

Variations individuelles des traits
reproducteurs chez le flamant rose : de
l'appariement à l'envol des jeunes

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Résumé

Dans les populations naturelles, on observe une grande variabilité du succès reproducteur des individus. Cette variabilité est structurée par l'âge et le sexe mais aussi par des facteurs individuels non directement observables. Dans cette thèse, je me suis intéressée à l'influence de l'âge et du sexe sur les traits reproducteurs à différentes étapes de la reproduction du flamant rose ainsi qu'à l'influence d'une mesure potentielle de qualité phénotypique, la condition corporelle des parents avant leur envol, sur la condition corporelle de leur poussin. Enfin, j'ai examiné l'effet de l'âge de première reproduction sur le nombre de jeunes produits par un individu durant sa vie.

Durant la parade nuptiale, nous avons montré que les séquences comportementales ne différaient pas entre les sexes. Cependant les femelles exhibent une coloration plus intense et les mâles présentent des comportements agonistiques de plus en plus fréquents entre eux avec l'âge. Nous avons aussi mis en évidence un effet quadratique de l'âge sur la complexité des séquences comportementales, suggérant un processus de maturation chez les jeunes et de la sénescence chez les plus âgés. Cette complexité apparaît être impliquée dans le choix du partenaire et être un signal honnête de la qualité de l'individu. Durant la période d'élevage, nous avons trouvé une relation positive de la seule condition corporelle des pères quand ils étaient poussins sur la condition corporelle de leur poussin et ce, quel que soit leur âge. Ceci souligne les différences liées au sexe dans les conséquences à long terme de la condition corporelle juvénile sur les performances reproductives. Bien que les individus atteignent leur maturité sexuelle à 3 ans, mâles et femelles retardent leur première reproduction à un âge moyen de 6-7 ans. C'est l'âge optimal pour le nombre total de jeunes produits dans leur vie pour les femelles mais ce nombre diminue constamment avec l'âge chez les mâles. Des éléments semblent indiquer que les jeunes mâles pourraient être exclus de la reproduction par leurs aînés durant la formation des couples.

Cette thèse met en avant l'importance de l'âge et du sexe dans la variation des traits reproducteurs chez une espèce longévive. Nous soulignons la nécessaire prise en compte du sexe dans l'étude des comportements reproducteurs même chez les espèces monogames où les rôles parentaux sont peu différenciés. Enfin, nous attirons l'attention sur la nécessité d'intégrer l'accès à la reproduction en amont du cycle de nidification pour avoir une meilleure compréhension des facteurs menant à cette variabilité.

Abstract

In wild population, we observe a high variability of reproductive success between individuals. This variability is generally structured by age and sex but also by individual factors not directly observable. In this thesis, I focused on the age and sex influence on reproductive traits at different times of reproduction in the greater flamingo as well as on the influence of a potential measure of phenotypic quality, the early body condition of parents, on the body condition of their chick. Finally, I examined the effect of age at first reproduction on lifetime reproductive success.

During courtship display we showed that behavioral sequences were similar between sexes. However, females express a more intense coloration and males display more and more interaction between themselves with age. We also highlighted a quadratic effect of age on sexual display complexity suggesting of a maturation process in young individuals and senescence in old individuals. In addition, this complexity seems involved in mate choice and being an honest signal of individual quality. During rearing period, we found a positive relationship between early body condition of males and body condition of their chicks and this whatever their age. This highlighted sex-related differences in long-term consequences of early body condition on reproductive performances. Although individuals reach sexual maturity at 3 years, males and females delay reproduction to a mean age of 6-7 years. This correspond to the optimal age to maximize lifetime reproductive success in females, while in males more they breed early for the first time, more their lifetime reproductive success is high. Elements suggest that young males could be excluded by their elders during pairing formation.

Our work emphasizes the role of sex and age on the variability of reproductive traits in a long-lived species. We emphasize the importance to take into account the sex of individuals in the study of reproductive behaviors even for monogamous species where sex roles are poorly differentiated. Finally, we draw attention to the need to integrate breeding access upstream of nesting cycle to have a better understanding of the factors leading to this variability.

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Introduction

Variabilité entre individus

L'une des plus grandes avancées de Darwin fut de reconnaître qu'au sein d'une même espèce, les individus expriment une grande variation dans leurs traits morphologiques, physiologiques ou comportementaux (Darwin 1871). Cette variabilité interindividuelle est la résultante d'interactions complexes entre les caractéristiques génétiques et les conditions environnementales expérimentées par les individus. Elle conduit à une hétérogénéité dans les traits d'histoire de vie tels que la survie et le succès reproducteur et donc à une capacité différentielle des individus à transmettre leurs gènes à la génération suivante (aptitude phénotypique, « fitness en anglais »). Cette hétérogénéité est constituée d'une composante stable, c'est-à-dire que les différences entre individus sont fixées en début de vie puis constantes au cours de la vie (ex. facteurs génétiques, environnementaux, parentaux), et d'une composante dynamique pouvant varier au cours de la vie des individus (ex. expérience, senescence, etc.).

Diversité génétique

Le génotype est la composition allélique de tous les gènes d'un individu. Il est hérité des parents. Les gènes sont des séquences nucléotidiques (en l'occurrence, d'ADN) qui codent pour des protéines affectant les propriétés des cellules, unités de base des organismes multicellulaire dont l'organisation produit l'ensemble des attributs de l'organisme appelé phénotype. Le phénotype des individus est ainsi l'expression visible du génotype dans un environnement donné. Au sein d'une espèce, les gènes codant peuvent varier d'un individu à l'autre et ce degré de variété est défini comme la diversité génétique de l'espèce. Chaque variant de ce gène correspond à un allèle. Les allèles d'un même gène peuvent aboutir à des protéines fonctionnellement identiques, sans conséquence sur le phénotype, mais aussi à des protéines différentes et donc à des phénotypes différents. La diversité génétique génère donc une diversité phénotypique au sein de l'espèce et est par conséquent responsable, du moins en partie de la diversité des traits morphologiques, physiologiques, et comportementaux liés à la reproduction.

Plasticité phénotypique

La plasticité phénotypique est définie comme la gamme de variations des phénotypes que peut prendre un génotype sous les effets de l'environnement (Clobert and Sinervo 2016). L'hétérogénéité de l'environnement dans l'espace et dans le temps, qu'il soit biotique ou abiotique génère alors une diversité phénotypique au sein d'une même espèce et par conséquent une diversité des traits individuels liés à la reproduction. Cette plasticité peut être le reflet d'une réponse adaptative à l'environnement, mais pas toujours. Parfois il s'agit simplement du reflet de contraintes imposées par l'environnement sur l'organisme de l'individu (Monaghan 2008). Les génotypes peuvent tous répondre de la même manière à l'environnement ou différemment. Si les réponses diffèrent, il y a alors variation dans la plasticité phénotypique, ce qui peut là aussi mener à de la diversité phénotypique. Le génotype, l'environnement dans lequel évoluent les individus ainsi que leurs interactions sont ainsi source de variation des phénotypes au sein d'une population.

La plasticité phénotypique peut être réversible ou non et se manifester à divers moments de la vie de l'individu. La plasticité prenant place au moment du développement a fait l'objet d'un intérêt particulier ces dernières années. Il en ressort que les conditions pré- et post-natales dans lesquelles se développent les individus influent directement sur le phénotype des jeunes, mais qu'elles ont aussi des conséquences à long-terme, notamment sur les caractéristiques reproductrices des individus et par conséquent sur leur succès reproducteur (Lindström 1999; Van De Pol et al. 2006; Monaghan 2008).

Patrons généraux de variabilité individuelle des traits reproducteurs

La variabilité individuelle des traits reproducteurs due à des différences latentes entre individus est parfois difficile voire impossible à mesurer (« hétérogénéité cachée »). Néanmoins dans les populations animales, on observe généralement une structuration de la variation des traits reproducteurs en fonction de divers attributs individuels dont les plus reconnus sont le sexe et l'âge (Short and Balaban 1994; Forslund and Pärt 1995; McNamara and Houston 1996).

Age

Les patrons de variation des traits reproducteurs liés à l'âge sont fréquents dans les populations naturelles, en particulier chez les espèces d'oiseaux et de mammifères longévifs (Curio 1983; Clutton-Brock 1988; Lunn et al. 1994; Forslund and Pärt 1995; Martin 1995; Hewison and Gaillard 2001; Reid et al. 2003; Mauck et al. 2004; Beauplet et al. 2006). Ces espèces partagent généralement le même patron : la performance reproductrice augmente durant les premières années de vie jusqu'à atteindre un plateau, puis parfois on observe une diminution de ces performances pour les âges les plus avancés. Par exemple, chez le Grand Albatros *Diomedea exulans*, le succès reproducteur, augmente progressivement et linéairement entre l'âge de première reproduction (8 ou 9 ans) et l'âge de environ 12 ans ; puis, il semble se stabiliser ou décliner faiblement progressivement entre l'âge de 12 ans et 25/30 ans, déclin qui s'accroît après 25-30 ans (Weimerskirch et al. 2005).

Ce patron peut résulter de plusieurs mécanismes non exclusifs. Premièrement, l'amélioration des compétences reproductrices au cours de la vie à travers des processus de maturation et ou d'expérience conduit à une augmentation des performances reproductives en début de vie (Curio 1983). Les jeunes individus moins expérimentés et souvent de plus petite taille expriment généralement une plus faible compétitivité en comparaison de leurs aînés et auront ainsi plus de difficultés à acquérir un partenaire, un site de reproduction, ou à nourrir efficacement leur descendance (Martin 1995).

La sénescence, définie comme le déclin des performances individuelles à partir d'un certain âge et/ou la détérioration progressive de l'organisme (Kirkwood and Austad 2000; Monaghan et al. 2008), est également susceptible d'entraîner le déclin des performances reproductrices à un âge avancé. Par exemple, il a été montré que la sénescence pouvait affecter tout aussi bien l'attractivité sexuelle d'un individu en influençant négativement l'expression des signaux sexuels (Torres and Velando 2007; Pavlova et al. 2010; Balbontín et al. 2011; Cooper et al. 2012), que la qualité des soins parentaux (Beamonte-Barrientos et al. 2010).

Enfin, une augmentation de l'investissement dans la reproduction avec l'âge est attendue en raison d'un lien fort entre l'âge et la valeur reproductive des individus, en particulier chez les espèces longévives (« restraints hypothesis », Pianka 1976; Forslund and Pärt 1995). La valeur reproductive d'un individu est définie comme l'espérance de production

actuelle et future de descendants. Typiquement, un jeune reproducteur à une valeur reproductrice élevée puisqu'il aura de nombreuses occasions de se reproduire au cours de sa vie. En revanche plus un individu est âgé, plus les occasions de reproduction vont diminuer du fait du phénomène de sénescence. Ainsi il sera dans l'intérêt d'un jeune individu de s'investir modérément dans la reproduction actuelle afin de se préserver pour les événements de reproduction suivants. En revanche, un individu âgé aura tout intérêt à s'investir au maximum car ses chances de se reproduire à nouveau seront faibles. Par exemple, chez le fou à pieds bleus, *Sula nebouxii*, les individus âgés avec des perspectives de reproduction réduites vont accroître leurs efforts dans la reproduction lorsque leurs perspectives de survie sont menacées (activation expérimentale du système immunitaire), alors que les jeunes individus ayant de bonnes perspectives de reproduction s'investiront moins (Velando et al. 2006).

Enfin, les patrons liant âge et succès reproducteur peuvent aussi être expliqués par l'hypothèse de sélection qui se réfère à la disparition précoce des reproducteurs de basse qualité si leur survie est inférieure à celle des reproducteurs de haute qualité (Curio 1983; Forslund and Pärt 1995). Il est donc nécessaire d'interpréter les relations entre âge et performance reproductrice avec précaution.

Sexe

Les sexes sont définis par les différences dans le type de gamètes qu'ils produisent. La femelle produit un petit nombre de gros gamètes, généralement peu mobiles alors que le mâle produit en grand nombre de petits gamètes très mobiles. Cette asymétrie dans la taille des gamètes est appelée anisogamie. Les sexes expriment des différences fondamentales dans leurs traits phénotypiques. Les causes de ces différences font débat, notamment dans la part de variance expliquée par l'anisogamie, les effets environnementaux et les effets stochastiques (Schärer et al. 2012; Ah-King 2013; Kokko et al. 2013). Cependant un lien évident est reconnu entre les régimes d'appariements et la divergence des comportements entre les deux sexes (Andersson 1994; Dunn et al. 2001). Chez les espèces polygames, le dimorphisme entre les sexes est très prononcé tant dans les comportements que dans la morphologie. Généralement les mâles font face à une compétition intra-sexuelle plus forte pour l'accès au partenaire, exhibent des caractères secondaires sexuels extravagants et coûteux et sont peu investis dans les soins parentaux. Les femelles sont le sexe sélectif lors de l'appariement et

s'investissent davantage dans la descendance. Mais ces rôles peuvent être inversés chez certaines espèces (cf. polyandrie, Jenni and Collier 1972; Oring and Lank 1982). Chez les espèces monogames, un dimorphisme sexuel peut aussi exister en particulier au sein des espèces qui présentent des rôles bien définis pour chaque sexe. C'est-à-dire que les tâches liées à la reproduction (défense du territoire, incubation, nourrissage, etc.) sont sexe-spécifiques. Au contraire, chez les espèces monogames présentant des rôles reproducteurs similaires entre les sexes, ce dimorphisme sexuel est peu marqué, voir absent.

Cependant, l'étude des différences de traits reproducteurs liés au sexe est globalement biaisé en faveur des espèces polygames (Kim et al. 2011). Chez les espèces monogames où les sexes présentent des rôles semblables, il n'est pas rare que le sexe ne soit pas pris en compte, ou encore qu'un seul des deux sexes soit considéré. Pourtant, même chez ces espèces, les coûts liés à la reproduction sont susceptibles d'être différents (Tavecchia et al. 2001). Ce différentiel de coût peut conduire à des pressions de sélections différentes et donc à des différences dans les traits reproducteurs entre les sexes.

Variabilité des traits reproducteurs et étapes de la reproduction

Les traits individuels liés à la reproduction sont divers tels que la condition physique des individus, leurs caractéristiques morphologiques et physiologique, et leurs comportements. L'ensemble de ces traits va déterminer les performances reproductrices d'un individu ainsi que sa compétitivité. Ces différents traits liés à la reproduction pouvant être impliqués dans différentes étapes de la reproduction vont déterminer le succès reproducteur de l'individu. Il est donc indispensable d'étudier l'ensemble des étapes de la reproduction pour comprendre les mécanismes sous-jacents menant à cette hétérogénéité du succès reproducteur.

Ainsi nous pouvons nous demander quelles sont les étapes critiques au cours de la saison de reproduction et quels sont les traits individuels associés les plus déterminants quant à la réussite de la reproduction ? Ces étapes et ces traits sont-ils les mêmes pour les deux sexes ? Ou encore, comment l'âge influence ces traits reproducteurs. Agit-il sur la totalité des traits ? Et dans le même sens ?

Pour répondre à ces questions je me suis intéressé à une espèce longévive monogame, et présentant un fort investissement parental chez les deux sexes, système principalement présent chez les oiseaux. Les mâles et les femelles sont ainsi impliqués dans les différentes étapes de la reproduction, ce qui nous permet d'étudier les différences liées au sexe dans les traits reproducteurs au cours de la saison de reproduction. Quant à la longévité, elle permet de répondre à la question de la plasticité des traits reproducteurs au cours de la vie de l'individu.

La définition de la saison de reproduction diffère entre les études. Chez les oiseaux, la saison de reproduction correspond généralement au cycle de nidification (Etterson et al. 2011). Ainsi, la saison débute avec la construction du nid et un individu est considéré comme faisant partie du « pool » de reproducteurs dès qu'il est observé réaliser cette tâche. À mon sens la saison de reproduction comprend deux composantes : l'accès à la reproduction et la reproduction *per se*. L'accès au partenaire sexuel ou encore l'accès au site de nidification sont effectivement des étapes déterminantes quant au succès de la reproduction. La réduction de la saison de reproduction au cycle de nidification des reproducteurs est probablement la résultante d'une difficulté dans l'observation des comportements liés à l'accès à la reproduction (Etterson et al. 2011). Néanmoins elle conduit à l'exclusion du « pool » de reproducteurs des individus qui ont échoué lors de l'accès à la reproduction. C'est pourquoi nous considérerons ici la saison de reproduction comme débutant dès l'accès au partenaire sexuel et se terminant avec l'envol des jeunes.

Traits reproducteurs impliqués au cours de la saison de reproduction : quelques exemples

Accès au partenaire sexuel

L'étape de l'accès au partenaire sexuel est déterminante puisque non seulement un individu ne peut se reproduire sans partenaire, mais aussi parce que les compétences reproductrices du partenaire sexuel sont cruciales quant à l'issue de la reproduction. Chez les espèces monogames où les deux sexes contribuent à l'investissement parental, on s'attend à ce que mâles et femelles expriment de la sélectivité dans leur choix du partenaire. Il s'agit alors d'un choix mutuel (Andersson and Norberg 1981; Parker 1983; Jones and Hunter 1993; Bergstrom and Real 2000). Durant le processus d'acquisition du partenaire, les individus expriment généralement des traits coûteux dans leur production reflétant honnêtement leur

qualité reproductrice (Andersson 1994). Il s'agit des signaux sexuels. Ils peuvent être morphologiques, auditifs, comportementaux ou encore olfactifs. Si ces signaux sont honnêtes, leur variabilité permet aux individus de discriminer leurs congénères en fonction de leur performance reproductrice.

Sélection du site de reproduction

L'environnement étant hétérogène, la qualité des différents habitats dédiés à la reproduction est susceptible de varier. Par exemple, l'exposition aux prédateurs, aux parasites, aux intempéries, la proximité des aires d'alimentation offrent des perspectives différentes quant à la réussite de la reproduction. Les individus devraient donc sélectionner les habitats de meilleures qualités afin de maximiser leur aptitude phénotypique. Seulement la disponibilité des habitats de haute qualité est généralement limitée, ce qui conduit à de la compétition entre les individus. Chez certaines espèces, et principalement chez les mâles, les comportements territoriaux et de dominance provoquent l'exclusion d'individus de certaines zones conduisant à une distribution idéale despotiques des individus sur les aires de reproduction (Fretwell 1969; Fretwell and Calver 1969). Il en résulte une monopolisation des territoires de qualité par les individus les plus compétitifs, et l'installation des individus de moindre compétitivité dans des habitats défavorables. Taille, agressivité, et expérience de l'individu sont des traits individuels connus comme étant impliqués dans la compétitivité et les comportements territoriaux des individus (Margalida and Bertran 2005; Serrano et al. 2007) et sont donc des traits relatifs à la reproduction impliqués dans la sélection du site de reproduction.

Ponte et incubation

Au sein des populations, les individus expriment des comportements de ponte et d'incubation variés ayant des conséquences sur la réussite de la reproduction (Lack 1968). Le choix de la taille de la ponte impacte directement la condition et la survie des jeunes (Monaghan et al. 1995; Monaghan and Nager 1997). De même, la décision de quand initier l'incubation influence le succès d'éclosion et le phénotype des poussins (Stoleson and Beissinger 1995). Par exemple, chez les espèces dont la couvée est composée de plusieurs œufs, l'incubation peut être initiée dès la ponte du premier œuf ou seulement une fois tous œufs pondus. Le patron général est que plus l'incubation débute tôt, plus le succès d'éclosion sera élevé (Wang and Beissinger 2009). Enfin, la présence au nid et le partage du temps

d'incubation sont variables au sein d'une population. Chez le manchot pygmée, *Eudyptula minor*, plus la fréquence des rotations d'incubation entre parents est courte plus la probabilité de succès d'éclosion sera grande (Kerry 1999).

Elevage des jeunes

Par les soins parentaux, les individus augmentent la survie de leur descendance et ainsi leur propre aptitude phénotypique. Ces soins sont variés tels que l'approvisionnement alimentaire, la protection contre les parasites par les comportements d'épouillage, la protection contre les prédateurs (Klug and Bonsall 2014). La variabilité de l'approvisionnement alimentaire des jeunes est certainement le soin parental associé à l'élevage de jeunes le plus étudié. Il apparaît une variabilité de comportements de nourrissage entre les individus que ce soit dans la fréquence d'approvisionnement, la quantité et la qualité de la nourriture délivrée à chaque nourrissage, etc. (Wendeln and Becker 1999; O'Dwyer et al. 2007; Mullers and Tinbergen 2009), et qui serait due à une performance différentielle entre les individus dans la recherche alimentaire. Cette variabilité d'approvisionnement des parents montrée comme ayant des conséquences sur la condition et la survie des jeunes, impacte directement leur aptitude phénotypique.

Sélectivité des étapes de reproduction

Chaque étape de la reproduction a le potentiel d'agir comme un filtre, excluant les individus les moins performants du « pool » de reproducteurs. Selon les espèces, les différentes étapes sont plus ou moins sélectives selon leur coût énergétique (Clutton-Brock 1988), conduisant à divers patrons de sélectivité au cours de la saison de reproduction (Arnold and Wade 1984). Par exemple, la sélectivité peut être constante au cours du temps (Fig. 1A). Si l'accès au partenaire sexuel conduit à une forte exclusion des individus, la sélectivité sera intense en début de reproduction (Fig. 1B). A l'opposé, la sélectivité peut se produire en fin de reproduction si la période d'élevage est très coûteuse comparé au reste de la saison de reproduction (Fig. 1C). Par conséquent, pour comprendre l'hétérogénéité dans la réussite de la reproduction entre les individus au sein d'une population, il est nécessaire de comprendre à quel niveau s'effectue la sélection.

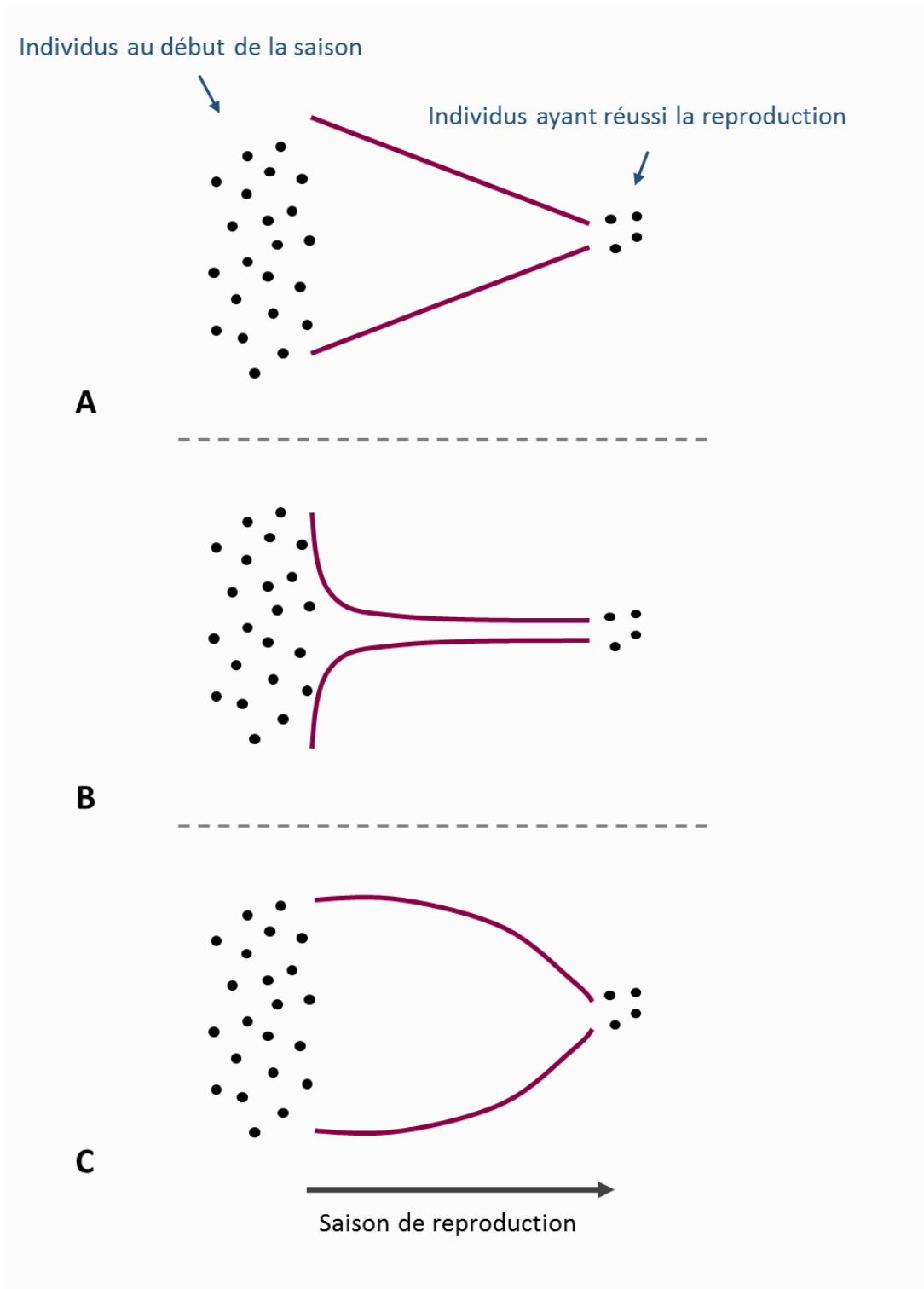


Figure 1 : Patron théorique de sélection des individus reproducteurs au cours de la saison de reproduction. A) Sélectivité constante des étapes de reproduction. B) Sélectivité très élevée en début de reproduction puis nulle C) Sélectivité élevée seulement en fin de saison.

Modèle biologique : le flamant rose (*Phoenicopterus roseus*)

Biologie générale

Le flamant rose est l'espèce la plus répandue géographiquement parmi toutes les espèces de flamants. On le trouve particulièrement dans les eaux saumâtres peu profondes et dans les étangs et lagunes salées de Méditerranées, de l'Afrique de l'ouest, du sud et de l'est, ainsi qu'au sud et au sud-ouest de l'Asie. Son régime alimentaire est composé d'invertébrés aquatiques, les artémies constituant leur principal apport alimentaire. Le flamant rose est une espèce coloniale, monogame et longévive avec un âge maximum enregistré de 40 ans dans la nature et de 68 ans en captivité (Johnson and Cézilly 2007). Cependant les estimations du taux de survie suggèrent que les flamants sauvages pourraient vivre jusqu'à 50 ans (Cézilly et al. 1996; Tavecchia et al. 2001). Les individus se reproduisent sur des îlots et forment des colonies très denses et constituées de plusieurs milliers d'individus. Un dimorphisme sexuel de taille est observé chez cette espèce, les femelles étant en moyenne plus petites que les mâles.

Les individus atteignent la maturité sexuelle à 3 ans, cependant la reproduction est rare à cet âge-là. Les flamants expriment une variabilité de l'âge à la première reproduction allant de 3 à environ 10 ans avec un pic de recrutement à 7 ans (Pradel 2005). Malgré le fait qu'il s'agisse d'une espèce monogame, la fidélité au partenaire sexuel d'année en année est proche de 0% (Cézilly et al. 1997). Ainsi afin de trouver un partenaire avant la saison de reproduction, mâles et femelles effectuent des parades nuptiales en groupes de quelques dizaines à plusieurs centaines d'individus. Ces parades sont constituées de séquence de mouvements formant des chorégraphies plus ou moins stéréotypées. La couleur du plumage semble impliquée dans le choix du partenaire, car durant la saison des parades, les individus intensifient le rose de leur plumage en étalant leur sécrétion uropygienne composée de caroténoïdes (Amat et al. 2010). En plus de son rôle dans l'accès au partenaire sexuel, la parade nuptiale est suspectée d'avoir un rôle dans la synchronisation temporelle de la reproduction entre individus.

