MHC variation in birds and reptiles. *Genetica*, **104,** 301-309.

Wooller, R. D. 1978. Individual vocal recognition in the kittiwake gull, *Rissa tridactyla* (L.). *Zeitschrift für Tierpsychology*, **48,** 68-86.

Wooller, R. D. 1979. Seasonal, diurnal and area differences in calling activity within a colony of kittiwakes *Rissa tridactyla* (L.). *Zeitschrift für Tierpsychology*, **51,** 329-336.

Yamazaki, K., Beauchamp, G. K., Imai, Y., Bard, J., Phelan, S. P., Thomas, L. & Boyse, E. A. 1990. Odortypes determined by the major histocompatibility complex in germfree mice. *Proceedings of the National Academy of Sciences USA*, **87,** 8413-8416.

Yamazaki, K., Singer, A. G. & Beauchamp, G. K. 1999. Origin, functions and chemistry of H-2 regulated odorants. *Genetica*, **104,** 235-240.

Ydenberg, R. C. 1989. Growth-mortality trade-offs and the evolution of juvenile life histories in the Alcidae. *Ecology*, **70,** 1494-1506.

Yu, H.-T., Liao, Y.-Y. & Kao, C.-H. 2001. Relatedness structure and individual identification in a semi-fossorial shrew (Soricidae: *Anourosorex squamipes*) - an application of microsatellite DNA. *Zoological Studies*, **40,** 226-232.

Zoorob, R., Béhar, G., Kroemer, G. & Auffray, C. 1990. Organization of a functional chicken class II B gene. *Immunogenetics*, **31,** 179-187.