La femelle pond un seul œuf. La période d'incubation dure 29 jours et l'envol des jeunes se fait autour de 80 jours. Les deux parents sont impliqués dans l'incubation et dans le nourrissage des jeunes, et s'organisent à l'aide de rotations. A environ 10 jours les poussins quittent l'îlot de reproduction, pour aller dans l'eau et former des crèches pouvant aller jusqu'à plusieurs milliers d'individus.

Programme de baguage et de suivi

Depuis 1977 la colonie de flamants roses établie en Camargue (France), fait l'objet d'un suivi à long-terme. Après chaque événement de reproduction, entre 600 et 900 poussins sont bagués avec des bagues portant un code alphanumérique unique (Fig. 2), ce qui représente entre 7 et 30% de la totalité de la crèche. Lors du baguage, la masse et la longueur du tarse du poussin bagué sont relevés.

La bague de l'individu peut être par la suite, lue à distance, informant de l'identité, de l'origine et de l'âge de l'individu. La saison de reproduction est le moment où l'effort de lecture est le plus fort. En Camargue, une tour d'observation a été construite sur l'étang du Fangassier pour permettre le suivi quotidien de la colonie (Fig. 3). Les lectures de bagues couplées aux méthodes de capture-marquage-recapture, permettent d'estimer les paramètres démographiques de la colonie tels que les probabilités de survie, de reproduction et de mouvements des flamants roses.



Figure 2 : Poussin venant d'être relâché après avoir été bagué. Colonie du Fangassier (Camargue)



Figure 3 : Installation de la colonie sur l'îlot du Fangassier (Camargue), et sa tour d'observation

Variabilité des traits reproducteurs chez le flamant rose

Facteurs environnementaux

Le succès reproducteur est variable entre individu et entre saisons de reproductions. Par exemple, en 1987 le succès reproducteur de la colonie de Camargue a été estimé à 12.7% alors qu'en 1969 la colonie a atteint une réussite de plus de 85% (Johnson and Cézilly 2007). Cette forte variabilité de succès entre saisons de reproduction suggère fortement la responsabilité d'effets environnementaux. Entre les facteurs environnementaux connus comme ayant une influence sur la productivité de la colonie, la dynamique hydrologique locale est considérée comme l'un des plus importants (Cézilly et al. 1995; Béchet and Johnson 2008; Béchet et al. 2009; Schmaltz et al. 2011). En effet l'assèchement des étangs entourant l'îlot de reproduction conduit à l'exposition de la colonie aux prédateurs terrestres pouvant mener les flamants à abandonner la reproduction. En revanche, des niveaux trop élevés conduisent à l'inondation des nids situés en périphérie sur l'îlot. En Camargue, les fortes pluies, et les épisodes venteux sont en grande partie responsables de la variation du niveau d'eau entourant l'îlot. Ces intempéries sont aussi susceptibles de rendre difficile les activités de recherche alimentaire des reproducteurs, les poussant parfois à abandonner leur nid (Johnson and Cézilly 2007) .

Facteurs intrinsèques

L'âge des flamants roses est une importante source de variation du succès reproducteur (Johnson and Cézilly 2007). Pour les deux sexes, on observe une augmentation du succès reproducteur avec l'âge (Fig. 4). L'effet de l'âge a été plus précisément regardé au cours de la saison de reproduction. Une étude menée il y a 20 ans chez le flamant rose (Cézilly et al. 1997) a montré une homogamie pour l'âge dans la formation du couple reproducteur. Celle-ci serait le résultat d'un choix directionnel envers les individus les plus âgés et donc les plus expérimentés. Cependant, l'âge maximum des individus de l'échantillon analysé était de 15 ans seulement, et cette relation entre l'âge des partenaires mériteraient d'être à nouveau investiguée avec une gamme d'âge plus représentative.

L'installation des individus sur les sites de reproduction ne se fait pas non plus aléatoirement en fonction de l'âge. Rendón et al. (2001) ont étudié la répartition des flamants en fonction de l'âge sur deux sites voisins de reproduction différant dans leur qualité. Le premier offre une protection plus efficace contre les prédateurs et un succès reproducteur plus élevé que le second. Leur étude a montré que les jeunes individus se reproduisaient en majorité sur le second site mais seulement après avoir essayé de s'installer sur le site de plus haute qualité. Ceci suggère une exclusion des sites de haute qualité des jeunes individus par leurs aînés, soulignant une distribution idéale despotique des individus sur les habitats de reproduction.

Enfin, durant la nidification, la probabilité de succès d'éclosion augmente avec l'âge, alors qu'une fois le poussin né, l'âge des parents ne semble plus avoir d'effet sur la probabilité d'élever un jeune jusqu'à l'envol (Schmaltz et al. 2011).

Chez les flamants, l'âge apparaît ainsi comme un facteur influençant de nombreux comportements impliqués dans la reproduction. L'amélioration des performances reproductives et l'augmentation de l'habileté compétitive avec l'âge étant probablement la conséquence de la maturation physique des individus et de l'expérience acquise (Pradel et al. 2012).

Mâles et femelles participent à toutes les étapes de la reproduction de sorte que leurs rôles sont peu différenciés. Ils expriment cependant quelques différences dans leurs caractéristiques reproductrices. Les femelles se reproduisant avant 7 ans affichent une mortalité élevée reflétant probablement une capacité réduite à faire face aux coûts physiologiques de la reproduction (Tavecchia et al. 2001). Durant la période d'élevage, les mâles semblent investir plus d'énergie dans le nourrissage et cela est d'autant plus fort que le poussin est âgé (Cézilly et al. 1994; Rendón et al. 2014). Toujours durant l'élevage des jeunes, les comportements associés au nourrissage diffèrent entre mâle et femelle. Les deux sexes n'utilisent pas les mêmes secteurs d'alimentation, et les mâles délivrent de la nourriture d'un plus haut niveau trophique à leur poussin (Rendón et al. 2014).

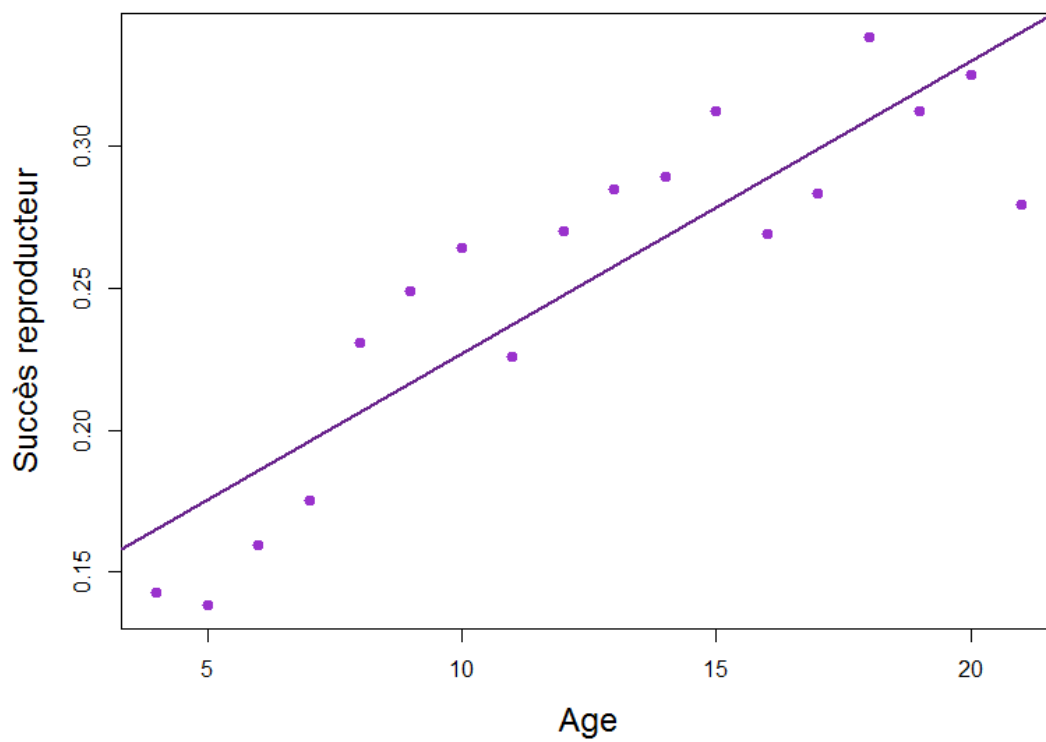


Figure 4 : Succès reproducteur en fonction de l'âge des individus de la colonie de Camargue, calculé à partir de 15 cohortes différentes (individus vus en succès / individus vus en incubation)

Les objectifs de la thèse

Le but de cette thèse est d'explorer l'effet de l'âge et du sexe sur la reproduction des flamants roses, et ce, à plusieurs étapes de la reproduction en couplant des approches utilisées en écologie comportementale et en dynamique des populations.

Dans un premier temps, nous nous sommes intéressés à l'accès au partenaire sexuel. L'homogamie pour l'âge entre les partenaires sexuels peut résulter d'une préférence liée à l'âge (Kokko and Lindstrom 1996) ou liée à un attribut étroitement associé à l'âge comme l'expérience (Jouventin et al. 1999). Les parades nuptiales étant un moment privilégié d'échanges d'informations entre individus, on s'attend à ce qu'il y ait un lien entre signaux sexuels et âge des individus, menant à cette homogamie pour l'âge. Nous nous sommes donc interrogés sur la variabilité entre individus des comportements associés aux parades nuptiales, et s'il existait un lien entre cette variation et l'âge des individus, potentiellement façonné par le sexe. Pour cela nous avons relevé la fréquence des différents comportements, décrit la structure de séquences comportementales, et mesuré la coloration des oiseaux, puis nous avons regardé comment l'âge et le sexe des individus influaient sur ces composantes (chapitre 1). Ensuite nous avons poussé plus loin l'exploration de la structure des séquences comportementales des parades nuptiales en nous intéressant à leur complexité (chapitre 2). La complexité des parades est considérée comme indicateur des performances motrices des individus (Hebets et al. 2011; Barske et al. 2011), et par conséquent comme signal honnête de la qualité reproductrice des individus. Nous avons donc étudié la variabilité de cette complexité entre individus, et comment elle pouvait être expliquée par l'âge et le sexe des individus. Par la suite, nous avons examiné l'implication de la complexité des séquences comportementales dans l'appariement et sur l'accès à la reproduction.

Dans le chapitre 3 nous nous sommes intéressés à la fin de la reproduction en nous focalisant sur la condition corporelle du poussin avant l'envol, mesurée lors du baguage. Les comportements liés à la période d'élevage ont été déjà étudiés chez cette espèce, mais l'influence des caractéristiques intrinsèques des parents sur la condition corporelle du poussin n'a jamais été regardée. Chez les espèces longévives, la condition du poussin peut avoir des conséquences à court et à long terme sur sa vie future (Lindström 1999; Nowicki et al. 2000; Lummaa 2003), en influençant positivement sa survie après l'envol, sa probabilité de recruter dans la population, sa future attractivité sexuelle et ses futures performances reproductives,

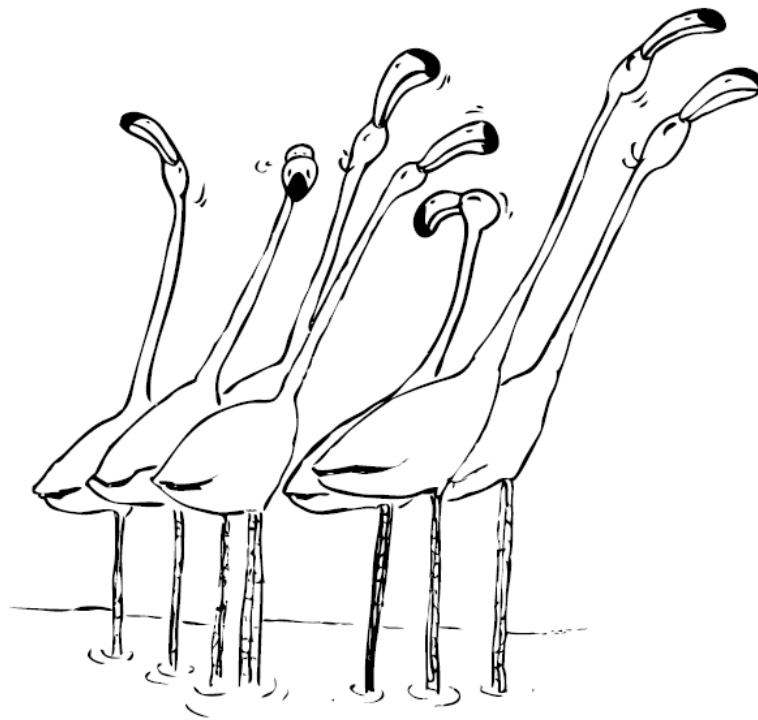
etc. Ainsi les parents devraient optimiser la condition des poussins afin d'optimiser leur propre aptitude. Nous avons examiné si les parents plus âgés et donc plus expérimentés produisaient des poussins en meilleure condition que les jeunes parents. De même, la condition avant l'envol pouvant influencer les futures compétences reproductrices, nous avons testé l'influence de la condition des parents lorsqu'ils étaient poussin sur la condition de leur poussin, et ce en interaction avec leur âge en tant que parents. Et comme précédemment, nous avons regardé si ces relations entre condition des poussins, âge des parents et leur condition lorsqu'ils étaient poussin étaient différentes en fonction du sexe des parents.

Dans le chapitre 4, nous nous sommes placés à l'échelle de la vie de l'individu. Nous avons étudié la variabilité de l'âge à la première reproduction, paramètre démographique clé façonnant les stratégies d'histoire de vie (Stearns 1976). En effet, l'âge auquel un individu se reproduit pour la première fois est susceptible d'influencer la survie et le succès reproducteur global de l'individu (McGraw and Caswell 1996; Oli et al. 2002; Krüger 2005; Aubry et al. 2009). Le but de ce chapitre est donc de comprendre quelles sont les relations entre âge à la première reproduction, durée de vie et succès reproducteur global et comment ces relations peuvent différer entre les deux sexes.

Chapitre 1

In prep.

Sex-related differences in communal courtship displays of greater flamingos, *Phoenicopterus roseus*



Sex-related differences in communal courtship displays of greater flamingos,

Phoenicopterus roseus

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Abstract

In monogamous species without differentiation of sexes concerning parental care, monomorphic signaling is expected in a context of mate choice. However, because of fundamental differences between sexes due to sexual selection, subtle difference in sexual behaviour could remain. We examined sex-related differences in courtship display of the Greater flamingo (*Phoenicopterus roseus*), a monogamous species where both parents share parental duties. For that we recorded behavioural sequence of courtship display for 100 individuals, as well as their plumage coloration known to be an important and honest signal of reproductive quality. We found that courtship displays followed the same pattern regardless of sex but with slight differences. Interactions between males were more frequent than between females and increased with age. In addition, females exhibit a more intense pink coloration. Our study suggests therefore a higher intrasexual competition in males than in females in the Greater flamingo, and that differences in sexual behaviors involved in mate choice could remain even if a priori there is no or slight differentiation in parental roles between sexes.

Introduction

When the two sexes contribute equally to parental care, both of them are predicted to show selectivity in mate choice, leading to mutual mate choice (Darwin 1871; Andersson and Norberg 1981; Parker 1983; Jones and Hunter 1993; Johnstone et al. 1996; Bergstrom and Real 2000). This is particularly true of monogamous species, when the encounter rate between potential mates is high (Kokko and Johnstone 2002).

Parents can share parental care in two ways. First males and females can express sex-specific task specialization as parental care includes various behaviors (Barta et al. 2014). For instance in raptors, females typically incubate the eggs and chicks, while males hunt for food for their mate and their offspring (Andersson and Norberg 1981). Consequently, necessary reproductive skills could differ between sexes, so that males and females should express different sexual signals reflecting their sex roles and their reproductive quality during mate choice process.

Second, parents can divide parental care equally, by sharing each reproductive task. In this case, reproductive skills necessary to breed successfully are similar between sexes, what should lead to similar selective pressures in both sexes in the context of sexual selection, and, thus, to sexual monomorphic signaling (Kokko and Johnstone 2002; Kraaijeveld et al. 2007). Hence, divergence in sexual behaviors between sexes during mate choice process should be minimal in strictly monogamous species where parent duties are shared equally. However, we can expect that some fundamental differences between sexes linked to the action of sexual selection contribute to maintain differences in sexual behaviors between sexes (Kelley 1988; Schärer et al. 2012). For example, only females lay and invest in egg in oviparous species, or performed gestation and lactation in mammal's species, whereas only males are facing sperm competition.

In that respect, flamingos (Phoenicopteridae) might be a particularly well-suited species to investigate sex-related variation in courtship behavior. In all flamingo species, birds are strictly monogamous and both sexes share each reproductive tasks so that mutual choice is strongly expected in these species. Nevertheless, flamingos express a sexual dimorphism, females being generally smaller than males, suggesting that sexual differences remain. Individuals perform conspicuous mixed-sex group (or communal) displays, which are supposed to stimulate synchronous breeding and facilitate pair formation. Up to several

thousand individuals then form dense aggregations and perform in synchrony a variety of 20 movements in a more or less stereotyped succession for several hours per day during the prebreeding period. This kind of complex courtship display offer therefore a good opportunity to investigate sex-related differences in sexual behavior in a monogamous species where sexual roles seem *a priori* similar. Several studies described courtship displays and sex-related behavior, but only in captive flamingo. Indeed, in captive Caribbean flamingos, displays are initiated by males, which display longer and more vigorously than females (Rooth 1965; Kahl 1975) while O'connell-Rodwell et al. (2004) did not detect any differences between sexes in the frequency of displays in an other captive flock. In addition, although there are slight differences, the general pattern of behavioral sequences is similar between sexes in Caribbean and Greater flamingo (Studer-Thiersch 1975). However even if studies in captive individuals give precious information on animal behavior, differences can be expected with wild populations, especially in a high colonial species like flamingos. For example, from studies on captive flocks, the greater flamingo was described as monogamous, with pair bonds extending over consecutive seasons (Studer-Thiersch 1975; Pickering 1992) although wild birds express a mate-switching rate between consecutive breeding seasons of 98.3% (Cézilly and Johnson 1995).

We therefore aimed to investigate sex-related differences in sexual behaviors in wild flamingos to know if a monogamous mating system without differentiation of sexes, leads to a monomorphic signaling in a context of mate choice or if subtle differences persist. For that we investigated sex-related differences in courtship behavior of wild greater flamingos (*Phoenicopterus roseus*), a long-lived colonial and monogamous species displaying multimodal signals. The fact that individuals divorce systematically between two consecutive breeding seasons means that individuals must invest each year in group displays in order to find a new mate and be able to breed. During group displays, greater flamingos typically exhibit their underwing colorations which offer a bright contrast between carotenoid-based crimson remiges and melanin-based black ones. Carotenoid-based plumage is dependent on diet (as birds cannot synthesize carotenoids *de novo*), and is thought to reflect current condition. Moreover, in birds, female carotenoid-based plumage has been shown to inform about its capacity to invest in progeny via maternal effects (McGraw et al. 2005). Indeed carotenoids present in egg yolks and transmitted by female, play a central role during embryo development and at hatching (Möller et al. 2000; Saino et al. 2003; Romano et al. 2008;

Newbrey and Reed 2009). In the greater flamingo carotenoid-based plumage is known to be an important and honest signal of reproductive quality during courtship display (Amat et al. 2010; Freeman et al. 2016). We therefore take into account plumage color as a sexual signal in addition to courtship dance in our study. During courtship display flamingos express aggressive behaviors between same-sex individual (personal observations) which could reflect intrasexual competition for mate access (Weir et al. 2011; Rosvall 2011).

We first investigated sex-related variation of courtship display, focusing on structure of behavioral sequences and posture occurrences. We examined also the frequency of aggressiveness during courtship display to assess the intrasexual competition occurring in both sexes. As sex differences in sexual display can take place along a maturation process, we evaluated the influence of sex on courtship dance in interaction with age. We then explored variations of carotenoid-based plumage coloration between sexes.

Methods

Behavioral observations

Observations were made in the Camargue, Southern France, one of the most important breeding sites of the Greater flamingo in the Mediterranean region (Balkız et al. 2007), during two consecutive seasons of courtship displays (November to March in both 2014 and 2015). Since 1977, on average, 12 % (7-30%) of the chicks fledged in the Camargue have been marked with PVC plastic rings engraved with a three or four digit alphanumeric code (Johnson 1997), allowing individual identification at distance and providing information about the age of individuals. In addition, the sex of ringed birds has been regularly ascertained, either through behavioral observations or through blood sampling and molecular analyses (Balkız et al. 2007). Ringing and sample collection of greater flamingo chicks were authorized through the personal permit (number 405) of Alan Johnson and Arnaud Béchet delivered by the Centre de Recherche sur la Biologie des Populations d'Oiseaux (CRBPO, Muséum national d'histoire naturelle, France).

Using a FullHD video camera equipped with a 60x zoom (20-1200 mm, Panasonic Lumix FZ72), we recorded the behavior of ringed individuals during displays. On each occasion, we attempted to follow a single displaying individual for up to five minutes. However, many observations were interrupted before the 5-min time-period due to movements of individuals

or because the focal bird stop displaying. We thus selected 100 focal-individual sequences of different individuals (50 females, 50 males) where the display behavior had been recorded continuously for five minutes. For each individual sequence, we recorded the group size of the courtship display, the hour of the day and the date (because such variables were previously found to influence display behavior in flamingos (Johnson and Cézilly 2007)). Behavioral sequences were then coded using the JWatcher software (Blumstein and Daniel 2007). Following previous studies of the display repertoire of the Greater flamingo (Johnson and Cézilly 2007), nine different postures were recognized (Table 1). We also recorded aggressive behaviors displayed and initiated by the focal individual. Aggressive behaviors correspond to an aggressive contact between two individuals where a bird extended its neck and pecked at another bird. The time spent in *head flagging* and *preening* was recorded (in seconds). The seven other behaviors and aggressive behaviors were considered as instantaneous. The frequency of *false feeding* and *twist-preen* were recorded. As *wing salute*, *inversed wing salute*, *scratching*, *marching*, *wing-leg-stretch* movements and aggressive behaviors are relatively rare over courtship boots, we focused on the absence/presence of these behaviors rather than on their frequency.

Neck color estimate

We estimated the color of neck feathers as a proxy of the overall intensity of plumage coloration. We scored the color intensity of displaying bird following Amat et al. (2010) but using four color levels for more precision: (0) white (1) very pale pink (2) pale pink and (3) pink. There was a close agreement between the two observers (CH and CP) in the assignment of neck color scores to 26 individual with a good repeatability (intra-class correlation between CH and CP: $r = 0.805$, $p < 0.001$). Although we used a coarse color scoring method, human vision may provide a valid proxy for avian perception of inter-individual differences in plumage coloration (Hill et al. 1999; Seddon et al. 2010).

Statistical analyses

We first investigated whether the sex of individuals had an influence on the general structure of courtship dance. For that, we combined all recorded sequences of the same sex in a unique sequence of 250 minutes. Using the JWatcher software we inserted a break code between each sequences in order not to create non-existent transitions between movements so that

the order in which the sequences were put together did not influence the result of the analysis. For each sex we calculated the probability of posture occurrence. We then arranged the data in one transition matrix for each sex separately. In order to reduce the number of cells that contained zeros, we removed the *marching* and *wing-leg stretch* movements which were very rare (probability of occurrence < 0.01 %), from the dataset by replacing them with a break code. We used log-linear models implemented in the MASS package of R to calculate expected transition matrix under the hypothesis of random transitions between movements in order to see which transitions were significant (Bakeman and Gottman 1997). A transition implies a change, so the structural zeros in matrices (i.e. empty diagonal) were taken into account for the calculation of expected transitions matrices. For each transition we then used z-scores to identify which transitions did not result from a random process. We finally compared the behavioral transition matrix of both sexes using the Mantel's permutation test for similarity of the two matrices using the *ape* package in R (Hemerik et al. 2006; Paradis et al. 2016). The Mantel's permutation test permutes the rows and columns of the two matrices randomly and calculates a Z-statistic. The significance of the correlations was based on 2,000 permutations in order to assess how extreme the actual Mantel's Z value was, relative to the Z values generated under the null hypothesis of no association between the compared matrices (Dietz 1983).

Then we investigated if the age and sex of individuals had an influence on the occurrence and duration of courtship movements within courtship bouts. During the 5 minutes focal bouts, individuals spent on average 192 ± 6 seconds performing *head flagging* and 56 ± 4 seconds *preening* so that 83% of display duration was composed of these two postures. Moreover, *head flagging* and *preening* durations were strongly negatively correlated ($r = -0.87$, 95% CI = $-0.92 - -0.81$) such that these two variables were not independent. Therefore, we performed multivariate multiple linear regression to determine if there were significant changes in *head flagging* time and *preening* time according to sex and age and their interaction. The complete model contained the interaction between sex and age, the quadratic effect of age (thus testing for a potential effect of senescence), as well as the hour of the day, the date of the year, the year and the interaction between year and date as covariates. We used the *mStep* function from the *qtlmt* package in R (Cheng 2015) to drop terms from the model sequentially, using Akaike Information Criterion (AIC) (Cheng, 2013). We conducted stepwise procedures for backward stepwise regression, starting with all of the

covariates listed above. We report the model with the lowest AIC from the stepwise procedure.

Finally, we investigated the influence of the age and sex on the occurrence of courtship movements within courtship bouts. We first used from generalized linear model with a Poisson distribution to analyze the occurrence of *false feeding*, but model assumptions (normality and homoscedasticity of data and residuals) were not meet. To solve it, we applied a square root transformation and we used linear model. Variation in the occurrence of *twist preen* was analyzed from generalized linear model with a Poisson distribution corrected for overdispersion. We used generalized linear model with a binomial distribution to analyze the occurrence of *wing salute*, *inverse wing salute*, *scratching*, *marching* and aggressive acts during courtship bouts. For each movement, the complete model contained the interaction between sex and age, the quadratic effect of age, and hour of the day, date of the year, year, and the interaction between year and date as covariates. In the same way, we used linear models to test the influence of sex and age on neck feather color, with the complete model including the same variables as above. Model assumptions (i.e. normality and homoscedasticity of residuals) were checked. From the complete model we derived a set of all possible submodels. As date of the year and size of display group were significantly correlated ($r = -0.475$, 95%CI = -0.643; -0.274), for all complete models, we did not include both variables to avoid collinearity. In this paper we showed only results with models containing date of the year, as the same analyses run with the group size as co-variable provided similar results.

Following recent recommendations to produce model estimates comparable between and within studies (Schielzeth 2010; Grueber et al. 2011), we standardized all explanatory variables by centering and dividing by two standard deviations using the *arm* package (Gelman et al. 2015). To prevent overparameterization, we respected the sample size rule-of thumb of 10 : 1 subjects to predictors in multiple regression (Harrell 2015). Model selection was based on Akaike Information Criterion corrected for small sample size (AICc) (Burnham and Anderson 2002). When several models were within a $\Delta AICc$ of 2 from the best model, we employed a model averaging approach, using the so-called zero method (Burnham and Anderson 2002) implemented in the *MuMIn* package of R (Bartoń 2015) on models within two points of AICc from the best one. This allows accounting for model selection uncertainty in order to obtain robust parameter estimates (Grueber et al. 2011).

All analyses were conducted with R 3.0.3 (R Development Core Team 2014).

Results

General structure of courtship display

When combining behavioral sequences by sex over a total duration of 250 minutes, posture occurrences seemed to follow the same pattern regardless of sex (Fig. 1). However, females performed more *preening* and *false feeding* postures than males.

Out of 42 possible transitions between movements, 29 were observed in females but only 11 of these were statistically more frequent than expected by chance at the 0.05 probability level (Fig. 2a). In males, 27 different transitions were observed and only 10 transitions were statistically more frequent than expected by chance (Fig. 2b). 10 significant transitions were common to both sexes. Occurrences of courtship movements and their transition probability indicate that the underlying pattern of display was similar between sexes. In addition, transitional matrices were highly similar between sexes (Mantel test: $Z = 249772$, $p = 0.001$).

Posture duration and occurrence within 5 min courtship bouts

The final model explaining *head flagging* and *preening* duration variability included the age and the sex of individuals and their interaction as explanatory variables — none of the other covariates significantly improved the model (*i.e.*, no other covariates reduced AIC). In female, *head flagging* and *preening* duration remained constant with age (*head flagging*: $\beta = -1.06$, 95% CI = $-2.98 - 0.85$; *preening*: $\beta = 1.20$, 95% CI = $-0.38 - 2.78$). In contrast, *head flagging* duration increased with age in males while *preening* time decreased (*head flagging*: $\beta = 3.15$, 95% CI = $1.28 - 5.03$; *preening*: $\beta = -2.46$, 95% CI = $-3.79 - -1.13$; Fig 3). 4 yrs. old males spent around 161 seconds to do head flagging and 79 seconds to preen while 30 yrs. old males spend around 249 seconds to do head flagging and 10 seconds to preen. The duration of *head flagging* and *preening* in females were more close to those of young males (*head flagging*: mean = 181.14 ± 8.03 SE, *preening*: mean = 65.48 ± 6.71 SE)

For *scratching* probability, 12 models were retained (Table 2), but model averaging did not indicate any significant effect (Table 3). The top model set attempting to explain variation in the occurrence of the five other behaviors (*false feeding*, *wing-salute*, *inverse wing salute*,

marching, twist-preen) includes the null model (S1), so none of the variables considered seemed to influence the occurrence of these movements.

Aggressiveness

Five models explained the presence of aggressiveness almost equally well within courtship bouts (Table 2). Model averaging indicated an influence of sex in interaction with age on aggressiveness probability (Table 3). Aggressiveness probability increased with age in males while it remained constant for females (males: $\beta = 0.13$, 95% CI = 0.02 – 0.26; females: $\beta = -0.03$, 95% CI = -0.10 – 0.04; Fig. 4). A 4 yrs. old male is predicted to express an aggression rate of 0.5 while a 30 yrs. old male is predicted to express an aggression rate of 0.96. Females display aggressiveness at similar rate than young males (mean = 0.5 ± 0.07).

Coloration

Five models explained the intensity of neck color almost equally well in individuals participating in courtship bouts (Table 2). Model averaging indicated an influence of sex on the intensity of neck color plumage (Table 3), with a more intense color in females than males (Fig. 5). Variance between male and female coloration were similar ($F = 0.647$, $df = 49$, $p = 0.13$).

Discussion

Our study shows that females and males Greater flamingo performed quasi-similar courtship choreographies during their communal displays. Indeed, postures occurrences and their transitions making up the courtship dances followed the same pattern regardless of sex. Nevertheless, with age, males increased time spent doing *head flagging*, their rate of aggressiveness and decreased the time spent *preening* while no change in courtship display with age was observed for females. Moreover, females display a more intense pink plumage than males.

The fact that courtship dance follows the same pattern in both sexes suggests that courtship dance has the same signaling function for females and males. This is in accordance with a previous study on complexity of courtship dance in this species. Indeed, in addition to explain observed pairing patterns, the complexity of courtship dance seems to be an honest signal of individual quality as it positively influences the probability to become a breeder

regardless of sex (Perrot et al. in press). Due to the energy and time cost associated with their realization, courtship dances have often been considered to reflect individual motor performance such as coordination and balance (Byers et al. 2010; Barske et al. 2011 Apr 20). Besides, motor skills are regularly considered in the study of behaviors that are linked to survival, to feeding or locomotion and thus reflecting individual vigor, but also the capacity for an individual to perform parental care (Byers et al. 2010). In the greater flamingo both sexes are involved in parental care as well during the incubation stage as during chick rearing. On the breeding site, nest density in colony can reach 3 nests per m² (Johnson and Cézilly 2007) so that it is essential for individuals to present good motor coordination and dexterity to avoid breaking their egg or hurting their chick. Motor performance during courtship dance could therefore be an honest signal of the individual reproductive quality for both sex, explaining why females and males displayed similar pattern of courtship dances.

Nevertheless, we found between sexes some differences in courtship display with an increase of *head flagging* duration and aggressiveness rate and a decreased of *preening* duration in males when they get older while in female these behaviors remain constant. *Preening* is a solitary and static behavior often performed on the periphery of the courtship group while *head flagging* is performed in interaction with conspecifics. Indeed during *head flagging*, individuals move close to each other (approximately 1 meter) and call loudly. Intuitively, *head flagging* seems to require more energy than *preening* behavior. Hence with age, males appear to invest most efforts in sexual display than females and interact increasingly with conspecifics, more precisely with other males, and sometimes in an agonistic way. This result is in accordance with those found in the Caribbean flamingo, a species performing similar sexual displays. In Caribbean flamingo, males initiate courtship display and display longer and more vigorously than females (Rooth 1965); and Hinton et al. (2013) showed that, in captive individuals, males are more aggressive than females. This suggests that intra-sexual competition for mate access is probably more intense in males in flamingo species which could be the result of a biased operational sex ratio (OSR) in the population, defined as the ratio of the numbers of sexually receptive males and females (Emlen and Oring 1977; Kvarnemo and Ahnesjo 1996). Indeed, a biased OSR is determinant for the direction of mating competition and for the intensity of sexual selection. More precisely, theory predicts that a biased OSR will make the most abundant sex compete for mates (Emlen and Oring 1977; Clutton-Brock and Parker 1992; Kvarnemo and Ahnesjo 1996; Grant and Foam 2002). The OSR

is primarily determined by the potential reproductive rates of the two sexes, but is also influenced by mortality rates in the two sexes (Clutton-Brock and Parker 1992; Kvarnemo and Ahnesjö 1996; Parker and Simmons 1996). In the Greater flamingo, females starting to breed early in age have a lower survival probability compared to males (Tavecchia et al. 2001), so that a males are expected to outnumber females in the population, leading to a biased OSR, potentially explaining the more intense competition between males than between females for mate access. In year 2014 the sex ratio of displaying individuals was balanced (68 females and 62 males), although in 2015, the sex ratio was biased in favour of males (50 females and 78 males). In addition, the effort of resighting displaying individuals was usually low so that it is difficult to conclude about OSR in our population. So, it would be interesting to investigate whether there is a biased OSR in favour of males at the time of courtship display in Camargue.

Alternatively, higher aggressiveness in males during courtship display could be linked with other breeding steps. Because of fundamental differences in initial parental investment and risk of extra-pair copulation resulting in uncertain paternity, males are expected to invest heavily in breeding territorial acquisition and defence and in mate guarding in monogamous species (Burger 1981). In the greater flamingos, to our knowledge, acquisition of nest on the breeding island is not documented, but mate guarding is known to last several weeks before egg laying (Johnson and Cézilly 2007) and extra-pair copulations events were related in captive birds (Pickering 1992). Consequently competitive abilities should be more required in males to succeed reproduction, which is reflected in courtship display by their higher rate of aggressive behaviors.

Regarding the increase with age of *head flagging* duration and aggressiveness in males, it could be the result of an age-related dominance process during courtship display like in other bird species (Collis and Borgia 1992; Magaña et al. 2011). More precisely, in the case of the Greater flamingo, older and more experienced males would dominate through aggressive behaviors younger males which would then stop displaying or perform less intense display. Age-related behavioral dominance in greater flamingos has been found to explain the monopolization of the best nest sites by older males in a densely populated breeding colony in Spain (Rendón et al. 2001). In addition, a positive correlation between age and aggression in males has been observed during the pair-bonding period in the Caribbean flamingo (Hinton et al. 2013) such as dominance hierarchy through agonistic behaviours in captive flocks (Royer and Anderson 2014; Rose and Croft 2015). Study of interactions between individuals within

courtship group, through social network for example, would help to better understand the role of age in aggressiveness.

Finally, our results show that both sexes present a similar variability in their plumage coloration but females had a more intense pink coloration. Amat et al. (2010) found that flamingos strengthen the intensity of their plumage color using uropygial secretions containing carotenoids pigments through a *preening* behavior during the period of courtship display. In our study females both had a more intense coloration and spent more time in preening than males, which is consistent with their findings. This higher coloration intensity in females could thus be the result of different processes which are not mutually exclusive. First, in addition to reflecting current condition, carotenoid-based plumage is also thought to inform about the female capacity to invest in progeny (Blount et al. 2002; McGraw et al. 2005; Midamegbe et al. 2013), so that females should invest a lot in their plumage coloration to be chosen. Alternatively, males had a higher aggression rate and spent more time displaying head flagging, which are two behaviors strongly suspected to be costly. Hence males could be facing two different trade-offs. First, the increase in time spent in *head flagging* could come at the expense of the time spent in *preening*, leading to a lower intensity of plumage coloration in males. Second, the lower coloration in males could result from a trade-off in the allocation of carotenoids to self-maintenance or to plumage color (Svensson and Wong 2011). Due to the cost of *head flagging* and aggressive behaviors, carotenoids obtained through the diet could therefore be preferentially used by males for their antioxidant function and their other physiological uses in order to maintain health.

In conclusion, despite the many similarities in sexual displays between males and females Greater flamingos, both sexes express light differences probably reflecting sex-specific reproductive competences or the intensity of intrasexual selection. Hence, even in a monogamous mating system where both sexes share parental duties, sexes remain an intrinsic individual characteristic driving sexual behaviors during mate access process.

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Figures captions:

Figure 1. Occurrence of courtship postures on a total of 250 minutes of recording for each sex (black bars: females, grey bars: males). HF correspond to *head flagging* movement, P to *preening*, FF to *false feeding*, WS to *wing salute*, IS to *inversed wing salute*, TP to *twist-preen* and S to *scratching*.

Figure 2. Flow diagram to illustrate the significant transitions of (a) female postures, (b) male postures. The thickness of the lines is proportional to the probability of each of the possible transitions between postures. HF correspond to *head flagging* movement, P to *preening*, FF to *false feeding*, WS to *wing salute*, IS to *inversed wing salute*, TP to *twist-preen* and S to *scratching*.

Figure 3. Relationship between *head flagging* (full line), *preening* (dashed line) duration and age of in male greater flamingo (*Phoenicopterus roseus*) within a courtship sequence of 5 minutes. Points correspond to observed values (full circles for *head flagging* duration, open circles for *preening* duration).

Figure 4. Relationship between aggressiveness probability and age in the greater flamingo (*Phoenicopterus roseus*) in males (full line and black circle) and females (grey circles). Points correspond to observed values. Point size correspond to the density of individuals having the given value.

Figure 5. Mean (\pm SE) color score of neck feathers of females (N = 50) and males (N=50) in the greater flamingo (*Phoenicopterus roseus*).

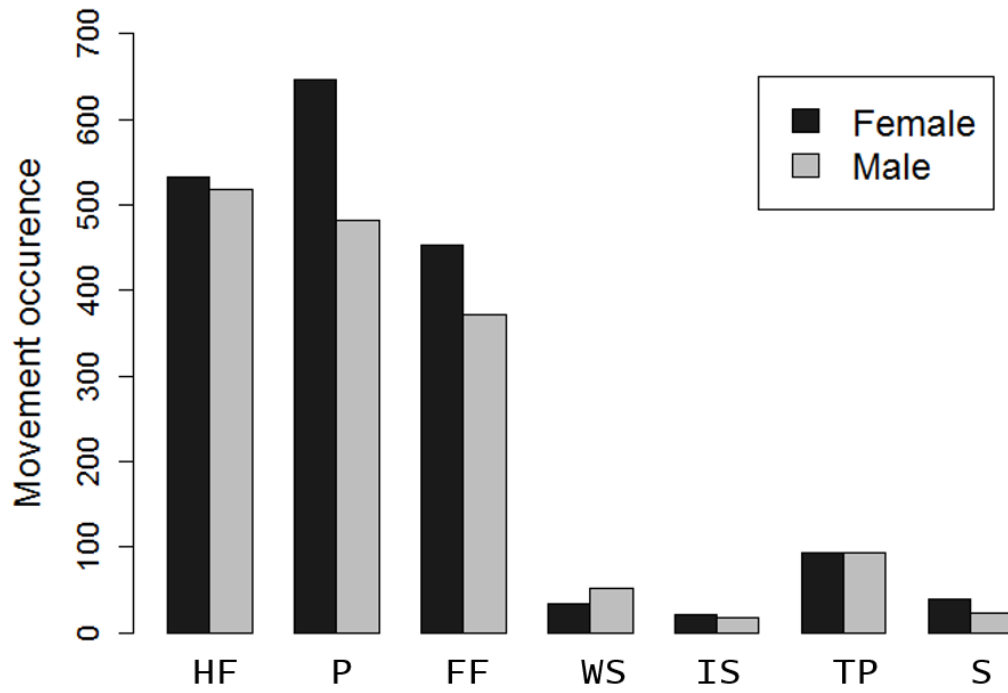


Fig. 1

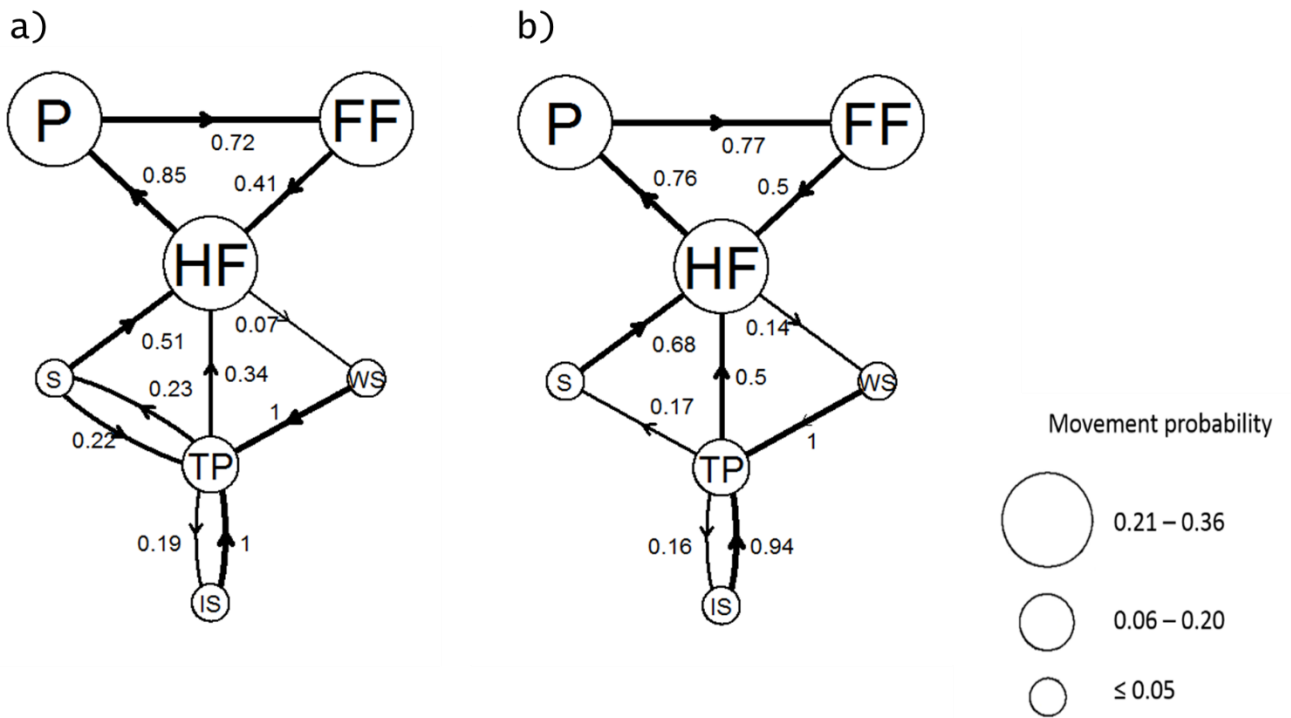


Fig. 2

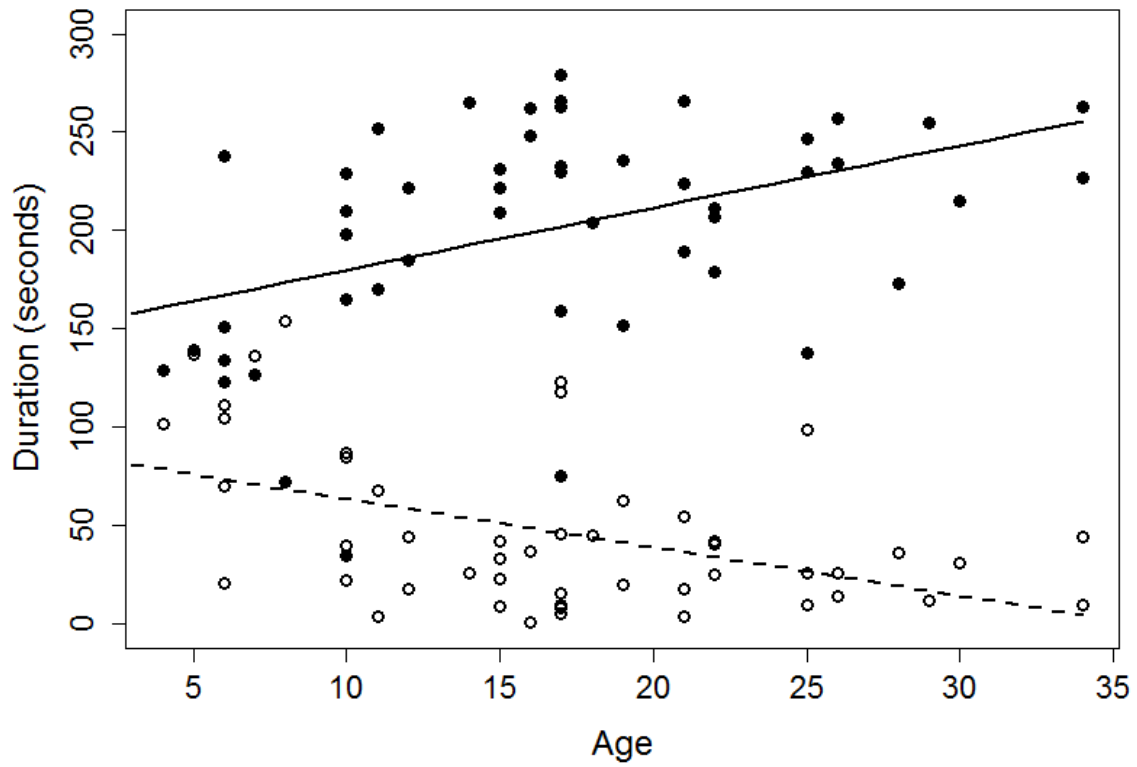


Fig. 3

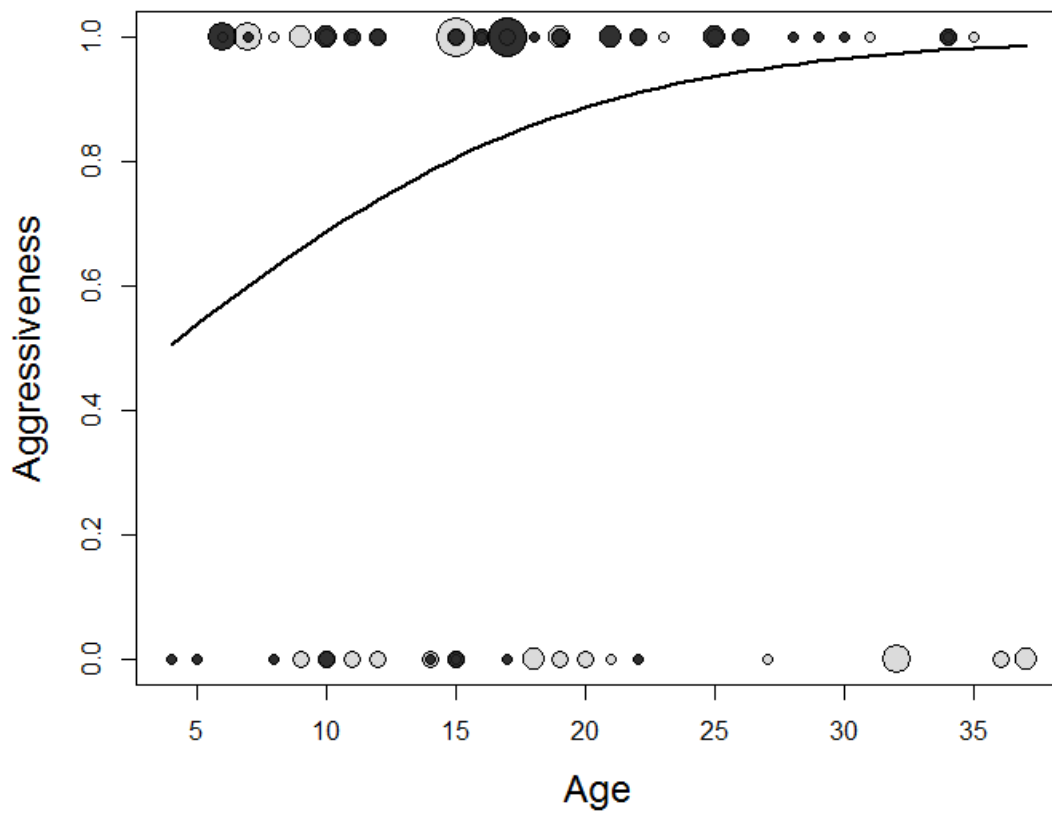


Fig. 4

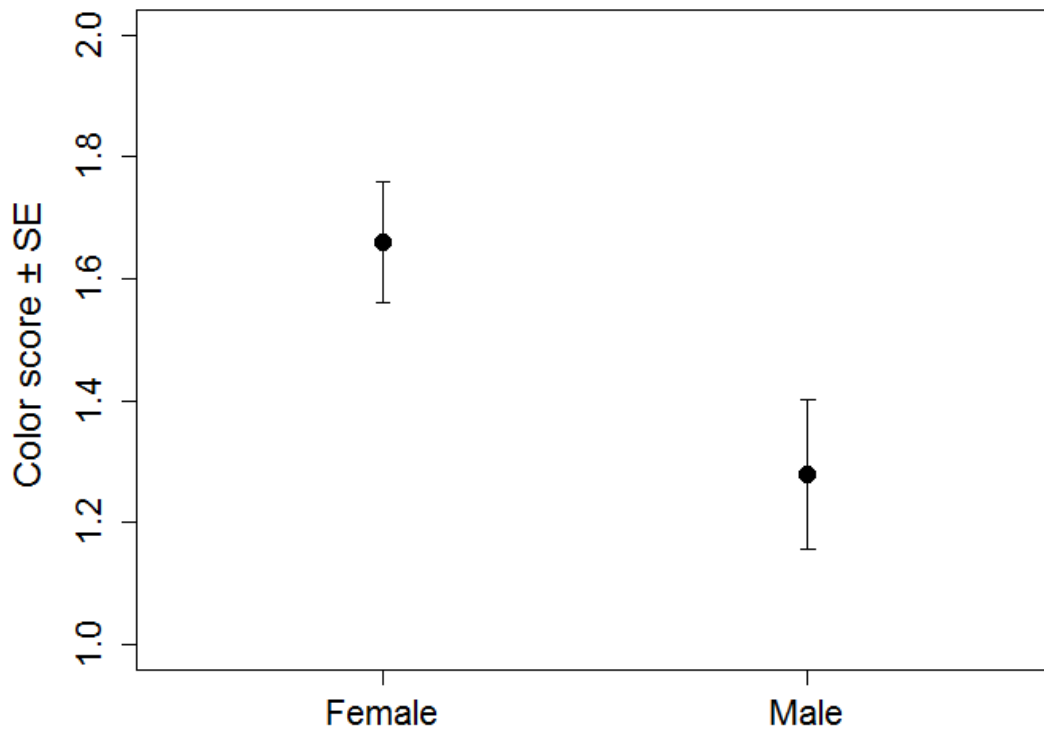


Fig. 5

Table 1. Behavioural repertoire of courtship display in the greater flamingo derived from Johnson and Cézilly (2007)

Behaviours	
Head Flagging	Flamingos walk with their neck stretched and flag their head from side to side. This movement is the most prolonged of the many which constitute a full display.
Preening	Flamingos preen their feather during several seconds, sometimes minutes. This behavior is often followed by a false-feeding.
False feeding	Flamingos dip their bill in water before taking it out immediately.
Wing Salute	In a sudden, rather butterfly like movement, flamingos throw open their wings to their full extent, holding them as much as 40° behind the plane of the body, for 1-2 seconds. Since the inside of wings is composed of black remiges and crimson wing-coverts, this movement produces a flash of colour.
Inversed Wing Salute	Flamingos bend forward from the erect position in a “bowing stance” so that the cocked tail is held higher than the chest. The neck is extended straight forward and in line with the body. At the same time, the wings are flashed partially open, providing a flash of red.
Twist-preen	In a sudden movement, flamingos twist their head and neck back to one side, drop down the wing on the same side and appear to preen behind the wing. An individual may repeat this movement several times in a quite rapid succession.
Scratching	In a lowered position, flamingos scratch their neck with their feet just behind the chin.
Marching	Flamingos move together in group, first rushing in one direction, then in another.
Wing-leg-stretch	Flamingos stretch outward one wing and the leg on the same side for 1-2 seconds, much as during comfort movement.

Table 2. Model selection of the factors influencing scratching and aggressiveness probability and the score of the neck color intensity in the greater flamingo (*Phoenicopterus roseus*): age, date, sex, group size, hour and year were tested on each response variable. Models with a $\Delta\text{AICc} \leq 2$ from the best model are represented.

Response variable	Models	df	logLik	AICc	ΔAICc	weight
Scratching						
	sex + year	3	-62.66	131.57	0	0.15
	sex + date + year	4	-61.58	131.58	0	0.15
	sex + date	3	-63.21	132.66	1.09	0.08
	sex + age + year	4	-62.19	132.81	1.24	0.08
	year	2	-64.36	132.84	1.27	0.08
	sex + age + sex : age + date + year	6	-59.99	132.89	1.31	0.08
	date + year	3	-63.35	132.94	1.37	0.07
	sex + age + date + year	5	-61.21	133.07	1.49	0.07
	sex + hour + year	4	-62.4	133.22	1.64	0.06
	sex + group size + year	4	-62.42	133.26	1.68	0.06
	sex + age + sex : age + year	5	-61.33	133.3	1.73	0.06
	sex + date + hour + year	5	-61.4	133.43	1.85	0.06
Aggressiveness						
	sex + age + sex : age	4	-56.52	121.47	0	0.34
	sex + age + sex : age + date	5	-55.96	122.56	1.09	0.2
	sex + age + sex : age + group size	5	-56.12	122.88	1.42	0.17
	sex + age + sex : age + age ²	5	-56.22	123.07	1.6	0.15
	sex + age + sex : age + hour	5	-56.38	123.4	1.93	0.13
Neck color						
	sex + age + sex : age + age ² + year	7	-112.1	239.41	0	0.33
	sex + age + sex : age + age ²	6	-113.83	240.57	1.15	0.19
	sex + year	4	-116.14	240.71	1.3	0.17
	sex + age + sex : age + year	6	-113.93	240.77	1.35	0.17
	sex	3	-117.42	241.1	1.69	0.14

Table 3. Model-averaged estimates \pm SE and 95%CI of parameters explaining variations in scratching probability, aggressiveness probability and the score of the neck plumage in greater flamingos. The relative importance of each factor is calculated by summing the AIC weights across the top models (Table 2) where the given factor appears (last column).

Response variable	Parameters	Estimate	SE	Confidence interval	Sum of weights
Scratching					
	intercept	-0.32	0.22	(-0.75 ; 0.11)	
	year	-0.95	0.55	(-2.03 ; 0.13)	0.92
	sex	-0.69	0.50	(-1.67 ; 0.29)	0.85
	date	0.42	0.57	(-0.70 ; 1.55)	0.51
	age	-0.10	0.29	(-0.68 ; 0.47)	0.29
	sex : age	0.18	0.57	(-0.94 ; 1.31)	0.14
	hour	-0.04	0.18	(-0.40 ; 0.33)	0.12
	group size	-0.02	0.14	(-0.29 ; 0.35)	0.06
Aggressiveness					
	intercept	0.85	0.28	(0.29 ; 1.40)	
	sex	1.72	0.55	(0.64 ; 2.81)	1
	age	0.79	0.58	(-0.35 ; 1.94)	1
	sex : age	2.72	1.20	(0.34 ; 5.10)	1
	date	0.11	0.31	(-0.50 ; 0.72)	0.2
	group size	-0.08	0.26	(-0.60 ; 0.45)	0.17
	age ²	0.10	0.42	(-0.72 ; 0.93)	0.15
	hour	-0.03	0.19	(-0.42 ; 0.35)	0.13
Colour					
	intercept	1.53	0.11	(1.31 ; 1.74)	
	sex	-0.38	0.16	(-0.69 ; -0.08)	1
	sex : age	-0.44	0.40	(-1.24 ; 0.35)	0.68
	age	0.17	0.19	(-0.20 ; 0.53)	0.68
	year	0.18	0.18	(-0.17 ; 0.54)	0.67
	age ²	-0.26	0.31	(-0.87 ; 0.36)	0.52

Supplementary materials

S1. Model selection of the factors influencing false feeding and twist preen occurrence as well as wing salute, inversed wing salute, marching probability in the greater flamingo (*Phoenicopterus roseus*): age, date, sex, group size, hour and year were tested on each response variable. Models with a $\Delta\text{AICc} \leq 2$ from the best model are represented.

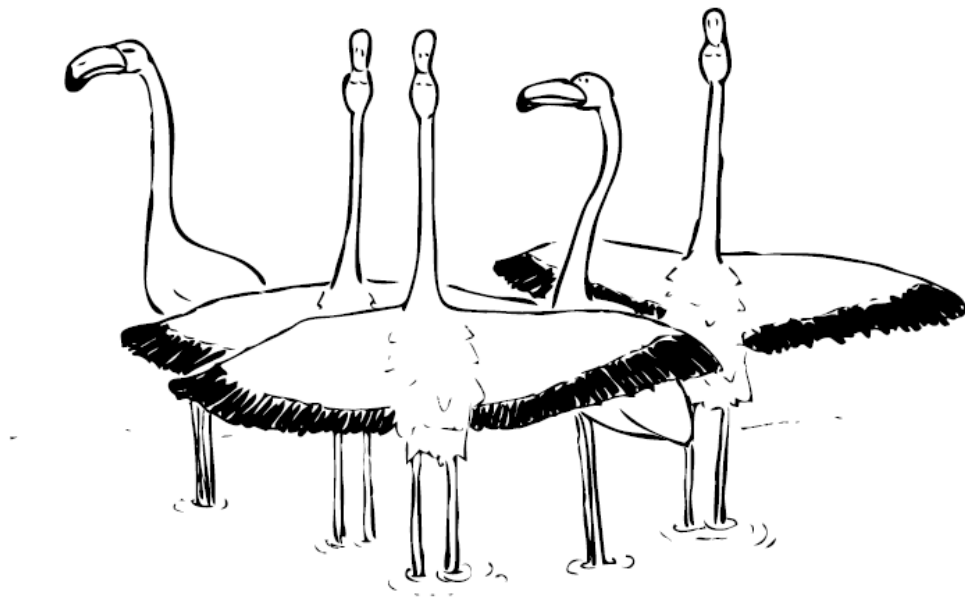
Response variable	Models	df	logLik	AICc	ΔAICc	weight
False feeding						
	year + date + year : date + hour	6	-125.77	264.45	0	0.14
	year + date + year : date	5	-127.07	264.78	0.33	0.12
	year + sex + age + date + hour + year:date + sex : age	9	-122.43	264.85	0.4	0.11
	year + sex + age + date + year:date + sex : age	8	-123.64	264.87	0.42	0.11
	hour	3	-129.43	265.11	0.66	0.1
	hour + group size	4	-128.35	265.12	0.66	0.1
	null	2	-130.86	265.84	1.39	0.07
	sex + age + sex : age + hour	6	-126.48	265.86	1.41	0.07
	year + sex + date + hour + year:date	7	-125.36	265.94	1.49	0.07
	year + sex + date + year:date	6	-126.56	266.02	1.57	0.06
	sex + date	4	-128.93	266.28	1.83	0.06
Wing salute						
	null	1	-69.23	140.51	0	0.15
	sex + age + sex : age + date	5	-65.25	141.14	0.63	0.11
	sex + age + sex : age	4	-66.38	141.18	0.67	0.11
	date	2	-68.67	141.47	0.96	0.09
	sex + age + sex : age + age ² + date	6	-64.29	141.48	0.97	0.09
	year	2	-68.72	141.57	1.06	0.09
	sex + age + sex : age + year	5	-65.56	141.75	1.24	0.08
	sex	2	-68.91	141.95	1.44	0.07
	age	2	-69.12	142.37	1.86	0.06
	age + age ² + date	4	-67.01	142.43	1.92	0.06
	sex + age + sex : age + age ²	5	-65.9	142.45	1.93	0.06
	age + age ²	3	-68.13	142.5	1.99	0.05
Inversed wing salute						
	age + age ² + group size	4	-53.52	115.46	0	0.16
	group size	2	-55.83	115.78	0.32	0.14
	sex + age + age ² + group size	5	-52.86	116.37	0.9	0.1
	sex + group size	3	-55.07	116.38	0.92	0.1
	null	1	-57.31	116.65	1.19	0.09
	sex	2	-56.36	116.85	1.39	0.08
	sex + age + age ² + date	5	-53.19	117.02	1.56	0.07
	age + age ² + date	4	-54.35	117.13	1.67	0.07
	date	2	-56.56	117.24	1.78	0.07
	age + age ² + group size + hour	5	-53.32	117.28	1.82	0.06
	sex + date	3	-55.59	117.43	1.97	0.06

Marching						
	null	1	-48.62	99.29	0	0.34
	hour	2	-48.05	100.23	0.94	0.21
	year	2	-48.25	100.62	1.34	0.17
	group size	2	-48.48	101.08	1.79	0.14
	age	2	-48.49	101.11	1.83	0.14
Twist preen						
	age + age ² + date	4	-245.92	173.41	0	0.37
	date	2	-254.42	174.65	1.24	0.2
	null	1	-258.58	175.28	1.86	0.15
	age + age ² + date + sex	5	-245.33	175.29	1.88	0.14
	age + age ² + date + hour	5	-245.37	175.32	1.9	0.14

Chapitre 2

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Sexual display complexity varies non-linearly with age and predicts breeding status in greater flamingos



Sexual display complexity varies non-linearly with age and predicts breeding status in greater flamingos

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Abstract

The long-lived greater flamingo (*Phoenicopterus roseus*) is famous for performing conspicuous group displays during which adults try to acquire a new mate each year with varying success. We examined variation in the sexual display complexity (SDC) of wild flamingos aged between 4 and 37 yrs. SDC was defined as the product of richness (the number of different display movements) and versatility (the number of transitions between movements) within a 5 min behavioral sequence. In both sexes, date in the pairing season had a linear and positive effect on SDC, whereas age had a quadratic effect, with SDC increasing until about age 20yrs, and declining afterwards. SDC better explained pairing patterns than age, and positively influenced the probability of becoming a breeder. Our results thus support the idea that SDC is an honest signal of individual quality and further suggest that senescence in display could be an overlooked aspect of reproductive decline in species with no or weak pair bonding.

Introduction

Various animal species, including both vertebrates and invertebrates, perform sexual displays. Such displays are considered to be complex (or elaborated) when they combine several different movements organized in a more or less stereotyped and repetitive sequence, in contrast with more simple displays consisting of only one or a few single movements^{1,2}. For instance, the relatively simple display of male magnificent frigatebirds, *Fregata magnificens*, consists in outstretching and vibrating wings rapidly with head thrown back, and red gular pouch fully blown out³. In contrast, males of several *Anas* duck species typically perform complex, highly ritualized sexual displays that include several distinct motor patterns organized in a fixed and ordered sequence^{4,5}.

Due to the energy and time cost associated with their production, complex displays have often been regarded as honest signals of individual quality⁶. For instance, in several lek-breeding bird species, males perform complex sexual display involving different movements such as wing beats, foot stamping and high jumps⁷, with displays effort predicting male mating success^{8,9}. However, the energetic cost of a display may not necessarily be related to its complexity. For instance, a display can consist of a single movement that is performed repeatedly², such that it is energetically costly but not particularly complex. Therefore, some particular benefits must be associated with complexity *per se*. Accordingly, various benefits of complex signaling have been suggested, independently of its energetic cost¹. For instance, different components of a complex display may signal different aspects of an individual's quality^{10,11} or work as different signals that serve to match the variable preferences of different receivers¹². Alternatively, complexity in display might be a form of redundancy (i.e. the different components convey the same information) that allows for an increased accuracy of the receiver response^{10,11,13}, or a strategy to cope with the variable transmission and reception efficiency of different signals across different environments^{14,15}.

Although each of these interpretations have received some empirical support¹, little is known about the causes and consequences of inter-individual variation in the complexity of “complex” sexual displays (but see¹⁶). So far, quantitative studies of sexual display complexity (SDC) have examined co-variation between the multiple components of the display and the signaling value of each one (e.g.^{17,18}). To the best of our knowledge, no empirical study to date has quantified SDC and related it to individual characteristics or fitness consequences. This

might be due to the recurring difficulty in defining and quantifying complexity in biology¹⁹ (but see²⁰), particularly when considering multimodal displays. In addition, most, if not all, studies of complex sexual displays concern species where one sex (usually the male) is displaying to the other one (usually the female)^{21–24}. However, complex displays can also be observed in species with mutual mate choice, particularly among socially monogamous bird species^{25–29}.

It has been further suggested that large and dense social groups demand more complexity in signaling because of the need to transmit information to a large number of individuals²⁰. In that respect, flamingos (Phoenicopteridae) might be a particularly well-suited species to investigate inter-individual variation in SDC. Flamingos are obligate colonial breeders, and perform conspicuous mixed-sex group (or communal) displays³⁰, which are supposed to stimulate synchronous breeding and facilitate pair formation. Up to several thousand individuals then form dense aggregations and perform in synchrony a variety of movements in a more or less stereotyped succession for several hours per day during the pre-breeding period^{26,31–33}. The group display of flamingos is therefore a good example of “communicative complexity” (*sensu*²⁰), as it contains several structurally distinct ritualized elements^{26,31}. Still, the display repertoire of flamingos remains limited in size, such that it can be reasonably used to quantify SDC³⁴, defined here as the product of display richness (i.e. the number of different acts in a sequence) by display versatility (i.e. the number of transitions between acts in a sequence).

Using a cross-sectional approach, we investigated inter-individual variation in SDC in the greater flamingo, *Phoenicopterus roseus*, taking advantage of a long-term study that today results in the observation in the Camargue (southern France) of on average 3000 individually marked birds of known sex and age each year²⁶. Although the species is socially monogamous, it is characterized by the total absence of long-term pair bonding, with all pairs divorcing systematically between two consecutive breeding seasons³⁵. This means that all sexually mature individuals must invest each year in group displays in order to find a new mate and be able to breed. However, some birds succeed to acquire mates whilst others remain unpaired. Most of the plumage of greater flamingos is pale pink but during group displays, they typically exhibit their underwing colorations which offer a bright contrast between carotenoid-based crimson remiges and melanin-based black ones (Fig. 1). Carotenoid-based plumage is dependent on diet (as birds cannot synthesize carotenoids *de novo*), and is thought to reflect current condition, whereas melanin-based plumage is synthesized as a by-product of amino-

acid catabolism, is under genetic control, and is likely to reflect genetic quality of individuals¹. In addition, such ornaments may act to enhance the apparent skill and vigor of individual motor performance during group displays⁶. We provide for the first time evidence for a quadratic age effect on SDC, suggestive of early improvement and senescence, assortative mating for SDC among pairs of greater flamingos, and a positive influence of SDC on the probability of becoming a breeding individual.

Results

The age of the focal individuals ranged from 4 to 34 years for males and from 5 to 37 for females. Within a five-minute courtship sequence, the number of postures varied from 2 to 8, while the number of transitions between postures (versatility) varied between 2 and 17. SDC scores consequently varied from 4 to 136.

Three models explained SDC almost equally well (Table 1). Model averaging indicated a quadratic effect of age on SDC (Table 2), with SDC being higher in individuals of intermediate age compared to young and old ones. For instance, SDC was 1.7 times higher in 20-year-old individuals compared to 6 and 34 year-old individuals (Fig. 2). SDC increased over the courtship season (Table 2), but was not influenced by either sex or year. In the same way, quadratic effects of age and date of courtship display were also retained in the best models explaining variability of repertoire size and versatility of sexual display, and model averaging indicated both higher repertoire size and higher versatility in individuals of intermediate age compared to young and old ones. Repertoire size was 1.2 times higher in 20-year-old individuals compared to 6 and 34 year-old individuals and versatility was 1.3 times higher in 20-year-old individuals compared to 6 and 34 year-old individuals (Fig. 2b and Fig. 2c). As for SDC, repertoire size and versatility increased throughout the courtship season (Table 2).

SDC was a good predictor of the future breeding status of individuals (Wilcoxon–Mann–Whitney test: $W = 146.5$, $p = 0.001$), with confirmed breeders having a mean SDC score of $61.23 (\pm 6.76 \text{ SE})$ compared to a mean SDC score of $41.14 (\pm 4.33 \text{ SE})$ in individuals not confirmed as breeders (Fig. 3). In addition, SDC was retained in the three best models explaining the probability that an individual was observed at the colony (Table 1). Model averaging indicated a positive influence of SDC on breeding status (Table 2). There was no effect of sex or age on breeding status after accounting for SDC.

In 2015, the age difference between mates ranged between 0 and 27 yrs, with a mean value of 7.905 (± 1.793 SE; $n = 21$) yrs. There was no evidence for males being older than females in pair, or the reverse (Wilcoxon matched-pairs test: $W = 115.5$, $p = 0.708$). The observed mean age difference between mates was within the confidence interval of the simulated distribution based on random mating ($\mu_{\text{obs}} = 7.905$; $95\%CI_{\text{distribution}} = [7.429; 13.048]$; Fig. 4a), indicating that age did not significantly affect pairing patterns. In contrast, the observed mean difference in age-related SDC between mates lied outside of the confidence interval of the simulated distribution based on random mating ($\mu_{\text{obs}} = 7.020$; $95\%CI_{\text{distribution}} = [7.721, 12.779]$, Fig. 4b), thus suggesting homogamy for SDC.

Discussion

Our study provides strong support for the hypothesis that SDC *per se* is an honest signal of individual quality involved in mate choice¹ in the greater flamingo. SDC, defined here as the product of display richness by display versatility, varied extensively between individual flamingos, with no difference between sexes, and this variation was partly explained by variation in both the date of observation and the age of individuals. In turn, SDC positively influenced the probability of becoming a breeding individual, being about 50% higher in confirmed breeders vs. not confirmed breeders. Taken together, our results suggest that high SDC in greater flamingos signals high individual quality and current vigor⁶, and, hence, superior competitive ability to secure a nest site on a crowded breeding island where access to nesting space is very limited²². SDC in flamingos could then play a role analogous to song complexity in songbirds, where males with high song complexity have been shown to obtain high quality territories and be more efficient at defending them²⁴.

The signal value of SDC and its energy cost may further explain the observed increase in SDC through time, independently of age. Although high quality individuals might be able to pay the full energy cost of complex displays early in the pairing season, lower quality individuals might not be able to perform costly complex displays, particularly at the beginning of the pairing season, between November and January, when temperatures are at the lowest in the Camargue (average temperatures of 9.4°C with minima near 1°C). However, towards the end of the winter, as temperatures increase (average temperature of 11.2°C with minima near 7°C in March 2015) individuals still unpaired may ultimately increase their display effort in a final attempt to attract a breeding partner. This interpretation does not rule out other

phenomena, such as an increase in hormone levels due to sustained social stimulation³². The existence of such a mechanism could be investigated in captive flocks of flamingos.

The fact that age-related variation in SDC better explains the observed pairing patterns than age itself further reinforces the idea that SDC signals individual quality. As incubation and chick provisioning duties are equally shared between the male and the female in pairs of greater flamingos, mutual mate choice for quality is expected, thus leading to assortative mating for quality as reflected in SDC. Age-assortative mating had previously been reported in the Camargue population of greater flamingos³⁶, contrasting with the present results. However, in that earlier study, the age of individuals ranged only from 3 to 15 yrs, whereas in the present one the age of paired birds that were ringed varied between 4 and 37 yrs. The pattern of age-assortative mating previously reported could then simply result from the positive association between age and SDC in younger age classes. Our results show that very old individuals can actually be paired with young ones, as they happen to be similar in terms of display complexity.

Our most important result may however lie in the observed quadratic effect of age on SDC, suggestive of improved motor performance with increasing age, followed by a period of senescence. This is, to the best of our knowledge, the first evidence for senescence affecting a sexual motor display. It is however in accordance with some previous results on the relationship between age and sexual display. For instance, a quadratic relationship between lek attendance (but not fighting rate or distance to the center of the lek) and age has been reported in male black grouse⁴¹, while a concave relationship between song consistency (but not repertoire size) and age has been observed in a free-living population of great tits, *Parus major*⁴². In the present study, a quadratic effect of age was detected on both repertoire size and the number of transitions between movements, indicating that both components of motor display are affected by senescence.

The observed increase in SDC during early life suggests that flamingos may acquire their motor competences progressively through a maturation process³⁷. However, this result is based on a cross-sectional study, with each individual having been sampled only once, in one of two consecutive breeding seasons. As within-individual variation has not been taken into account, the observed pattern could have been generated by the disappearance of poor quality individuals and/or the appearance of high-quality individuals with age³⁸. A longitudinal study of SDC, involving the collection of repeated display sequences of the same individual at

different ages, would be necessary to discriminate between the two processes. However, this approach would not be easy to apply, as the collection of field data across several years would be labor intensive, and the probability of re-observing the same individuals over multiple seasons rather low, given the high dispersal of flamingos between breeding colonies in the Mediterranean region and their irregular breeding at that regional scale³⁹. Such an approach might however be possible using captive flocks of flamingos.

Symmetrically, the observed decrease in SDC after age 20 likely reflects reproductive senescence in wild flamingos. Alternatively, old birds might be more experienced at successfully acquiring a reproductive partner, and a lower SDC could simply correspond to a modulation of their investment in sexual display⁴⁰. However, this explanation is unlikely as the probability to become a breeder increased with increasing SDC for both males and females. On the other hand, competing for mates may incur substantial costs, particularly when individuals need to invest heavily in the production of sexual signals to attract a reproductive partner. As engaging in group displays must be energetically demanding in flamingos (as it has been shown in other bird species^{41–43}), it may increase metabolic rate and, hence the production of reactive oxygen species that can damage biomolecules, unless regulated by enzymatic and non-enzymatic antioxidant systems⁴⁴. Interestingly, a quadratic age effect has been found in resistance to oxidative stress in a captive population of greater flamingos⁴⁵. Similar to SDC in the present study, resistance to oxidative stress increased for age between 0 and 15yrs to reach an asymptote between 16 and 25yrs, and finally slightly decreased at older ages. Oxidative stress may then limit display effort in wild greater flamingos and could explain the observed quadratic relationship between SDC and age observed in the present study.

Previous studies on greater flamingos in the wild reported an increase in survival, breeding propensity and breeding success with age, but failed to detect any pattern of senescence^{46–48}. However, the maximum age of individuals included in these studies was 20 yrs. Still, as flamingos divorce each year²⁶, senescence may actually take place early in the reproductive season, at the time of pairing and acts as a filter. More precisely, only the best individuals among the older ones could manage to find a partner and consequently, senescence might not be detected afterwards. Our results thus suggest that the influence of the dynamics of pair bonding and that of costly and complex sexual displays on patterns of reproductive senescence in the wild deserve further consideration.

Methods

Behavioral observations

Observations were made in the Camargue, Southern France, one of the most important breeding sites of greater flamingos in the Mediterranean region⁴⁹, during two consecutive seasons of courtship displays (November to March in both 2014 and 2015). Since 1977, on average, 12 % (7-30%) of the chicks fledged in the Camargue have been marked with PVC plastic rings engraved with a three or four digit alphanumerical code⁵⁰, allowing individual identification at distance and providing information about the age of individuals. In addition, the sex of ringed birds has been regularly ascertained, through behavioral observations or through blood sampling and molecular analyses⁵¹. Ringing and sample collection of greater flamingo chicks were authorized through the personal permit (number 405) of Alan Johnson and Arnaud Béchet delivered by the Centre de Recherche sur la Biologie des Populations d'Oiseaux (CRBPO, Muséum national d'histoire naturelle, France).

Using a FullHD video camera equipped with a 60x zoom (20-1200 mm, Panasonic Lumix FZ72), we recorded the behavior of ringed individuals during displays. On each occasion, we attempted to follow a single displaying individual for up to five minutes. To that end, we first located a display group at a distance of less than 300 meters, at which the code engraved on a flamingo ring is readable²⁶ and at which good quality videos can be recorded. We then looked for a ringed individual displaying within the courtship group and started recording its behavior. In addition, we recorded, for each individual sequence, the size of the display group (ranged from 9 to 130 individuals), the hour of the day and the date (as such variables were previously found to influence display behavior in flamingos²⁶). However, many observations were interrupted before that time due to movements of individuals, agonistic interactions, or because the focal bird stopped displaying. We thus randomly selected 100 focal-individual sequences of different individuals (50 females, 50 males) where the display behavior had been recorded continuously for five minutes. Behavioral sequences were then coded using the JWatcher software⁵² in order to estimate courtship complexity. Following previous studies of the display repertoire of the greater flamingo²⁶, nine different postures were recognized (Fig. 5).

Display complexity

We analyzed sexual displays of greater flamingos as sequences of discrete postures from a finite repertoire, and relied on a simple method, widely used in the study of bird song, to assess complexity. Following^{53–55}, we defined sexual display richness as the number of different postures in a sequence (i.e. repertoire size), and versatility as the number of transitions between different postures in a sequence. SDC was then calculated as the product of display richness and display versatility. Thus, complex sexual displays correspond to sequences where numerous transitions occur between a maximum number of postures, whereas simple ones correspond to monotonous sequences with high continuity and low versatility.

Statistical analyses

We first investigated if the age and sex of individuals had an influence on the variability of display complexity, repertoire size and versatility, using generalized linear models. The complete model contained the interaction between sex and age, the quadratic effect of age (thus testing for a potential effect of senescence), as well as group size, hour of the day, date of the year, year and the interaction between year and date as explanatory variables. Model assumptions (i.e. normality and homoscedasticity of residuals) were checked. From the complete model we derived a set of all possible submodels. As group size and date were significantly correlated ($r = -0.475$, 95%CI = -0.643 ; -0.274), we removed models containing both variables from the set of models to avoid collinearity.

To investigate the influence of courtship complexity on the subsequent breeding probability we assigned a score to each individual according to their observed reproductive status at the Fangassier breeding colony in the Camargue. An individual was considered to have been breeding if it was seen at the same place on the breeding island for at least 48 hours, or if it was seen with an egg or rearing a chick (see²⁶ for details). Any flamingo not seen at the colony, or seen at the colony but not in one of the previously described states, was considered as a non-breeding individual. The analysis was restricted to the 2015 data set, because flamingos bred at a different location in 2014, where continuous monitoring was not possible. First we compared SDC between non-breeding (N=43) and breeding individuals (N=13), using a two-sided Wilcoxon–Mann–Whitney test. Next, we used generalized linear models with a binomial distribution to test the influence of courtship complexity on breeding

probability. The complete model contained courtship complexity, sex, age and the quadratic effect of age as explanatory variables. Model assumptions (i.e. normality and homoscedasticity of residuals) were checked.

Following recent recommendations to produce model estimates comparable between and within studies^{56,57}, we standardized all explanatory variables by centering and dividing by two standard deviations using the *arm* package⁵⁸. To prevent overparameterization, we respected the sample size rule-of thumb of 10 : 1 subjects to predictors in multiple regression⁵⁹. Model selection was based on Akaike Information Criterion corrected for small sample size (AICc)⁶⁰. When several models were within a Δ AICc of 2 from the best model, we employed a model averaging approach, using the so-called zero method⁶⁰ implemented in the *MuMIn* package of R⁶¹ on models within two points of AICc from the best one. This allowed us to account for model selection uncertainty in order to obtain robust parameter estimates⁵⁷.

Analyses on assortative mating were performed on a sample of pairs (N=21) with both partners ringed observed in 2015. We first examined age-assortative mating using the absolute value of age difference between members of the same pair. We examined where the observed mean age difference was situated within its theoretical distribution under the assumption of random pairing with respect to age (1000 simulations). As courtship complexity had not been measured on the same individuals, we used the predicted values of complexity according to age from the previous model to allocate a score of complexity to each individual. We then relied on the same procedure to test for assortative mating for courtship complexity.

All analyses were conducted with R 3.0.3⁶².

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Author Contributions

C.P., C.H. and A.A. participated in data collection. C.P., A.B., R.P. & F.C. analyzed the data. C.P., F.C. and A.B. wrote the manuscript. All authors were involved in the design of the protocol.

Additional Information

Competing financial interests: The author(s) declare no competing financial interests.

Figure legends:

Figure 1. Group displays of greater flamingos in the Camargue (Photography by Benjamin Vollot).

Figure 2. Quadratic relationship between age and a) SDC b) courtship repertoire size c) courtship versatility of individuals in the greater flamingo according to model 1 ($\text{age} + \text{age}^2 + \text{date}$) for the tree variable, with date fixed at February 3. Individual points correspond to the arithmetic mean of observed SDC, repertoire size or versatility per age \pm SE when there was more than 1 observation.

Figure 3. Mean (\pm SE) SDC of individuals confirmed (43) and not confirmed (13) as breeders at the breeding colony in the year 2015.

Figure 4. a) Distribution of mean age difference under the assumption of random pairing with respect to age. Full lines correspond to the upper and lower 95% confidence limits, dashed line corresponds to the observed mean of age difference between mates in our sample of flamingo pairs. b) Distribution of mean SDC differences under the assumption of random pairing with respect to complexity. SDC values were inferred from age according to the relationship $\text{SDC} \sim \text{age} + \text{age}^2 + \text{date}$ (model 1, table1). Full lines correspond to the upper and lower 95% confidence limits, dashed line corresponds to the mean of inferred SDC difference between mates in our sample of flamingo pairs.

Figure 5. Behavioral repertoire of SDC in the greater flamingo derived from Johnson and Cézilly (2007). (Drawings by Samuel Hilaire).



Fig.1

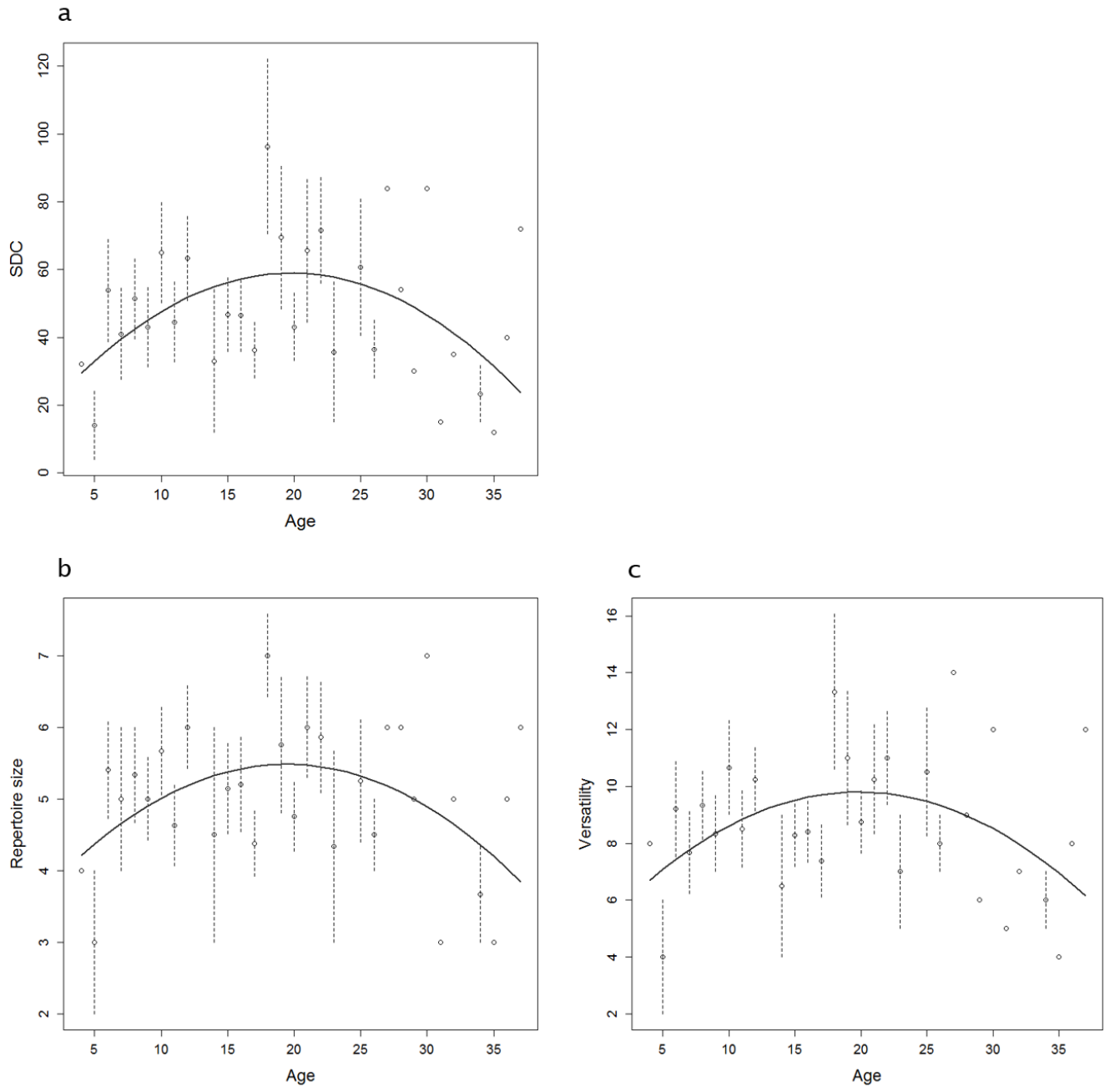


Fig. 2

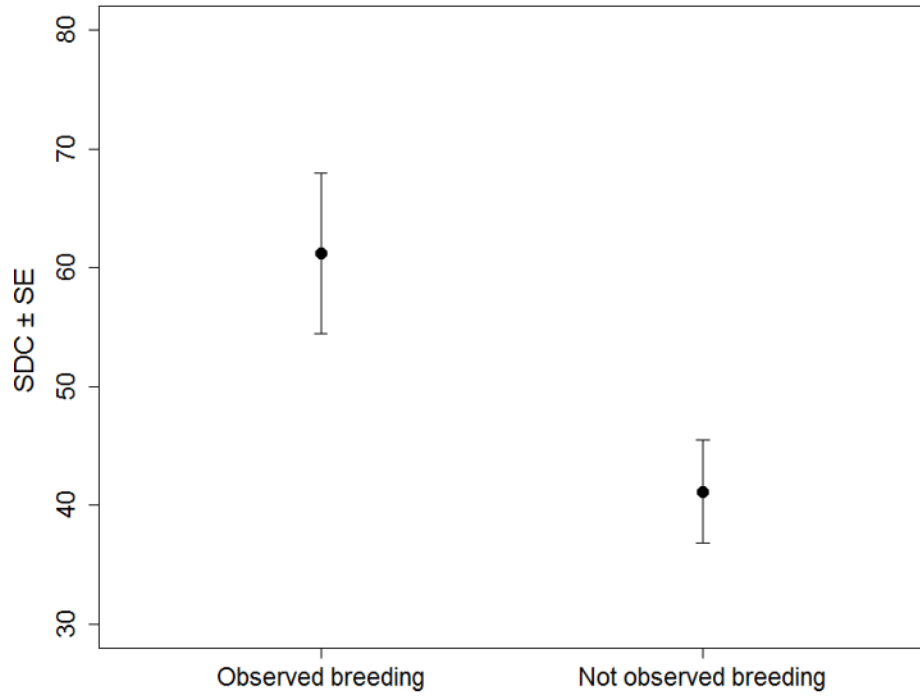


Fig.3

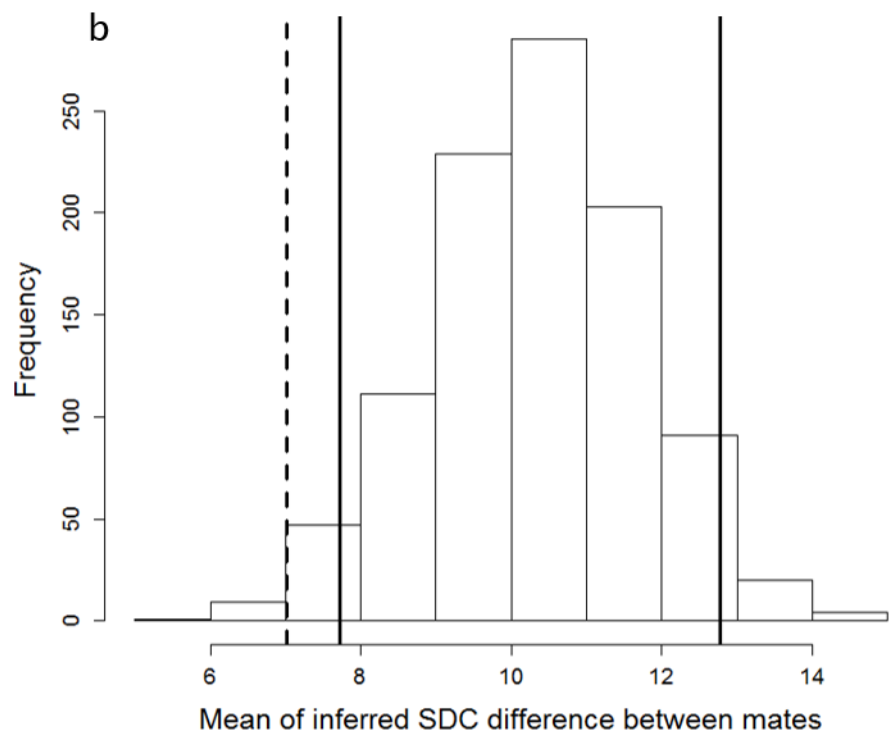
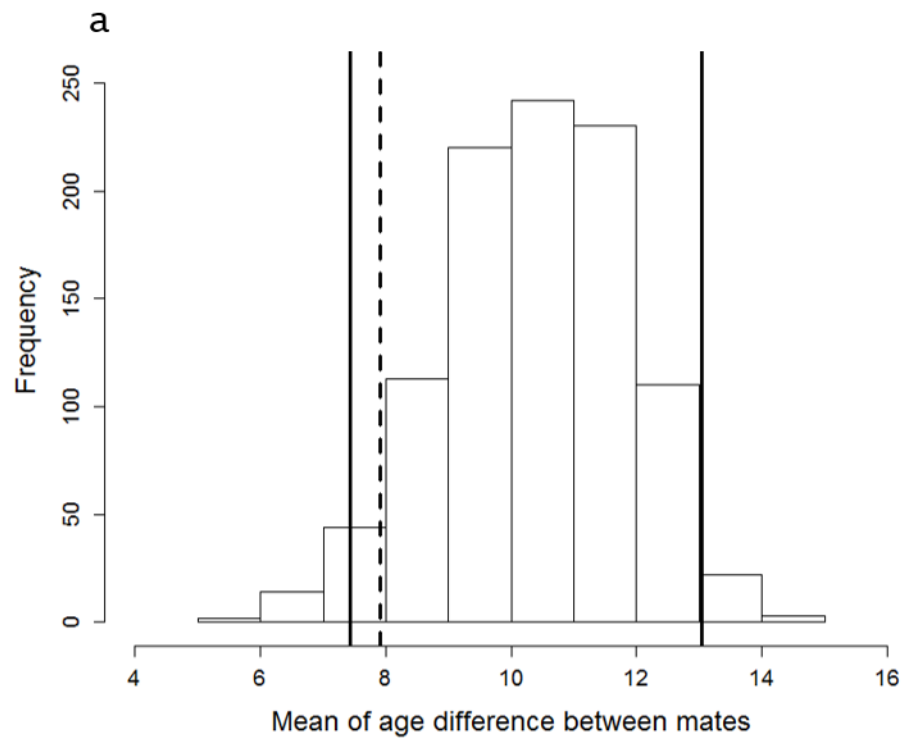


Fig. 4

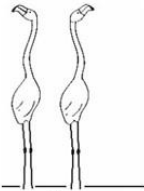

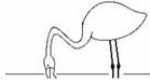
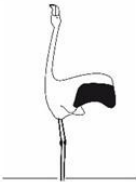





Behaviors		
Head Flagging		Flamingos walk with their neck stretched and flag their head from side to side. This movement is the most prolonged of the many which constitute a full display.
Preening		Flamingos preen their feather during several seconds, sometimes minutes. This behavior is often followed by a false-feeding.
False feeding		Flamingos dip their bill in water before taking it out immediately.
Wing Salute		In a sudden, rather butterfly like movement, flamingos throw open their wings to their full extent, holding them as much as 40° behind the plane of the body, for 1-2 seconds. Since the inside of wings is composed of black remiges and crimson wing-coverts, this movement produces a flash of colour.
Inversed Wing Salute		Flamingos bend forward from the erect position in a "bowing stance" so that the cocked tail is held higher than the chest. The neck is extended straight forward and in line with the body. At the same time, the wings are flashed partially open, providing a flash of red.
Twist-preen		In a sudden movement, flamingos twist their head and neck back to one side, drop down the wing on the same side and appear to preen behind the wing. An individual may repeat this movement several times in a quite rapid succession.
Scratching		In a lowered position, flamingos scratch their neck with their feet just behind the chin.
Marching		Flamingos move together in group, first rushing in one direction, then in another.
Wing-leg-stretch		Flamingos stretch outward one wing and the leg on the same side for 1-2 seconds, much as during comfort movement.

Fig. 5

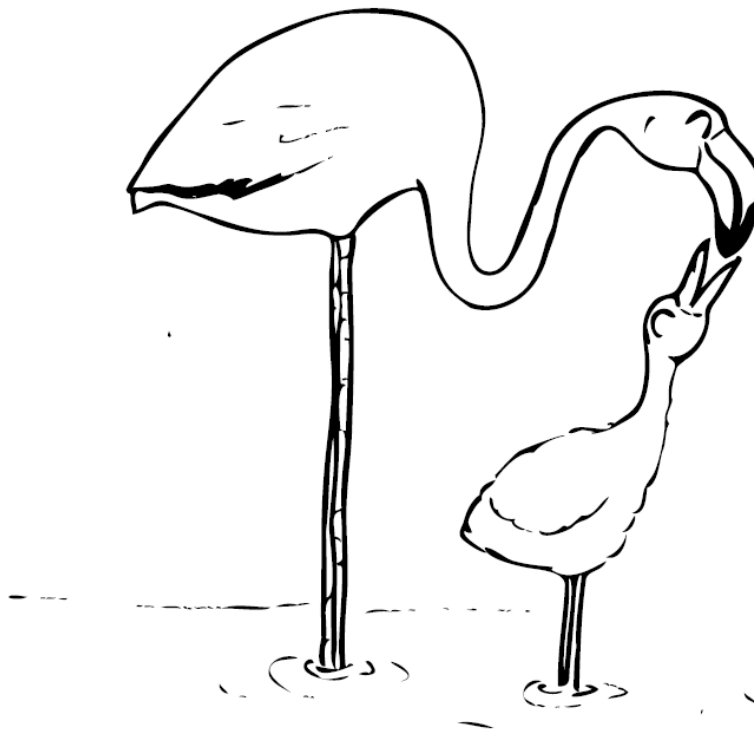
Table 1. Model selection of the factors influencing sexual display complexity (SDC), repertoire size, and versatility of sexual display and the subsequent probability of observation at the breeding colony in the greater flamingo: age, age², date, sex, group size, hour and year were tested on SDC, repertoire size and versatility; age, age², SDC, and sex on probability of observation at the colony. Models with a $\Delta\text{AICc} \leq 2$ from the best model are represented. For sexual display variables, models under the dashed line are shown for comparison with the best model (SDC ~ age + age² + date) for a better view of the strength of age, age² and date effects.

Response variable	Models	df	logLik	AICc	ΔAICc	weight	
SDC	Age + age ² + date	5	-479.75	970.14	0	0.52	
	Age + age ² + date + sex	6	-479.27	971.44	1.3	0.27	
	Age + age ² + date + year	6	-479.57	971.44	1.9	0.20	
	Date	3	-483.84	973.93	3.79		
	Age + date	4	-483.81	976.05	5.91		
	Age + age ²	4	-485.29	979.00	8.86		
	Models	df	logLik	AICc	ΔAICc	weight	
	Repertoire size	Age + age ² + date	5	-177.51	365.67	0	0.57
		Age + age ² + date + sex	6	-177.31	367.52	1.85	0.22
Age + age ² + date + age : sex		7	-176.21	367.65	1.98	0.21	
Date		3	-180.97	368.18	2.52		
Age + date		4	-180.97	370.35	4.69		
Age + age ²		4	-182.54	373.50	7.83		
Models	df	logLik	AICc	ΔAICc	weight		
Versatility	Age + age ² + date	5	-260.95	532.53	0	0.66	
	Age + age ² + date + sex	6	-260.46	533.82	1.29	0.34	
	Date	3	-264.50	535.25	2.72		
	Age + date	4	-264.47	537.36	4.94		
	Age + age ²	4	-265.53	539.48	6.95		
Models	df	logLik	AICc	ΔAICc	weight		
Probability of observation at the colony	SDC	2	-27.96	60.15	0	0.45	
	SDC + sex	3	-27.15	60.76	0.61	0.33	
	SDC + age	4	-27.52	61.50	1.35	0.23	

Table 2. Model-averaged estimates \pm SE and 95%CI of parameters explaining variations in SDC, repertoire size and versatility of sexual display and probability of observation at the colony in greater flamingos. The relative importance of each factor is calculated by summing the AIC weights across the top models (Table 2) where the given factor appears (last column).

Response variable	Parameters	Estimate	SE	Confidence interval	Sum of weights
SDC	Intercept	58.097	3.981	(50.195 ; 65.998)	
	Age	9.778	6.749	(-3.62. ; 23.175)	1
	Age ²	-29.926	10.591	(-50.948 ; -8.903)	1
	Date	20.623	6.559	(7.606 ; 33.638)	1
	Sex	-1.581	4.062	(-9.604; 6.442)	0.27
	Year	-0.836	3.546	(-7.856; 6.184)	0.20
	Repertoire size	Intercept	5.449	0.194	(5.064; 5.833)
Age		0.385	0.328	(-0.267; 1.036)	1
Age ²		-1.318	0.519	(-2.347; -0.288)	1
Date		0.988	0.308	(0.377; 1.598)	1
Sex		-0.079	0.212	(-0.499; 0.342)	0.43
Age : sex		0.185	0.456	(-0.712; 1.083)	0.21
Versatility		Intercept	9.718	0.445	(8.834; 10.602)
	Age	1.035	0.756	(-0.466; 2.536)	1
	Age ²	-3.144	1.182	(-5.490; -0.797)	1
	Date	2.138	0.705	(0.739; 3.537)	1
	Sex	-0.883	0.673	(-1.213; 0.767)	0.34
	probability of observation at the colony	Intercept	-1.334	0.354	(-2.042 ; -0.623)
SDC		1.418	0.658	(0.099; 2.736)	1
Age		0.145	0.419	(-0.687; 0.978)	0.33
Sex		0.289	0.579	(-0.860; 1.436)	0.23

Male early body condition explains chick body condition at fledging in the greater flamingo (*Phoenicopterus roseus*)



**Male early body condition explains chick body condition at fledging in the greater flamingo
(*Phoenicopterus roseus*)**

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Abstract

Early body condition is known to influence individual fitness through short- and long-term effects on individual life history traits. Consequently, parents should optimize offspring body condition in order to increase their own fitness. Parents commonly express variability in their reproductive quality (protection, food provisioning, etc.) influencing directly conditions in which their offspring grows and thus affecting their chick phenotype. We investigated the influence of the parent factor on chick body condition in the greater flamingo, *Phoenicopterus roseus*, a long-lived and monogamous species where both parents are involved in parental care. Using a dataset of 81 “ringed parent-chick” pairs, we modeled chick body condition as a function of the age and the early body condition of their parents.

We found a positive relationship between chick body condition and male early body condition but not with female early body condition. Our results suggest that early body condition has long-term reproductive consequences in male flamingos. Parental age did not affect chick body condition. The fact that young individuals tend to fail at an early stage of the reproduction may explain the lack of parental age influence on chick body condition.

Introduction

Early body condition (EBC) has been shown to influence individual fitness through short- and long-term effects on various life-history traits (Lindström 1999). Early body condition may affect metabolic rate (Verhulst et al. 2006), disease resistance (Worthman and Kuzara 2005), growth (Metcalf and Monaghan 2001; Johnsson and Bohlin 2006), dispersal (Nunes and Holekamp 1996; Belthoff and Dufty 1998; Edelman 2011) sexual attractiveness (Nowicki et al. 2000; Ohlsson et al. 2002) or survival and reproductive success (Lindström 1999).

Inter-individual variations in EBC may result from different non-exclusive processes. EBC variation could be attributed to some part of genetic variation (Küttner et al. 2011; Gillingham et al. 2012). However, individual's genetic make-up likely interacts with both parental reproductive ability and environmental conditions at growth so that similar genotypes may produce a variety of phenotypes resulting in different EBC (Monaghan 2008).

Parents typically vary in their reproductive performance, hence impacting differentially the early developmental conditions of their offspring. Age, experience, or body condition are known to positively influence parental breeding skills and more precisely such parental behaviors as the timing of breeding (Pyle et al. 1991; Bêty et al. 2003; Robinson et al. 2005), incubation attendance (Schmaltz et al. 2011), and foraging efficiency (Jansen 1990; Limmer and Becker 2009). Parental characteristics are also likely to influence how parents respond to environmental variations (Jansen 1990). Indeed, environmental conditions prevailing during chick development like food availability, weather, or population density, can affect directly offspring phenotype by imposing constraints on growth. Environmental conditions can also impact indirectly offspring phenotype by their influence on parental phenotype. These are generally referred to as parental effects. A compelling example of such indirect effects is the so-called maternal effects in birds. Maternal effects refer to the allocation by the mother of different components of eggs like carotenoids, antibodies and hormones, known to influence chick growth, immune system and behaviors (Groothuis et al. 2005). The allocation of such components in egg yolk have been shown to be mediated by the environmental conditions experienced by the mother before laying (Blount et al. 2000; Gasparini et al. 2007; Vergauwen et al. 2012). Such maternal effects highlight that female and male state could impact chick phenotype in different ways. But additional sex-specific effects influencing reproductive skills

and therefore chick body condition may result from differences in life-history trade-offs between sexes (Holtby and Healey 1990; Rice and Chippindale 2008; Han et al. 2015)

In the greater flamingo (*Phoenicopterus roseus*), chicks also appear to be in poorer body condition when breeding numbers are high, which could result from density-dependent effects and to have a genetic basis (Gillingham et al. 2012). However, the influence of parental state on chick body condition remains unknown. Greater flamingos are long-lived seasonally monogamous species with divorce occurring systematically between two consecutive breeding seasons (Cézilly and Johnson 1995). Both sexes share incubation duties and parental care with a slightly higher effort provided by males in provisioning chicks at the end of the breeding season (Cézilly et al. 1994; Rendón et al. 2014).

The aim of our study was first to investigate the influence of the age of parents on the body condition of their offspring. In the greater flamingo, breeding propensity increases with experience and breeding success with age suggesting an improvement of reproductive skills throughout life (Pradel et al. 2012, Schmaltz et al. 2011), so that chick body condition is expected to increase with the age of parents. Next, as early body condition is likely to shape future individual reproductive performance (Monaghan 2008), we explored the effect of early body condition of parents on the body condition of their chick. We took into account the sex of individuals in order to detect potential differences between male and female in the way their phenotype influences the condition of their chick.

Materials and methods

Species, behavioral observations, and dataset

Flamingos start to gather at the breeding site of the Fangassier lagoon in the Camargue in March. Egg laying usually begins in April and incubation lasts 29 days. Females lay a single egg and both parents share incubation and chick rearing duties. At about 10 days of age chicks form small crèches on the breeding island, before leaving the nesting island to join a single large crèche, which can hold several thousand individuals. Parental food provisioning usually takes place at dusk.

Since 1977, on average 12% of the chicks fledged in the Camargue have been ringed each year with PVC rings engraved with alphanumeric codes that can be read from a distance up to 300 m. Rings allow individual identification and give information about its age. The sex

of adults was determined through blood sampling and molecular analyses for some individuals (Balkız et al. 2007) or based on their relative size and sex-specific behaviour from multiple observations. This sexing procedure was accurate, as 96% of adults for which sex was determined using molecular methods were correctly classified when sex was assigned using phenotypic and behavioural characteristics (Rendón et al. 2014). Weight and tarsus length were recorded at the time of ringing, i.e. a few days before fledging.

After each ringing operation, efforts were made to record observations of ringed adults feeding ringed chicks in order to identify kinship between parents and chicks. As only 12% of the chicks are ringed each year, parent-chick pairs are difficult to observe so that records generally concern either a father or a mother-chick relationship. A total of 80 “parent-chick” pairs (45 mother-chick pairs and 35 father-chick pairs) could be identified from 20 different cohorts ranging from 1985 to 2005. Chicks came from 15 different cohorts ranging from 1991 to 2014. No chick has been reobserved often enough yet to be reliably sexed so that we did not take into account chick sex in the analyses.

Estimation of chick and ringed parent early body condition (EBC)

The scaled mass index was used as a proxy of EBC, as this index controls for the effects of body size on both the independent and dependent variables (Peig and Green 2009; Peig and Green 2010). Chick tarsus length was used as a body size indicator, which significantly correlates with chicks' mass (Cézilly et al. 1995; Barbraud et al. 2003; Béchet and Johnson 2008). The slope of the standard major axis (SMA) of the natural log-transformed mass against the natural log-transformed tarsus length was estimated using the scaling exponent using the R function SMATR. However, as slopes differed between cohorts ($p < 0.001$), we estimated the scaled mass index for each cohort separately ($N = 682 \pm 35$ SE chicks per cohort). Next, to control for cohort effects induced by environmental effects (Albon et al. 1987) and make individual EBC from different cohorts comparable, we standardized EBC within each cohort by centering and dividing by standard deviations.

Estimation of early body condition and age of the unknown parent

For each parent-chick pair, the age and early body condition of the second parent remain unknown. To deal with these missing data, rather than taking an overall average value, we approximated the age of the unknown parent from the relationship between male and female ages in a pair known to exist in this species characterized by close age assortative mating up to 15 years (Cézilly et al. 1997). After 15 years of age, a linear relationship no longer holds. Hence, we predicted the age of the unknown parent from a non-linear regression fitted to 298 pairs of ringed flamingos of known age (see supplementary material).

Finally, we approximated the EBC of the unknown partner by the mean EBC of all individuals of the same sex observed breeding successfully in the same year (i.e. seen feeding a chick at the colony). This accounts for potential differences among years in the average EBC of successful breeding adults in the colony.

Statistical analyses

We investigated the effects of age and EBC of mothers and fathers on the variations of chick EBC using linear models. The complete model contained the interaction between mother age and father age, the interaction between mother EBC and father EBC, the interaction between mother age and mother EBC, the interaction of father age and father EBC as well as the quadratic effect of age of both parents (thus testing for a potential effect of senescence). Model assumptions (i.e. normality and homoscedasticity of residuals) were checked. From the complete model we derived a set of all possible submodels. Following recent recommendations to produce model estimates comparable between and within studies (Schielzeth 2010; Grueber et al. 2011), we standardized all explanatory variables by centering and dividing by two standard deviations using the *arm* package (Gelman et al. 2015). To prevent overparameterization, we respected the sample size rule-of thumb of 10 : 1 subjects to predictors in multiple regression (Harrell 2015). Model selection was based on Akaike Information Criterion corrected for small sample size (AICc; Burnham and Anderson 2002). When several models were within a ΔAICc of 2 from the best model, we employed a model averaging approach, using the so-called zero method (Burnham and Anderson 2002) implemented in the *MuMIn* package of R (Bartoń 2015) on models within two points of AICc

from the best one. This allowed us to account for model selection uncertainty in order to obtain robust parameter estimates (Grueber et al. 2011).

Results

The age of mothers (observed and estimated) ranged from 5 to 24 years and the age of fathers from 4 to 29. Mean father EBC (0.14 ± 0.07) was higher than mean mother EBC (-0.15 ± 0.10) (Wilcoxon signed rank test for paired sample: $V = 2144$, $p = 0.01$); however, they had a similar variance (Bartlett's test: $\chi^2 = 1.80$; $p = 0.18$).

Five models explained chick EBC almost equally well (Table 1). Retained models (i.e. those within two points of AIC of the best model) included one or more of the following explanatory variables: male EBC, male and female age and the quadratic effect of male age. However, model averaging indicated only a significant and positive effect of male EBC on chick EBC (Table 2). Male EBC explained between 9.6% of chick EBC variance. An increase of 2 units in father EBC leads to an increase of 1 unit in chick EBC (Fig. 1).

Discussion

Our results provide evidence of sex-related long-term reproductive consequences of early body conditions in the greater flamingo. Males of higher EBC at fledging produced later in their life chicks with higher EBC than males with lower EBC at fledging. In contrast the EBC of females did not have any significant influence on the EBC of their chick. Besides, contrary to our expectation, we found no effect of parental age on chick body condition.

Sex differences in the long-term effect of EBC could result from several processes. First, sexes can differ in their sensitivity to conditions during the growth period (Lindström 1999; Monaghan 2008). In some species, offspring express a sexual dimorphism (Weimerskirch et al. 2000; Becker and Wink 2003; Merckling et al. 2012) so that males and females require a different amount of energy for their development. In flamingos, males are larger than females so that male chicks likely require higher energetic investment. Consequently they are suspected to be more vulnerable to poor environmental conditions and especially to food deprivation. Being more impacted by early developmental conditions,

males should express a higher EBC variability than females. Such variation would make the detection of effects of EBC on other life-history traits easier and hide similar effects on females. However, we did not find any difference in EBC variability between sexes so that it invalidates this hypothesis.

Second male EBC could reflect individual quality and be a good predictor of future reproductive competences. Our results suggest that male quality would be more determinant for chick body condition than female quality, because of a higher offspring investment by males during the rearing period (Cézilly et al. 1994). Reproduction has been found to be more costly in females (Perrot et al. chapitre 4; Cézilly 1993; Tavecchia et al. 2001) and during chick rearing, females seem to need to restore their body condition by spending longer time periods than males in the colony before returning to adult foraging areas and by feeding on areas closer to the breeding site (Rendón et al. 2014). By doing so, males seem to compensate females' energy shortage by increasing investment in their offspring through increased foraging efforts during the rearing period. Males of higher EBC may be more efficient at foraging, leading to an increase in the body condition of their chick before fledging, explaining this positive relationship between father EBC and chick EBC.

Third, male phenotype can affect indirectly chick body condition by its influence over its partner's reproductive decisions (Braun and Champagne 2014; Brommer et al. 2014). Indeed, females are expected to invest more in reproduction when they are paired with an attractive male (Horváthová et al. 2011). Hence if male EBC influences positively their future attractiveness and reproductive performances, females paired with males of high EBC should allocate more energy in chick development during egg formation or chick rearing for example, and consequently leading to the production of chick of higher body condition.

The lack of female EBC effect on chick body conditions does not mean that long term effects of poor EBC are absent for females. If female EBC affects its future reproductive quality, the influence of EBC should be detected in reproductive stage where females invest the most. As egg productions is suspected to be costly in female flamingo (Cézilly 1993; Tavecchia et al. 2001), we could expect for example a link between female EBC and its capacity to invest in egg reflected by hatching success and chick weight at birth.

Finally, we did not find any effect of parental age on chick body condition. This result is in line with the study of Schmaltz et al. (2011) which showed that chick attendance was independent of parental age. As they also found that incubation performance increased with

age, they proposed that incubation act as a bottleneck in the greater flamingo, eliminating younger breeders, so that the remaining parents are equally able to assume the chick rearing step. Actually, another bottleneck may occur even earlier, at mating. In flamingos, young and old individuals were shown to perform courtship display of lower complexity than individuals of intermediate ages (Perrot et al. in press, chapitre 2), and to have a lower probability to breed afterward.. Furthermore, during colony establishment, old individuals were found to hold breeding sites of higher quality (Rendón et al. 2001) giving them a better chance to succeed reproduction. Hence, mate access, settlement at the breeding site, and incubation period could filter out individuals younger birds with poor breeding competence so that age has no influence on the final step of the chick provisioning period.

Finally our study emphasizes the importance of long-term consequences for fitness of early development in long-lived species. We showed that this long-term consequences probably differ between sexes, and may be related to different reproductive costs experienced by males and females (Perrot et al. chapitre 4). More investigations are needed to understand if the reproductive consequences of early body condition in the greater flamingo result from environmental conditions experienced during development or from the gene makeup, or both.

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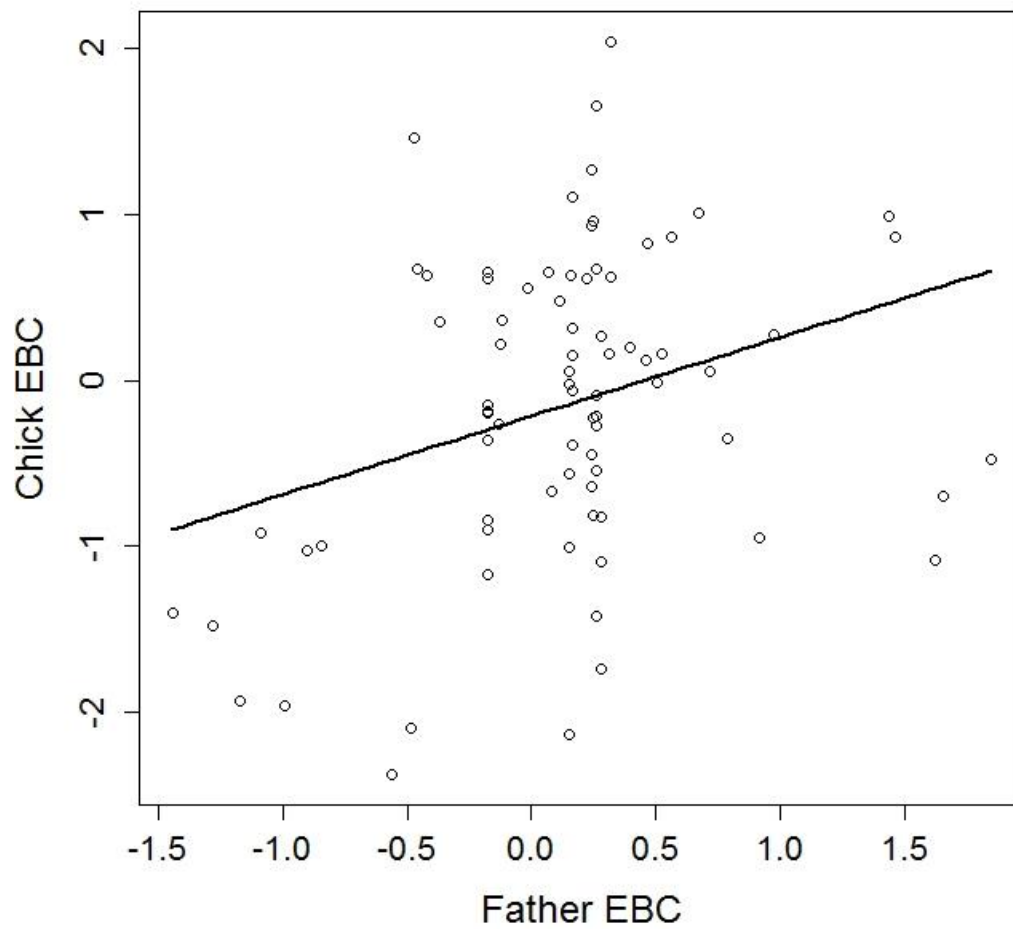


Figure 1. Relationship between father EBC and chick EBC in the greater flamingo according to model 1 ($\text{chick EBC} \sim \text{male EBC}$). Points correspond to the raw data.

Table 1. Model selection of the factors influencing chick body condition in the greater flamingo: age, age², early body condition (EBC) of both sexes and their interaction were tested on chick body condition. Models with a $\Delta AICc \leq 2$ from the best model are represented along with the null model.

Response variable	Models	df	logLik	AICc	$\Delta AICc$	weight
Chick EBC	Male EBC	3	-102.34	211.00	0.00	0.34
	Male EBC + Male age	4	-101.44	211.42	0.42	0.28
	Male EBC + Male age + Female age	5	-100.43	211.67	0.66	0.25
	Male EBC + Male age + Male age ²	5	-101.07	212.95	1.95	0.13
	Null	4	-106.39	216.93	5.93	

Table 2. Model-averaged estimates \pm SE and 95%CI of parameters explaining variations in chick body condition in greater flamingos. The relative importance of each factor is calculated by summing the AIC weights across the top models (Table 2) where the given factor appears (last column).

Response variable	Parameters	Estimate	SE	Confidence interval	Sum of weights
Chick EBC	Intercept	-0.14	0.10	(-0.35 ; 0.06)	
	Male EBC	0.59	0.20	(0.19 ; 0.99)	1
	Male age	-0.24	0.30	(-0.82 ; 0.35)	0.66
	Male age ²	-0.03	0.11	(-0.36 ; 0.57)	0.13
	Female age	0.10	0.23	(-0.25 ; 0.20)	0.25

Supplementary material

Relationship between male and female ages within pairs.

We aimed to get an estimation of male age according to female age, as well as of female age according to male age. For that, from a dataset of 298 pairs we investigated how female age vary according to male age, and reciprocally how male age vary according using non-linear using local polynomial regression using the loess function implemented the stats package of R. Relationship between age of mates are plotted in figure S1. We can observe that there is no differences between sexes concerning the age of the partner. Age of mate increase linearly with age until approximately 15 years old, next the slope became lower and age of mate reach a plateau around 25 years for finally decrease slightly in old ages.

Age assortative mating seems occurs only in young flamingos. Next, from 20 years, we detected any pattern between ages of mates.

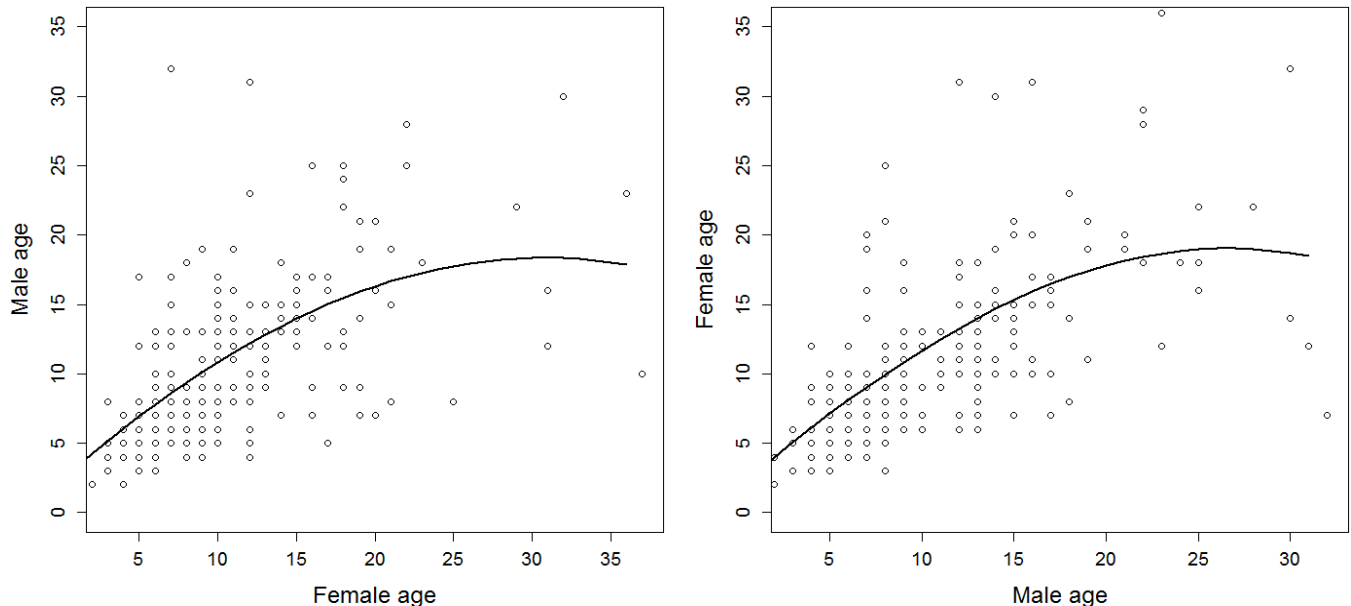
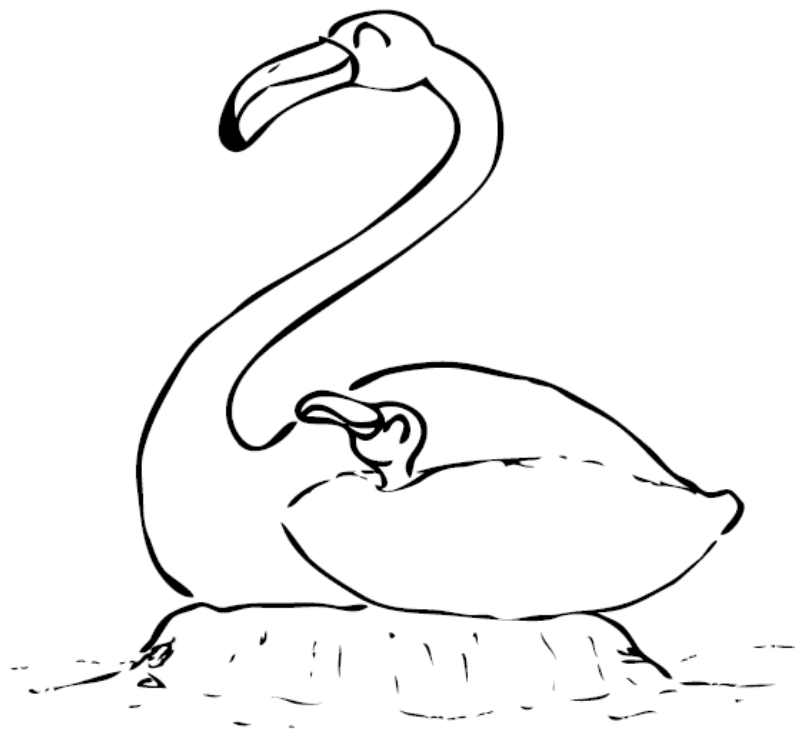


Figure S1: Relationship between age of mates within pair in the greater flamingo, a) male age according to female age, b) female age according to male age. Lines represent predictions from local polynomial regression with a degree of smoothing of 1.2. Points represent raw data.

**Sex-dependent consequences of age at first reproduction on
lifespan and lifetime reproductive success in the greater
flamingo (*Phoenicopterus roseus*)**



Sex-dependent consequences of age at first reproduction on lifespan and lifetime reproductive success in the greater flamingo (*Phoenicopterus roseus*)

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Abstract

Age at first reproduction (AFR) is a key life-history trait which is likely to be under selective pressure due to its direct influence on fitness. Early recruitment is expected to enhance fitness by increasing the number of reproductive events over the lifespan. However, the theory of ageing predicts that early reproduction should be associated with accelerated reproductive senescence and reduced longevity. We used long-term data from greater flamingo, *Phoenicopterus roseus*, a long-lived seabird, to address the relationship between AFR, lifespan and lifetime reproductive success (LRS) using multi-event models.

We found no AFR differences between sexes, however AFR consequences differ between males and females. In males, early breeder produce more chicks throughout their life and AFR had no influence on lifespan. Hence, males should breed as soon as possible in order to optimize their fitness, but they did not. Optimal AFR was 6-7 years in females. Females reproducing before 6 years expressed a higher mortality and produced fewer chicks during their life. Probably young females have a reduced ability to deal with the high physiological costs of reproduction. After 6 years, lifespan remained constant but LRS decreased for females reproducing later probably due to their shorter breeding career. We discuss the discrepancy between the theoretical optimal AFR for males and estimated male AFR. Our study shows that recruitment is a complex process driven by sex-related reproductive costs and highlights the need to investigate separately between sexes the AFR influence on fitness.

Introduction

Age at first reproduction is a key life-history trait which is likely to be under high selective pressure due to its direct influence on fitness (Stearns 1976; McGraw and Caswell 1996; Oli et al. 2002; Krüger 2005; Milot et al. 2011). Early recruitment should be selected if it increases the number of reproductive events over the lifespan (McGraw and Caswell 1996). Yet, breeding investment early in life may imply short-term costs by depressing subsequent survival probability or affecting subsequent breeding attempts. Indeed, early breeders, may not have gained enough experience yet in tasks required to master reproduction (Pradel et al. 2012) such as foraging for example (Greig et al. 1983), leading to an enhanced cost of reproduction. In this view, young individuals would delay recruitment because they are incapable to succeed reproduction at that time (i.e. constraint hypothesis; Curio 1983; Forslund and Pärt 1995) and in order to minimize costs of first reproduction (Aubry et al. 2009).

Early recruitment could also generate long-term costs causing premature senescence. Indeed, first reproduction is often assumed to initiate mechanisms that impede somatic repair, resulting in an association between early reproduction and accelerated senescence (Kirkwood and Rose 1991). The decision of when to start breeding would then be mediated by trade-offs between early recruitment, future reproduction and survival. Such trade-offs could be particularly important in long-lived species because of the high residual reproductive value in early life (Reid et al. 2003) and should lead to the evolution of an optimal age at first reproduction. Indeed, the restraint hypothesis (Pianka 1976; Forslund and Pärt 1995) predicts a positive correlation between age and reproductive investment. More precisely, more an individual age, more its reproductive prospects decrease. Consequently old individuals should invest a lot in reproduction in order to enhance their fitness. At the opposite, young individuals should restrain their reproductive investment by delaying maturation for example in order to ensure their self-maintenance and thus their future reproductive prospects, especially when reproduction is highly expensive.

Inter-individual variations in the age at first reproduction is widespread in long-lived species of different taxa (Congdon et al. 1993, Hadley et al. 2006, Becker and Bradley 2007, Fay 2016). Some individuals reproduce as soon as they have reached sexual maturity while others defer reproduction. This suggests that age at first reproduction is weakly canalized

(Stearns and Kawecki 1994), yet the causes and consequences of inter-individual variations remain poorly known. Temporal variability of the reproductive environment is likely to influence the age at recruitment by affecting the fitness expectations of commencing reproduction early in life (Tuljapurkar 1990). For instance, if the breeding site reaches its carrying capacity, it will be difficult for a young and inexperienced individual to acquire a breeding place. In that case intraspecific competition may push young individuals to postpone their first breeding (Ferrer et al. 2004). Environmental conditions and food availability variability may also explain the temporal variance in the age at first reproduction, recruitment being often related to body condition at the start of the breeding season (Gaillard et al. 2000; McMahon et al. 2003). However, these environmental effects do not explain why individuals sharing the same environment express variations in their age at first reproduction.

Intrinsic factors also play an important role on the decision of when to breed for the first time (Becker and Bradley 2007). Variation in age at first reproduction may be explained by heterogeneity in individual quality, i.e., “an axis of among-individual heterogeneity that is positively correlated with individual fitness” (Wilson and Nussey 2010). Inter-individual variation in quality could be consistent throughout life leading to positive correlations between life-history traits (Cam et al. 2002; Hamel et al. 2009; Fay et al. 2016). Hence, individuals of higher quality might incur smaller reproductive costs than others for equal reproductive investment so that they can afford to recruit earlier without important long-term consequences.

Finally, differences in life –history trade-off between sexes (Holtby and Healey 1990; Rice and Chippindale 2008; Han et al. 2015) may explain variance in age at first reproduction especially when there is an asymmetry in costs of early reproduction between sexes (Spear et al. 1995; Ludwigs and Becker 2003; Kim et al. 2011). Hence, individuals should opt for different strategies about when to reproduce for the first time according to the environment they experienced and their intrinsic characteristics.

A common approach to investigate the influence of age at first reproduction on fitness is to use lifetime reproductive success as a proxy of fitness (Brommer et al. 1998; Brommer et al. 1998; Krüger 2005; Descamps et al. 2006; Hawn et al. 2007; Kim et al. 2011). Indeed lifetime reproductive success of an individual i.e. the number of young raised during its lifespan, is likely to be a good indicator of its contribution to future generations and thus a rather reliable proxy of fitness (Clutton-Brock 1988; Newton 1989). Nevertheless, in wild populations, the

exhaustive monitoring of large number of individuals over a long time period is difficult; there are not only problems of detection but also problems in assessing reproductive status. Hence, the capture-recapture (CR) data are inevitably incomplete which is problematic for measuring age at first reproduction and lifetime reproductive success (Pradel et al. 1997; Rouan et al. 2009). Multi-event models (Pradel 2005) allow taking into account uncertainty in breeding state assignment and estimating directly composite indices of fitness such as age at first reproduction and lifetime reproductive success (Pradel et al. 1997; Rouan et al. 2009).

Here, we analyzed the ring resightings of 3769 greater flamingo (*Phoenicopterus roseus*) over a 36-year period to investigate (i) the inter-individual variability of age at first reproduction according to sex and cohort (ii) the presence of trade-off between early recruitment and survival and (iii) the effect of age at first reproduction on fitness. We developed a new approach using multi-event models implemented in a Bayesian framework to estimate for each individual its age at first reproduction (AFR), lifetime reproductive success (LRS) and lifespan.

The greater flamingo is a long-lived colonial bird that shows delayed maturity. While individuals reach sexual maturity at the age of 3, age at first reproduction is observed to vary between 3 and 9 years old (Pradel et al. 1997). Flamingos show no mate fidelity between consecutive breeding seasons, females lay a single egg, and both sexes are involved in courtship display, nest building, incubation and chick rearing duties (Johnson and Cézilly 2007) so that reproduction should be costly for both sexes. Nevertheless, females which reproduce for the first time before 7 years express a higher subsequent mortality than female which delay much later reproduction and than first breeder male whatever their age at recruitment (Tavecchia et al. 2001). This suggests that early breeding incurred a heavy cost in young females, but not in males. Hence, we expected different strategies between sexes in the age at first reproduction and in their consequences on individual fitness.

Material and methods

Species, study area and data collection

The greater flamingo can live up to 40 years in the wild and >60 years in captivity (Johnson and Cézilly 2007). Flamingos inhabit temporary and permanent coastal brackish wetlands and salt lakes all over the world where they feed on aquatic invertebrates and seeds (Johnson and Cézilly 2007). Since 1974, greater flamingos have bred on an artificial island located in the salt pans of Salin-de-Giraud (Camargue, southern France). Since 1977, on average, 12% (7–30%) of the chicks reared each year in the Camargue have been marked individually with PVC plastic rings engraved with a three- or four-digit alphanumeric code (Johnson and Cézilly 2007). Ring codes can be read through a telescope from a distance of up to 400 m. During the breeding season (from March to September), observers recorded breeding activities of ringed birds, from a nearby dike until 1982 and then between 1983 and 2013, from a tower hide built near the breeding island, 70 m from the closest nests (Johnson and Cézilly 2007). Flamingos were considered as breeders, when they were seen at the same location of the breeding island for ≥ 48 h, observed incubating an egg, or feeding a chick on the breeding island or in the crèche.

Multievent model design and parameters

We analyzed the resighting histories of 3769 flamingos (1916 females and 1853 males) ringed as chicks at the Fangassier colony of the Camargue from 1977 to 1987 and resighted at this colony between 1978 and 2014. Data collected during the breeding period (March to September) were analyzed using multievent capture-recapture models (Pradel 2005) set in a Bayesian framework. The Bayesian framework, unlike currently available frequentist implementations of multievent models, allowed us to estimate simultaneously age at first reproduction, lifespan, and LRS.

For each individual, we coded 5 distinct observation events at each breeding season : (0) *Not resighted* (flamingo not observed at the colony a given breeding season), (1) *Detected at the colony* (flamingo observed at the colony with no additional information regarding its breeding status), (2) *Detected only incubating* (flamingo observed incubating an egg or observed at the same location on the breeding island for at least 48 h but not observed afterward with a chick), (3) *Detected only with a chick* (flamingo observed feeding its chick on

the breeding island or later feeding its chick in the crèche outside the island but not observed at the incubation step), (4) *Detected in incubation and with a chick* (flamingo observed in incubation and later with a chick). Underlying those events, we distinguished 5 biological states: (1) Not yet Returned to the colony (NR); (2) Non-Breeder (NB); (3) Unsuccessful Breeder (UB); (4) Successful Breeder (SB); (5) Dead (D).

In the greater flamingo, breeding failure mainly occurs during incubation but after hatching, chicks mortality seems negligible (Schmaltz et al. 2011) so that we considered individuals observed with a chick as successful breeders, and those only seen during incubation as potential successful breeders. Hence, birds detected only during incubation are a mixture of birds that failed breeding and birds that were successful breeders but were undetected afterwards. It happens sometimes that individuals were observed at the colony, not detected during incubation but finally observed with a chick, yet this situation occurred rarely in the dataset. Thus, individuals observed at the colony but not re-observed during incubation or with a chick were considered as individuals that did not attempt to breed at all (Non-Breeders). Hence, individuals *detected at the colony* (1) are considered as non-breeders (NB), individuals *detected incubating* (2) are either unsuccessful breeders (UB) or successful breeders (SB), individuals *detected with a chick* are considered as successful breeders as well as individuals detected both in incubation and with a chick (SB). Finally, individuals that are not detected (0) can be in any of the five states (Fig.1).

We then defined three different kinds of parameters:

- p_t^r Resighting probability: probability that an individual is resighted, given that it is in state r at time t .
- φ_t^r Survival probability: probability that an individual in state r at time $t-1$ survives until t .
- ε_t^r Return probability: probability previously in state NR returns to its birth colony (Camargue) at time t , given that it survived.
- β_t Breeding probability: probability that an individual that is returning to the colony or has returned earlier breeds at time t , given that it survived.
- σ_t Success probability: probability that an individual breeding at time t breeds successfully.

From marking as a chick to its return to its birth colony an individual is set to be “Not yet Returned (NR)”. After its first encounter at the colony it integrates the pool of possible breeders and cannot reverse to the “NR” state. Individuals then move among states according

to a first-order Markov chain that can be described by the state-specific survival probabilities and the transition probabilities conditional on survival. The state process can be described by a transition matrix with departure states in rows and arrival states in columns and can be represented as the product PHI of a survival matrix by the conditional transition matrix:

$$PHI = \begin{bmatrix} \varphi_{NR} & 0 & 0 & 0 & 1 - \varphi_{NR} \\ 0 & \varphi_{NB} & 0 & 0 & 1 - \varphi_{NB} \\ 0 & 0 & \varphi_{UB} & 0 & 1 - \varphi_{UB} \\ 0 & 0 & 0 & \varphi_{SB} & 1 - \varphi_{SB} \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

$$* \begin{bmatrix} 1 - \varepsilon & \varepsilon * (1 - \beta) & \varepsilon * \beta * (1 - \sigma) & \varepsilon * \beta * \sigma & 0 \\ 0 & (1 - \beta) & \beta * (1 - \sigma) & \varepsilon * \beta * \sigma & 0 \\ 0 & (1 - \beta) & \beta * (1 - \sigma) & \varepsilon * \beta * \sigma & 0 \\ 0 & (1 - \beta) & \beta * (1 - \sigma) & \varepsilon * \beta * \sigma & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

The event process conditional on underlying states is described by an event matrix PSI (states in rows and events in columns) and the following detection probabilities:

- ***pNR***: the probability to detect an individual which has not yet returned to the colony. *pNR* is fixed to 1 at the time of the first capture as a chick. Later it is fixed to 0 as individuals that have not yet returned to the colony cannot be encountered.
- ***pNB***: the probability to detect non-breeders
- ***pINC***: the probability to see an individual in incubation
- ***pCHK***: the probability to see an individual with a chick. As the probability to see an individual with a chick (*pCHK*) is a non-identifiable parameter, we used external information (see S1 for explanations).

PSI

$$= \begin{bmatrix} 1 - p_{NR} & p_{NR} & 0 & 0 & 0 \\ 1 - p_{NB} & p_{NB} & 0 & 0 & 0 \\ 1 - p_{INC} & 0 & p_{INC} & 0 & 0 \\ (1 - p_{INC}) * (1 - p_{CHK}) & 0 & p_{INC} * (1 - p_{CHK}) & p_{CHK} * (1 - p_{INC}) & p_{INC} * p_{CHK} \\ 1 & 0 & 0 & 0 & 0 \end{bmatrix}$$

Age and sex effect on parameters

Considering the high computation time of Bayesian models we built one model. The choice of the different effect on parameters were decided based on our knowledge of the biology of flamingos.

In the greater flamingo, age has been found to influence survival, breeding propensity and breeding success (Tavecchia et al. 2001; Johnson and Cézilly 2007; Schmaltz et al. 2011; Pradel et al. 2012). Physiological maturity is reached at 3 years (Johnson and Cézilly 2007) but recruitment peaks at 8 (Pradel et al. 1997; Pradel et al. 2012). In addition, females starting to breed before age 7 show a reduced survival (Tavecchia et al. 2001). Therefore, we considered five age-classes: 0; 1; 2-6 ;7-14; 15+.

Survival (ϕ), breeding probability (β), breeding success probability (σ) and the probability to return at the colony (ϵ) were set to be age dependent.

Survival probability (ϕ), the probability to breed (β), and the probability to return at the colony (ϵ) were set to depend on sex. We considered that the probability to breed successfully was the same regardless of sex as the fate of breeding is linked for both sex of a pair. Finally, as the probability to detect an individual has also been found to be sex-dependent (Johnson and Cézilly 2007), we added a sex effect on detection (p).

Estimation of AFR (Age at First Reproduction), LRS (Lifetime reproductive success) and lifespan

We used the Bayesian modelling software JAGS (Plummer 2003) to implement our model using the *rjags* and the *R2jags* packages via R (version 3.2.0; R Development Core Team 2014). Model relies on a combination of two equations using the two matrices PHI and PSI defined above. Since there are more than two possibilities when moving from one state to the next and sometimes more than 2 events generated by a given state, the likelihood can no longer be based on the Bernoulli distribution, like in single-state capture-recapture models, but on the categorical distribution, which is a special case of the multinomial distribution with trial size equal to 1 (Kéry and Schaub 2012). We considered a matrix Z with elements $z[i,t]$ indicating the state of individual i at time t , and a matrix Y[i,t] the observed resighting history of an individual i at time t .

The state equation describes the state process, that is, it describes the state of an individual at time $t+1$, given its state at time t . To attain the state s at time t , an individual must survive from state r at $t-1$ to time t , and make the transition from r to s . This change of state is stochastic:

$$z[i,t] \sim \text{categorical}(\text{PHI} [z[i,t-1], t-1,])$$

The observation equation links the state to the event. It specifies the observation (or the lack of observation) of an individual at time t given its state at that time. This is also a stochastic process:

$$y[i,t] \sim \text{categorical}(\text{PSI} [z[i,t], t,])$$

As we had little prior knowledge, we used vague prior distributions for all the parameters. We chose uniform distributions (between $[0,1]$) for survival probability, return probability, breeding probability, breeding success probability and detection probability. We manually fixed the survival probability between 1985 and 1986 at 0.785 because of the cold spell which killed several thousand flamingos this winter (estimated in Lebreton et al. 1992) which allowed us to gain computation time.

To fit the multievent model we used MCMC simulations. We ran 30,000 iterations with 5,000 burn-in iterations. LRS was calculated for each individual at each MCMC iteration by counting the number of occasions where the state assigned was successful breeder along the estimated lifespan of the individual. We then calculated the mean LRS, as well as its standard variation, for each individual. In the same way, AFR was calculated for each individual, by calculating the age at which the breeder status was assigned to it for the first time. For each individual, we therefore obtained the mean AFR, as well as its standard deviation. Lifespan was also calculated for each individual at each MCMC iteration and corresponded to the age at which the state *Dead* was attributed for the first time to the individual. However as the maximum age of flamingo is higher than the number of occasions in our study, it happened that the state *Dead* was not attributed. When it was the case, the model attributed a lifespan which corresponded to the age of the individual at the last occasion. We therefore did not use the mean lifespan as it had no biological meaning. Rather we chose the most frequently estimated lifespan value and when the most frequently estimated lifespan value corresponded to the age of the bird at the last occasion, we considered that the individual was

still alive. Individuals considered as still alive were not considered for the subsequent analyses.

Finally, to assess convergence, we ran three chains and used Gelman and Rubin's convergence diagnostic from package coda. This diagnostic is based on a comparison of within-chain and between-chain variances (Gelman and Rubin 1992). We also checked visually the mixing of chains by looking at the traces of chains (with different initial values) and parameter densities.

Inter-individual AFR variability and its influence on lifespan and LRS.

We focused on individuals for which we had the entire lifespan and which had reproduced at least once in the Camargue. For that we removed individuals that were considered as still alive or had never bred. In addition even if the majority of individuals died before 20 year-old, several individuals survived over 30 years (Johnson and Cézilly 2007). So we selected individuals born in the first cohorts (i.e. between 1977 and 1984) to have a period of at least 30 years to resight them. Finally, Pradel et al. (1997) observed in the greater flamingo a marked stabilization of recruitment after age 10 yrs., suggesting that the individuals of more than 9 yr and observed breeding for the first time at their colony of birth were most likely to have bred in another colony before. So, to reduce the risk of AFR bias which would result from the heterogeneity of dispersal strategy in young birds, we removed individuals which reproduce at the Camargue colony for the first time after 9 yrs old, leading to a dataset of 674 individuals (314 females, 360 males).

We first investigated if the sex and cohort of individuals had an influence on AFR using Kruskal–Wallis tests by ranks. Next, we tested the influence of AFR on lifespan and the influence of AFR on LRS using generalized additive mixed models (GAMM) of the *mgcv* package implemented in R (Wood 2016). Generalized additive models (GAMs) were particularly adapted because we they allow to capture the shape of a relationship without prejudging of the issue. For each model, AFR was included as a fixed effect and cohort as a random effect to account for the non-independence of observations from individuals which fledged the same year. Analyses were performed separately between sexes in order not to miss differences in pattern between AFR and lifespan or between AFR and LRS.

Results

AFR ranged from 3 to 9 year-old with a peak at 6 and 7 year-old, the age at which 48 % of individuals recruited into the breeding population. Sex did not influence AFR (Kruskal-Wallis test : $\chi^2 = 0.04$, $df = 1$, $p = 0.85$), males and females having similar mean AFR (6.55 ± 0.08 SE and 6.58 ± 0.07 SE respectively). There was a clear cohort effect on AFR (Kruskal-Wallis test : $\chi^2 = 51.76$, $df = 7$, $p < 0.001$) with lower AFR for cohorts 1979 and 1980 (6.12 ± 0.12 SE and 6.15 ± 0.14 SE respectively) and higher AFR for cohorts 1983 and 1984 (7.09 ± 0.17 and 6.96 ± 0.12 , respectively). However the maximum difference in mean AFR between cohorts was lower than 1 year which may have few consequences in individual fitness in a species that can potentially live up to 50 years.

Lifespan ranged from 6 to 34 yrs in males and from 4 to 34 yrs in females. AFR had no effect on male lifespan ($df = 1$, $F = 2.38$, $p = 0.12$; Fig. 3a) but positively influenced females lifespan ($df = 2.64$, $F = 13.73$, $p < 0.001$) in a non linear way (Fig. 3b). Females which started to breed at age 3 appeared to die early at age around 13.5 yrs while with increasing AFR lifespan increases to reach a plateau at age 6 allowing a lifespan of about 22 years.

LRS ranged from 1 to 17 chicks in both sexes. In males, LRS variability was explained by AFR ($df = 1$, $F = 9.77$, $p = 0.002$). LRS was negatively and linearly influenced by AFR, with a production of two additional chicks for males which started reproduction at 4 yrs. old compared to males which started at 9 yrs. old (Fig. 4a). Female LRS was also influenced by AFR ($df = 2.62$, $F = 4.23$, $p = 0.009$) but following a doomed shape relationship (Fig. 4b). Females reproducing early produced fewer chicks during their life (less than four chicks on average for an AFR of 3 yrs.). The maximum LRS was obtained for an AFR around 6-7 yrs. where females are expected to produce around 6.5 chicks. However females which deferred too long saw their LRS decrease.

Discussion

Our results show that inter-individual variability of AFR is explained by cohort effects but not by sex in the greater flamingo. Nevertheless, our study suggests that even if AFR is similar between sexes, the cost-benefits balance of delayed recruitment differ between sexes. Males met the expectation that delayed breeding entails a cost in term of reduction of the overall reproductive value: males that started breeding late had a lower LRS than early starters. As male lifespan was independent of age at first reproduction, breeding early allows more breeding attempts and therefore gives the possibility to produce more fledglings. Hence, males should breed as soon as possible in order to optimize their fitness. However, few males reproduced early and their AFR ranged from 4 to 9 yrs. old. The negative covariations between AFR and LRS and the absence of trade-offs between AFR and lifespan suggest that heterogeneity in individual quality could be responsible for the AFR heterogeneity among males.

In females, relationship between AFR and LRS and between AFR and lifespan were more complex. Consistent with previous results (Tavecchia et al. 2001), females recruiting before 7 yrs express a higher mortality than later breeding females, highlighting a trade-off between early recruitment and self-maintenance. Probably young females would have a reduced ability to deal with the high physiological costs of reproduction, especially with the cost of egg production which can be energetically expensive (Monaghan and Nager 1997; Monaghan et al. 1998). Deferred reproduction could therefore allow females to gain more experience in reproductive-related tasks such as foraging (Greig et al. 1983) and therefore to minimize reproductive costs. The high mortality of early breeding females led to a shorter breeding lifespan and to fewer breeding occasions, which resulted in a lower LRS. In the same way, as from an AFR of 6-7 yrs. lifespan stabilizes, females which reproduced after 7 yrs. have also a reduced breeding lifespan compared to females with an AFR of 6-7 old, explaining the decrease of LRS. These relationships suggest the existence of an optimal AFR for females. Hence, for both males and females, the best way to maximize their LRS seems to increase their breeding career length as more as possible but with different constraints. In addition we found a strong positive correlation between LRS and breeding lifespan in both sexes (males: $R = 0.84$, $p < 0.001$; females: $R = 0.83$, $p < 0.001$).

Like in other species, differences in optimal AFR between males and females suggest that age at recruitment seems to be under different selective pressures between sexes (Pyle et al. 1997, Orell and Belda 2002, Hawn et al. 2007, Millon et al. 2010, Kim et al. 2011). However, even if optimal AFR seems to differ between sexes, like Pradel et al.(1997) we did not found any differences in mean age at recruitment between sexes. In addition, while in females the optimal AFR fits with our estimated mean AFR (6.55 ± 0.08 SE), it is not the case for males. Indeed, although the best strategy for males should be to breed as soon as sexual maturity is reached, males also delay reproduction at similar age than females (mean AFR: 6.58 ± 0.07 SE). Hence, males must face other constraints that prevent them from reproducing at younger ages. One could be the lack of competitiveness of young males during courtship display so that they fail to find a mate and are constrained to defer reproduction. Actually, during mate access process, males displays more interactions and aggressiveness among them than females, suggesting a higher competition among males (Perrot et al. chapitre 1). Hence, only the best individuals among young males could manage to find a partner and consequently the cost of first reproduction in young males might not be detected afterwards. A differential cost of reproductive effort has been demonstrated for other vertebrates such as Richardson's ground squirrel (*Urocitellus richardsonii*), in which the mating period is costly for males but not for females, and reproduction appears to be costly for females but not for males (Michener and Locklear 1990).

Environmental conditions that flamingos experienced seem also to affect age at first reproduction as individuals of different cohorts responded by recruiting at different ages, with in particular low AFR for cohorts 1979, 1980, and 1981. We suspect that this pattern is the result of a density-dependence process (Ferrer et al. 2004; Krüger 2005; Cooper et al. 2012). Greater flamingos nest on a breeding island which has limited carrying capacity so that the number of 'hopeful' breeders at the onset of colony settlement exceeds the number of nesting pairs that the breeding site can accommodate. In addition, the Camargue colony is a particularly large and competitive so that younger individuals queue to breed (Nager et al. 1996, Rendón et al. 2001, Balkız et al. 2010). In January 1985 due to severely cold spells in Camargue several thousands of flamingos died in the region, which could have reduced the competition for nest access the following seasons at the colony. Indeed, Pradel et al. (1997) found an increase of the recruitment rate for breeding season following the severe winter. Hence such favorable conditions may have prompted the recruitment in 1985 of younger

classes affecting therefore AFR of individuals of the cohorts 1979, 1980, and 1981 which had at that time 6, 5 and 4 years respectively. Consequently, the differences of AFR between cohorts were more likely to be the result of conditions at the time of breeding which were more or less favorable to recruitment, and thus to a temporal effect of environmental conditions.

To conclude, our results highlight complex sex-specific processes in the tuning of age at first reproduction in the greater flamingo. Sex-related reproductive costs, individual experience, intra-sexual competition and inter-individual competition for nest space explain variations in AFR which have important consequences for individual fitness. Our study emphasized also the need to investigate separately between sexes the links between age at recruitment, lifespan and lifetime reproductive success to highlight different selective pressures, even if *a priori* individuals of both sexes express no difference in the decision of when to start breeding.

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Figure legends

Figure 1. Links between events and states of the multievent model.

Figure 2. Mean (\pm SE) AFR of individuals according to cohorts in the greater flamingo (*Phoenicopterus roseus*) breeding in the Camargue.

Figure 3. Relationship between AFR and lifespan in a) males and b) females in the greater flamingo (*Phoenicopterus roseus*) according to the GAMM model (Lifespan \sim AFR + 1 | Cohort). Points correspond to the arithmetic mean (\pm SE) of observed AD grouped in classes of AFR.

Figure 4. Relationship between AFR and LRS in the greater flamingo (*Phoenicopterus roseus*) according to the GAMM models (LRS \sim AFR + 1 | Cohort), a) in males, b) in females. Points correspond to the arithmetic mean (\pm SE) of observed LRS grouped in classes of AFR.

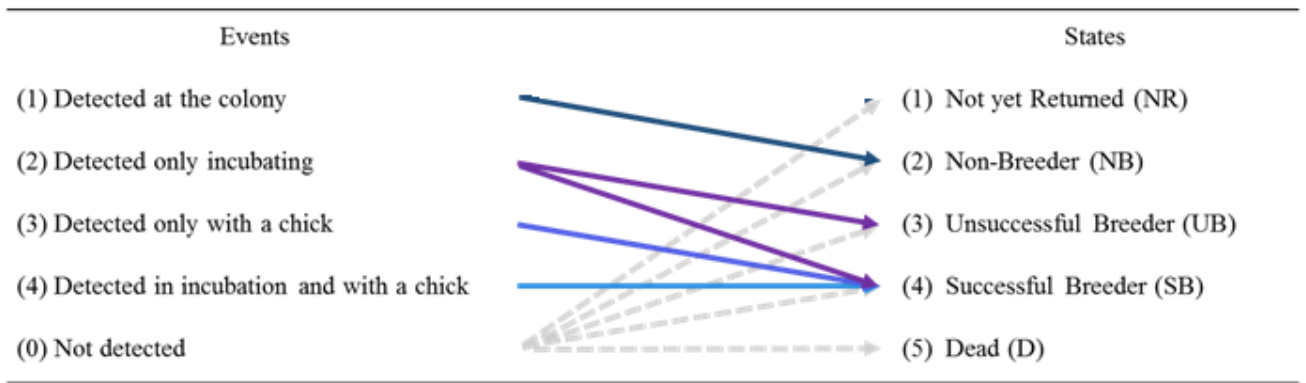


Fig.1

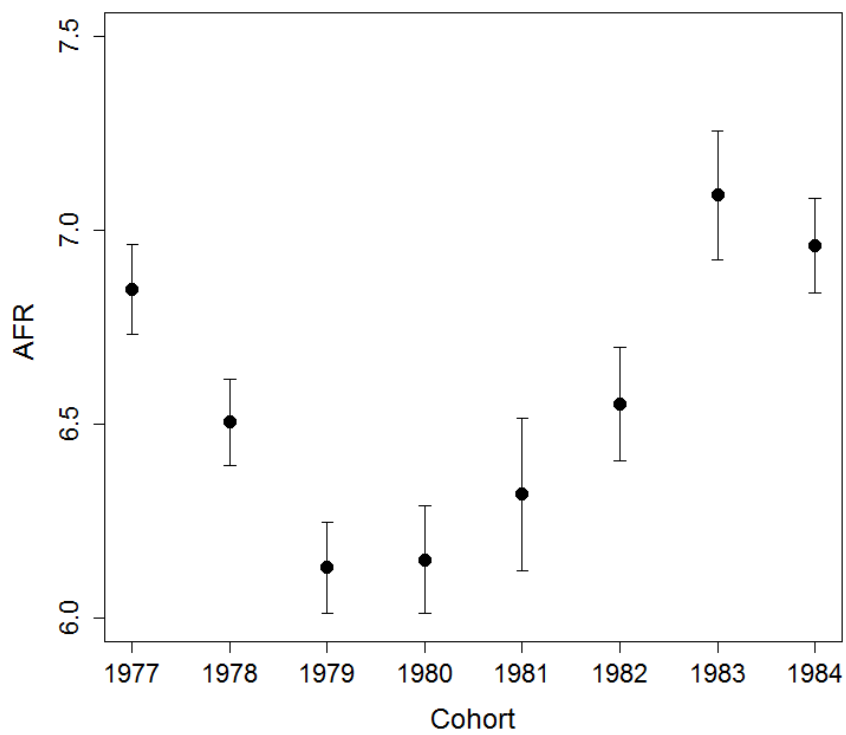


Fig.2

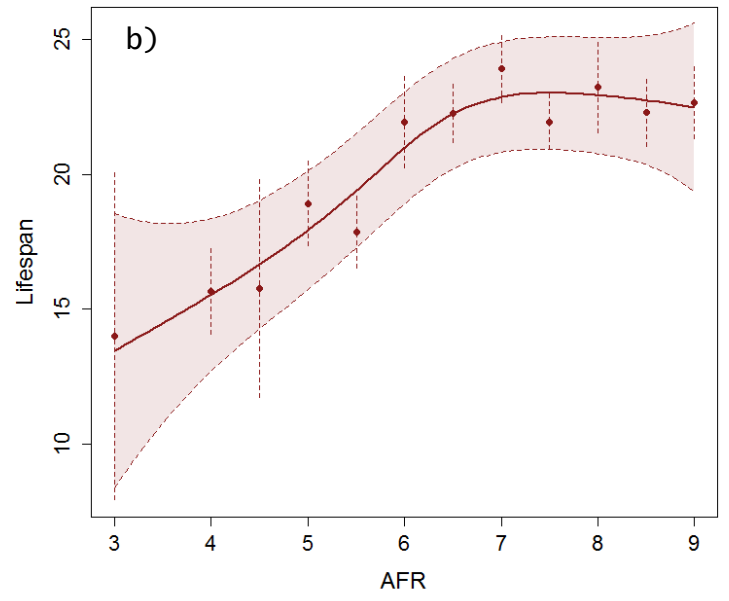
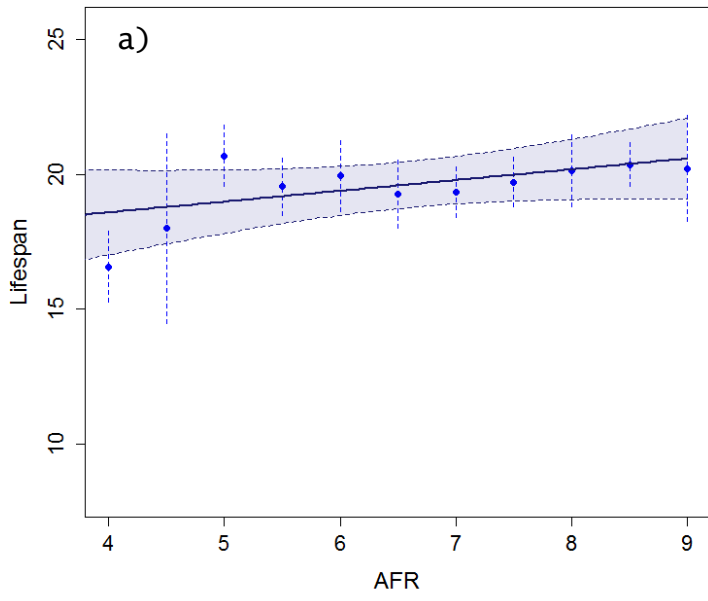


Fig.3

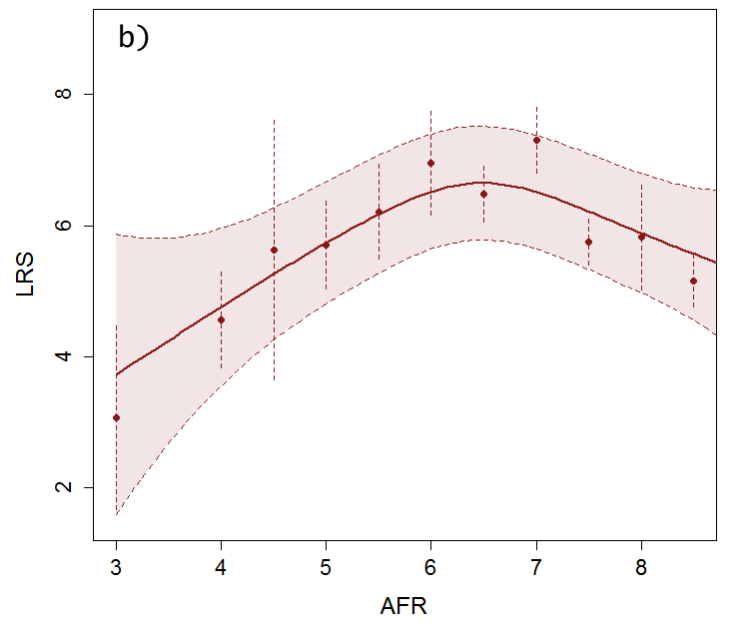
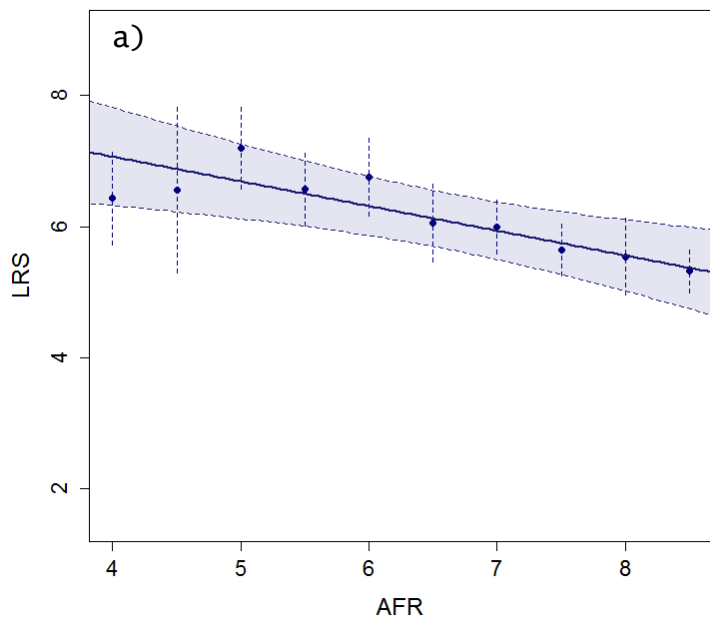


Fig.4

Supplementary material

S1 Estimation of the probability to see an individual with a chick (pCHK)

The proportion of successful breeders that were seen with a chick at time t can be calculated from the following equation:

$$pCHK(t) = 1 - \delta SB(t)$$

with $\delta SB(t)$ is the proportion of successful breeders that were only seen at the incubation stage a given year t (figure 2). Let us call $B(t)$ the number of ringed individuals observed with a chick and $A(t)$ the number of successful breeders only observed during incubation, a given year t . Hence,

$$\delta SB(t) = \frac{A(t)}{A(t)+B(t)}.$$

While $B(t)$, is perfectly known, we need to estimate $A(t)$. $A(t)+B(t)$ is the total number of successful breeders, observed, for a given year t .

$SB_{Tot}(t)$ is the total number of successful breeders at the colony for a given year and P_{marked} is the proportion of marked individuals in the population. We can indirectly estimate $SB_{Tot}(t)$ since we have an estimation of the total number of chicks at the crèche each year since 1947 (Johnson and Cézilly 2007, Béchet, unpublished data). Thus, for each year, $SB_{Tot}(t) = 2 * \text{Number of chicks at the crèche this year}$.

We have,

$$A(t) + B(t) = SB_{Tot}(t) * P_{marked}(t)$$

Then, what we need to estimate is the percentage of the population that is marked. Marking started in 1977. We know the number of chicks that are marked for each cohort $N_{cm_{coh}}$, and we have an estimation of the total number of chicks at the crèche $N_{ct_{coh}}$.

$s_{\rightarrow t}$ is the probability for an individual of a given cohort to survive until the year t .

bp_{t-coh} is the breeding propensity of an individual from a given cohort at year t .

To estimate this probability, we used results from a previous study of Lebreton *et al.* (1992) and results obtained by Pradel *et al.* (2012) to estimate breeding propensity.

Hence,

$$P_{marked} = \frac{\sum_{coh=1}^t Ncm_{coh} * s_{\rightarrow t} * bp_{t-coh}}{\sum_{coh=1}^t Nct_{coh} * s_{\rightarrow t} * bp_{t-coh}}$$

Finally,

$$\delta SB(t) = \frac{A(t)}{A(t) + B(t)} = \frac{SBTot(t) * P_{marked}(t) - B(t)}{SBTot(t) * P_{marked}(t)}$$

S2 Model outputs

Table 1: Estimation of return probabilities (ϵ), breeding probabilities (β), success probabilities (σ), and survival probabilities (φ) according to sexes, age classes and states

Return probability				
	Female		Male	
Age classes	mean	se	mean	se
0	0.1173	0.0003	0.1540	0.0004
1	0.0381	0.0004	0.0620	0.0005
2-6	0.2661	0.0002	0.2685	0.0002
7-14	0.1705	0.0002	0.2113	0.0003
≥ 15	0.2203	0.0006	0.2808	0.0009

Breeding probability				
	Female		Male	
Age classes	mean	se	mean	se
2-6	0.1577	0.0001	0.1846	0.0001
7-14	0.3890	0.0001	0.4485	0.0001
≥ 15	0.3573	0.0002	0.4352	0.0002

Success probability		
Age classes	mean	se
2-6	0.9962	0.0001
7-14	0.9988	0.0000
≥ 15	0.6101	0.0002

Survival probability - Not yet Returned to the colony				
	Female		Male	
Age classes	mean	se	mean	se
0	0.8224	0.0082	0.8134	0.0085
1	0.9986	0.0014	0.9982	0.0019
2-6	0.9995	0.0005	0.9993	0.0007
7-14	0.9987	0.0013	0.9981	0.0018
≥ 15	0.9936	0.0062	0.9874	0.0124

Survival probability - Non-Breeder

Age classes	Female		Male	
	mean	se	mean	se
1	0.9817979	0.01613704	0.9745568	0.01972414
2-6	0.9817122	0.003916951	0.976913	0.003913006
7-14	0.9545882	0.003210653	0.9362518	0.004166004
≥ 15	0.9325125	0.003508844	0.9325246	0.004721696

Survival probability - Unsuccessful Breeder

Age classes	Female		Male	
	mean	se	mean	se
2-6	0.5199855	0.2879461	0.5413196	0.281482
7-14	0.5670139	0.2845807	0.4922366	0.2872893
≥ 15	0.9835196	0.01083793	0.9376654	0.01361799

Survival probability - Successful Breeder

Age classes	Female		Male	
	mean	se	mean	se
2-6	0.9797904	0.01252169	0.9934446	0.005842572
7-14	0.9984005	0.001450296	0.9946883	0.003180254
≥ 15	0.997133	0.00249821	0.9911436	0.005309263

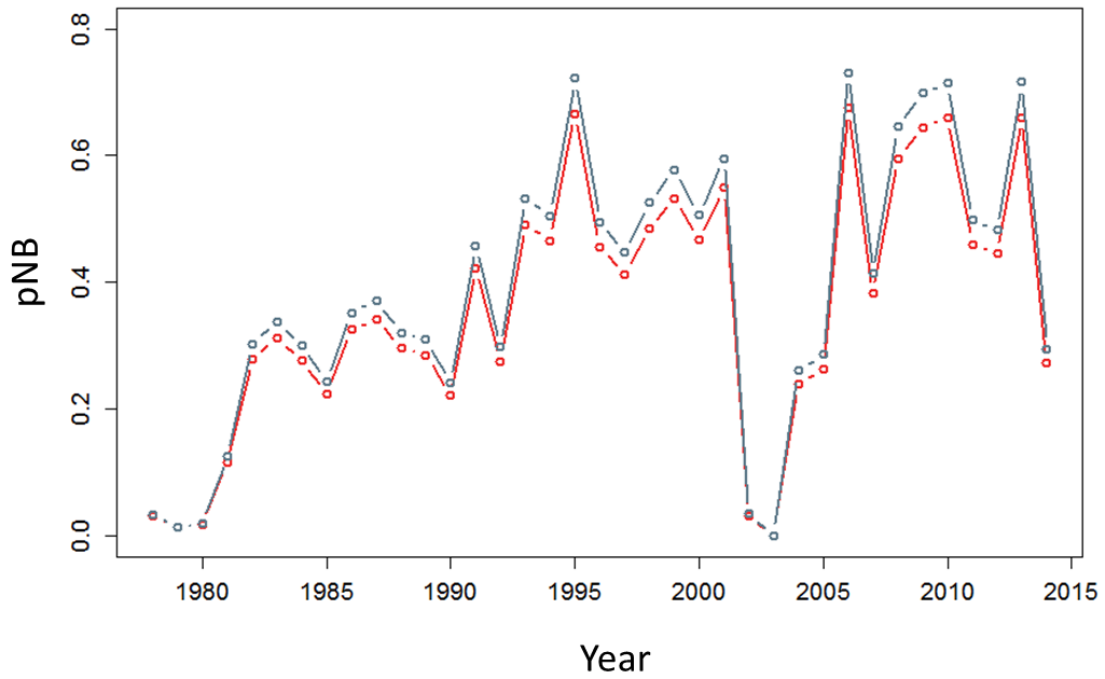


Figure S2 A: Probability to detect a non-breeder individual according to year and sexes (grey = males, red = females)

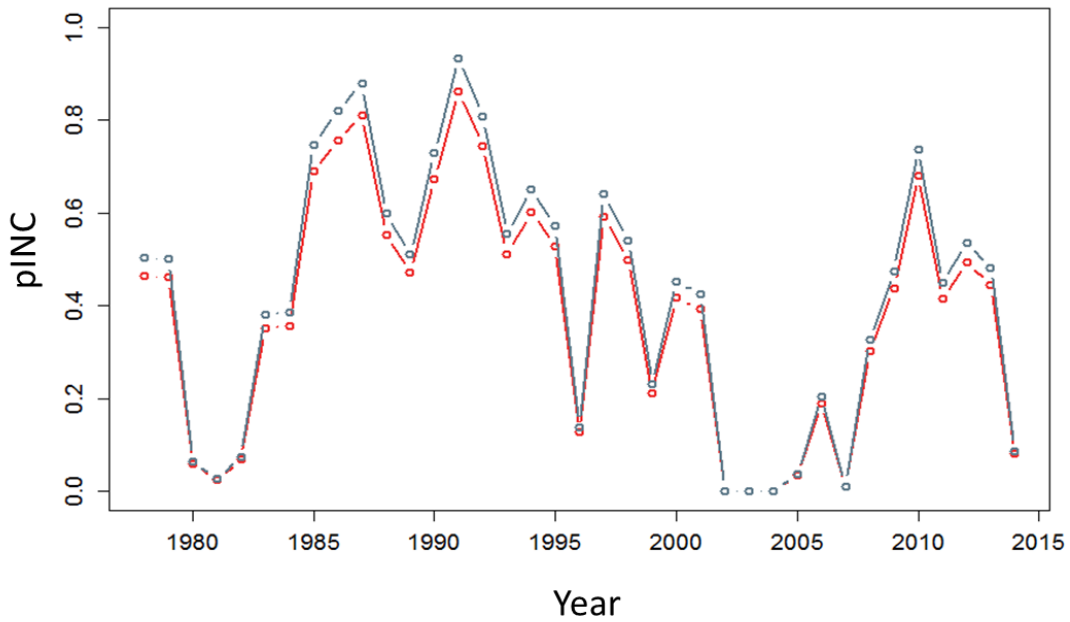


Figure S2 B: Probability to detect an individual in incubation according to year and sexes (grey = males, red = females)

Principaux résultats

Les différences liées au sexe dans les parades nuptiales collectives des flamants roses.

Dans le premier chapitre, notre but était de regarder si chez cette espèce monogame avec partage des soins parentaux, les signaux sexuels exprimés durant la parade nuptiale différaient entre les sexes. Les résultats exposés ici mettent en évidence une structure des séquences comportementales similaire entre les sexes. Cependant, avec l'âge on observe une divergence entre les sexes des interactions entre individus pouvant parfois être agonistiques. Les jeunes mâles et les femelles présentent ces interactions à un taux relativement faible. En revanche, le nombre d'interactions augmente avec l'âge chez les mâles. Ces résultats suggèrent une compétition intra-sexuelle plus intense chez les mâles que chez les femelles ainsi qu'une hiérarchie de dominance liée à l'âge lors de la parade nuptiale. Enfin, les femelles expriment une coloration du plumage plus intense que les mâles. La coloration rose du plumage étant due aux caroténoïdes, antioxydants indispensables au développement de l'embryon dans l'œuf, les femelles investiraient d'avantage dans la coloration afin d'informer de leur capacité à investir dans la descendance.

Notre étude montre donc une grande similarité des parades nuptiales entre les deux sexes, ce qui est en accord avec le monomorphisme des signaux sexuels attendus chez les espèces monogames où les rôles des sexes sont peu différenciés. Mais, malgré cette grande similarité, des différences persistent révélant une compétition intra-spécifique plus prégnante chez les mâles.

La complexité des parades nuptiales varie de façon non linéaire avec l'âge et prédit le statut reproducteur des flamants roses.

Dans ce second chapitre nous nous sommes intéressés à une composante particulière de l'organisation des séquences de mouvements composant la parade nuptiale, qui est la complexité. La complexité lors des parades est considérée chez certaines espèces comme un signal honnête de la qualité des individus et notamment de leurs performances

reproductrices. Nous avons montré un effet quadratique de l'âge sur cette complexité. Les jeunes individus et les individus âgés réalisent des séquences comportementales moins complexes que les individus d'âges intermédiaires. Nous proposons pour expliquer ce patron, un processus de maturation chez les jeunes individus et de la sénescence chez les individus âgés. De plus, la complexité des parades semble impliquée dans le choix du partenaire sexuel puisque les patrons d'appariement des partenaires révèlent une homogamie pour la complexité. Enfin, la complexité des parades nuptiales influence positivement la probabilité de devenir un reproducteur ce qui supporte l'idée que la complexité est un signal honnête de la qualité des individus.

Influence de la condition corporelle juvénile des mâles avant l'envol sur la condition corporelle de leur poussin

L'objectif du 3ème chapitre était de regarder l'influence des caractéristiques individuelles des parents sur la condition corporelle des jeunes, caractère connu pour influencer par la suite la valeur adaptative des individus. Nous nous sommes intéressés plus particulièrement à l'âge des parents ainsi qu'à leur condition corporelle lorsqu'ils étaient au stade de poussin, juste avant l'envol. La condition corporelle des poussins n'est pas affectée par l'âge des parents. En revanche, la condition corporelle juvénile des mâles influence positivement la condition corporelle de leur poussin (et ce quel que soit leur âge en tant que parent), alors que la condition corporelle juvénile de la femelle ne semble pas avoir d'impact. Si l'on part du postulat que la condition corporelle avant l'envol influence les futures performances reproductrices de l'individu, ce résultat suggère que les performances reproductrices des mâles jouent un rôle plus important que celle des femelles pour produire un poussin en bonne condition. Nous expliquons ce résultat par l'effort de nourrissage du poussin plus élevé chez les mâles durant la fin de la période d'élevage. La condition corporelle juvénile semble ainsi entraîner des conséquences reproductives à long terme différentes selon les sexes.

Conséquences de l'âge à la première reproduction sur la durée de vie et le succès reproducteur global chez le flamant rose : les différences entre les sexes

L'âge à la première reproduction est un trait d'histoire de vie clé susceptible d'être sous pression sélective en raison de son influence directe sur la valeur adaptative de l'individu. Le but de ce dernier chapitre était donc de comprendre qu'elles étaient les conséquences de l'âge à la première reproduction sur la valeur adaptative chez le flamant rose. Pour cela nous avons étudié le lien entre l'âge à la première reproduction et la durée de vie de l'individu ainsi qu'entre l'âge à la première reproduction et le succès reproducteur global.

L'âge moyen à la première reproduction ne diffère pas entre femelles et mâles (entre 6 et 7 ans). Les femelles se reproduisant avant 6 ans affichent une courte durée de vie reflétant probablement une capacité réduite à faire face aux coûts physiologiques de la reproduction. Après 7 ans, la durée de vie étant constante, plus une femelle se reproduit tard plus sa carrière de reproducteur diminue conduisant ainsi à une baisse du succès reproducteur global. Ainsi, l'âge optimal estimé de première reproduction des femelles est de 6-7 ans ce qui correspond à l'âge moyen de première reproduction.

Chez les mâles, l'âge de première reproduction n'impacte pas la durée de vie, mais influence négativement le succès reproducteur global. Ceci suggère que les mâles ne sont pas soumis à un compromis entre première reproduction et survie/reproduction future. Ainsi pour optimiser leur valeur adaptative, les mâles devraient se reproduire le plus tôt possible. Mais ce n'est pas le cas. En effet, les mâles se reproduisent en moyenne à 6-7 ans suggérant un accès difficile à la reproduction les contraignant à repousser leur première reproduction.

Notre étude montre l'importance d'étudier les deux sexes séparément quant aux conséquences à long termes de l'âge à la première reproduction. Car même si les deux sexes expriment les mêmes patrons d'âge au recrutement, les mécanismes sous-jacents ne sont pas nécessairement les mêmes.

Discussion

Patrons entre âge et traits reproducteurs chez le flamant rose

En début de vie

L'ensemble des études portant sur l'influence de l'âge sur les traits reproducteurs des flamants roses décrivent une faible performance des jeunes reproducteurs. Les jeunes individus réalisent des parades nuptiales de plus faible complexité (chapitre 2), se reproduisent sur des sites de moins bonne qualité (Rendón et al. 2001), et ont une plus faible probabilité d'achever la phase d'incubation (Schmaltz et al. 2011). Cette augmentation de la qualité des traits reproducteurs avec l'âge en début de vie peut résulter d'un processus de maturation et d'acquisition d'expérience dans les tâches liées à la reproduction (Pradel et al. 2012). Par exemple, l'augmentation de la complexité des parades nuptiales en début de vie suggère l'acquisition de compétences motrices avec l'âge.

L'une des conséquences de ces processus de maturation et d'acquisition d'expérience est l'augmentation de la compétitivité des individus avec l'âge. Cette variation âge-dépendante de la compétitivité peut engendrer une hiérarchie de dominance entre les individus qui, à son tour, peut entraver la reproduction de jeunes individus (Collis and Borgia 1992). Chez le flamant rose, l'augmentation des comportements d'agressivité avec l'âge chez les mâles durant les parades nuptiales (chapitre 1) suggère l'inhibition des comportements de parade des jeunes individus ou encore leur exclusion des groupes de parades par leurs aînés. De même, la monopolisation des sites de qualité par les individus âgés laisse penser à une exclusion des jeunes individus de ces sites (Rendón et al. 2001). De plus, le fait que les mâles retardent leur première reproduction par rapport à l'âge optimal estimé de première reproduction suggèrent qu'ils font face à des contraintes quant à l'accès à la reproduction (chapitre 4).

Cependant, les études chez le flamant rose à propos du lien entre traits reproducteurs et âge sont des études transversales. Il n'est donc pas possible de savoir si ces patrons sont le résultat d'un processus se jouant au niveau individuel (à savoir, la plasticité phénotypique, l'amélioration des compétences, la sénescence) ou au niveau populationnel (la disparition ou

l'apparition de certains phénotypes, voir « selection hypothesis » Curio 1983; Forslund and Pärt 1995; Cam and Monnat 2000). Seules les études longitudinales permettent de discriminer la part de variation intra-individuelle et la part de variation inter-individuelle expliquant ces corrélations entre traits reproducteurs et âge des individus. Le programme de suivi à long terme des flamants roses détient à ce jour des données qui permettraient de répondre à ces questions. Par exemple, en Camargue à chaque saison de reproduction, lorsqu'un individu bagué est observé en reproduction l'emplacement de son nid sur le site de reproduction est relevé. Ce site exprime une hétérogénéité dans la qualité des différents emplacements des nids. Tout d'abord les individus se reproduisant sur l'îlot sont moins exposés aux prédateurs que les individus se reproduisant sur la digue avoisinante et le succès reproducteur y est beaucoup plus élevé. Ensuite au sein de l'îlot les nids en périphérie sont plus exposés aux intempéries que les nids centraux. Nous avons donc 3 niveaux de qualités de l'emplacement du nid. Il serait donc possible de regarder l'évolution de la qualité de la localisation du nid au cours de la vie des individus et donc en fonction de leur âge.

La sénescence

Le déclin de la complexité des parades nuptiales pour les âges avancés suggère la présence de sénescence chez ces individus (chapitre 2). Il s'agit de la première trace de sénescence chez les flamants roses sauvages. En effet les études précédentes chez cette espèce ont mis en évidence une augmentation de la survie et une augmentation des performances reproductrices avec l'âge mais n'ont décelé aucun signe de sénescence (Tavecchia et al. 2001; Schmalz et al. 2011). Cependant, l'âge maximum des individus présents dans ces jeux de données était respectivement de 14 ans et de 19 ans. Si l'on considère que les flamants pourraient vivre jusqu'à 50 ans dans la nature (Cézilly et al. 1996; Tavecchia et al. 2001), la non-détection de signe de sénescence n'est pas étonnante. Le faible niveau de complexité des parades nuptiales chez les individus âgés suppose qu'une mise à jour des études liant âge, survie et performance reproductive devrait être envisagée. Aujourd'hui, le jeu de données comprend des individus âgés de 1 an à 39 ans. La gamme d'âges commence à devenir vraiment intéressante pour l'étude des variations des paramètres démographiques avec l'âge et en particulier pour l'étude de la sénescence.

Age et sélectivité des étapes de la reproduction

Chez le flamant rose, l'âge influence différents traits reproducteurs eux-mêmes impliqués dans différentes étapes de la reproduction. Dès l'accès au partenaire, l'âge influence la reproduction par son lien avec la complexité des parades nuptiales. Cette complexité est fortement suggérée comme étant un signal honnête de la qualité des individus impliqué dans le choix du partenaire sexuel (chapitre 2). Ainsi les jeunes et les individus âgés qui exécutent des parades de moindre complexité devraient plus difficilement trouver un partenaire sexuel, compromettant leur accès à la reproduction. L'accès au partenaire sexuel apparaît comme une première étape sélective susceptible d'éliminer les individus les moins performants qui seraient dans ce cas les jeunes individus et les âgés. Un deuxième filtre semble se produire lors de l'accès au site de reproduction, les jeunes individus étant exclus des sites de qualité (Rendón et al. 2001). L'incubation, comme chez d'autres espèces d'oiseaux longévives (Croxall and Ricketts 1983; Tveraa et al. 1997) s'avère être une étape critique de la reproduction éliminant les individus peu performants et en particulier les jeunes individus (Schmaltz et al. 2011). En revanche, une fois l'incubation passée, l'âge des individus ne semble plus avoir d'influence sur la capacité à réussir la reproduction. Aucun effet de l'âge n'a été décelé ni sur la capacité à élever le poussin jusqu'à l'envol (Schmaltz et al. 2011), ni sur la condition corporelle des poussins (chapitre 3). Ces résultats supposent une plus faible variabilité dans la performance reproductrice des individus, une fois l'incubation passée.

Ainsi, l'ensemble des étapes de la reproduction semble par leur sélectivité réduire progressivement la variabilité dans la performance reproductrice individuelle du pool de reproducteurs et en conséquence la gamme d'âges associée. Plus précisément on s'attend à ce que les jeunes individus et les individus les plus âgés (sous l'hypothèse de sénescence) soient progressivement éliminés du pool de reproducteurs. Cette sélectivité ne semble pas être constante au cours de la saison de reproduction puisque elle apparaît être élevée de l'accès au partenaire sexuel à l'incubation puis considérablement diminuer une fois l'éclosion passée.

Les données brutes de la saison de reproduction 2015 montrent effectivement une diminution de la proportion des jeunes individus observés au sein du pool de reproducteurs au cours de la reproduction (Fig. 5). La proportion des individus âgés de 3 à 6 ans diminue de 6.2% durant les parades nuptiales à 0.4% pour la période d'élevage des poussins. De même les individus âgés de 7 à 11 ans représente 20.8% des individus observés en parade et

seulement 13.5% des individus observés durant la période d'élevage. Mais contrairement à ce à quoi on pourrait s'attendre sous l'hypothèse de sénescence les individus âgés de plus de 30 ans restent très présents dans le pool de reproducteurs durant la période d'élevage (18.5 %). La diminution de la proportion relative des jeunes individus (< 12 ans) au cours de la saison de reproduction pouvant être responsable de l'augmentation de la proportion relative des autres classes d'âge, nous avons retiré ces jeunes individus du jeu de données. Et même dans ce cas-là, nous observons une augmentation de la proportion relative des individus âgés au cours de la saison (14% en parade nuptiale, 21 % pour la période d'élevage). Néanmoins ces résultats sont à interpréter avec précaution puisqu'il s'agit de données brutes ne prenant pas en compte la probabilité de détection et l'incertitude liée au statut reproducteur.

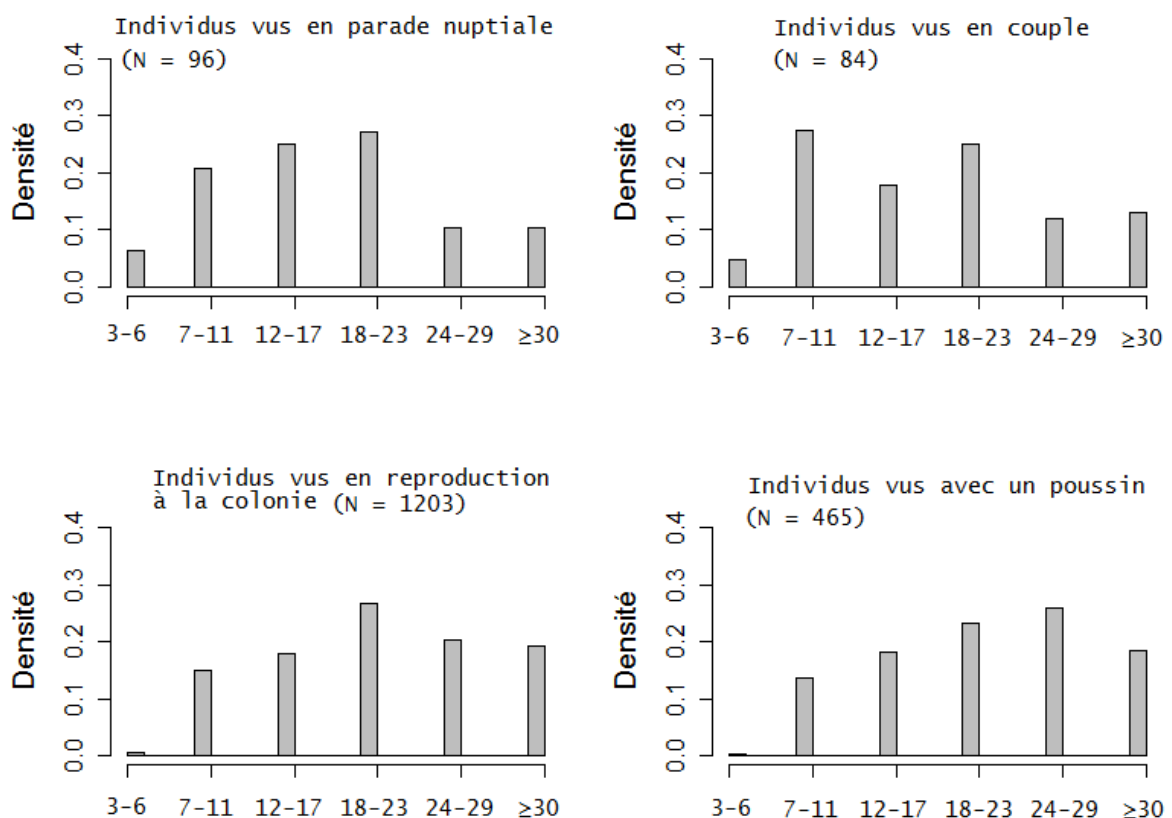


Figure 5 : Distribution en classe d'âge des individus observés en parade nuptiale, en couple, en reproduction à la colonie durant l'incubation, et avec un poussin

Accès à la reproduction et succès reproducteur

La présence probable de sénescence lors des parades nuptiales suggère qu'il est essentiel de prendre en compte les étapes menant à l'accès à la reproduction. Dans le cas des flamants roses, il est possible qu'il y ait une exclusion des individus sénescents dès la phase de l'accès au partenaire sexuel conduisant par la suite à une non détection de la sénescence au niveau des autres traits reproducteurs (succès d'éclosion, condition du poussin, comportements de nourrissage et succès reproducteur). L'accès à la reproduction est une étape importante de la reproduction qui agit comme un filtre éliminant les individus les moins aptes à la reproduction. Une grande partie des études sur l'influence de l'âge sur la reproduction des individus se focalisent principalement sur le succès reproducteur (Nussey et al. 2009; Kervinen et al. 2015), excluant ainsi une partie de la population et pouvant conduire à des conclusions erronées. Si les étapes menant à l'accès à la reproduction (accès au partenaire sexuel, accouplement, accès au site de reproduction) sont difficilement observables, s'intéresser à la probabilité de se reproduire à travers des études longitudinales est une méthode qui prend en compte la totalité des individus et pas seulement ceux présents sur le site de reproduction (Devries et al. 2008; Pradel et al. 2012; Souchay et al. 2014). En revanche cela ne nous dit pas si les individus considérés « non reproducteur » ont tenté de se reproduire et se sont heurtés à des obstacles pour accéder à la reproduction, ou si ces individus ont « décidé » de ne pas se reproduire.

Condition corporelle avant l'envol

Nous avons montré une influence positive de la condition corporelle des pères lorsqu'ils étaient juvéniles sur la condition corporelle de leur poussin avant l'envol. Ceci corrobore l'hypothèse de conséquences à long terme de la condition corporelle avant l'envol de l'individu. En effet, la condition corporelle juvénile est connue comme pouvant influencer de nombreux traits d'histoires de vie tels que la survie et le succès reproducteur (Lindström 1999). La condition corporelle juvénile est donc elle-même un trait d'histoire de vie clé que ce soit au niveau individuel par son influence sur la valeur adaptative de l'individu ou au niveau populationnel par son influence sur les paramètres démographiques de la population.

Le flamant rose, par le suivi à long-terme dont il fait l'objet est un modèle d'étude idéal pour explorer les conséquences de la condition corporelle juvénile sur les traits d'histoire de vie. Depuis 1977 pour tous les poussins bagués, la masse et la longueur du tarse ont été enregistrées ce qui nous permet par la suite de calculer un index de condition corporelle. Ainsi, il est possible de regarder l'effet de la condition corporelle juvénile sur les différents traits reproducteurs des individus au cours de leur vie à l'aide de méthode de capture-marquage-recapture. Ce type d'études longitudinales nous aiderait à déterminer l'importance des conséquences de la condition corporelle juvénile mais aussi de savoir si ces conséquences sont stables au cours de la vie ou au contraire si elles ont tendance à s'estomper par l'acquisition d'expérience (Thornton 2008).

Notre étude souligne également l'influence des traits parentaux sur la condition corporelle du poussin. 9% de la variation de la condition corporelle du poussin est expliquée par la condition juvénile du père mais les mécanismes sous-jacents restent inconnus. Il peut s'agir d'une héritabilité génétique de ce trait puisqu'il a été déjà montré que la condition corporelle du poussin avait une base génétique. En effet, environ 2% de la variation de la condition du poussin serait due au gène DRD4 codant pour le récepteur de la dopamine jouant un rôle important dans la régulation de la prise alimentaire et dans le métabolisme du glucose et des lipides chez les vertébrés (Gillingham et al. 2012). Mais il pourrait aussi s'agir d'un effet paternel, c'est-à-dire l'influence du phénotype du père sur le phénotype du poussin par des mécanismes autres que la transmission des allèles (Crean and Bonduriansky 2014). Ou plus probablement d'une combinaison de ces deux mécanismes.

Plus généralement, nous pouvons nous demander quelles sont les causes de la variabilité de la condition corporelle des poussins. Chez le flamant rose, la condition corporelle des poussins est aussi connue pour être négativement influencée par la densité des individus reproducteurs à la colonie ainsi que par la dynamique hydrologique locale (Béchet and Johnson 2008). Mieux comprendre les interactions entre génotype, facteurs environnementaux et facteurs parentaux conduisant à cette variabilité dans la condition des poussins aiderait certainement à mieux comprendre la dynamique de la population.

Les différences liées au sexe

Le flamant rose est une espèce monogame où les deux sexes sont impliqués dans les soins parentaux. Les rôles sexuels semblent peu différenciés puisque mâles et femelles participent à la parade nuptiale, à la construction du nid, à l'incubation et au nourrissage des jeunes. Dans ce contexte, la théorie prédit des pressions de sélection similaires sur les signaux sexuels pour les deux sexes ainsi qu'une compétition équivalente pour l'accès au partenaire conduisant à un monomorphisme dans les signaux sexuels (Andersson 1994; Kraaijeveld et al. 2007). Chez le flamant rose la structure comparable entre mâles et femelles de leurs séquences de mouvements composant les parades nuptiales corrobore cette hypothèse (chapitre 1, chapitre 2). Cependant, les interactions présentes entre individus du même sexe diffèrent. En effet, les mâles interagissent plus fréquemment entre eux à travers notamment des comportements agonistiques. Ceci suggère une compétition intrasexuelle pour l'accès au partenaire plus prononcée chez les mâles. De plus, les femelles exhibent une coloration rose plus intense que les mâles probablement pour indiquer leur capacité à investir dans les œufs. Ces résultats supposent qu'il existe bel et bien des pressions de sélection similaires entre les sexes conduisant à des signaux sexuels similaires (ex. complexité des parades nuptiales). Mais aussi que mâles et femelles investiraient différemment dans la reproduction suivant les étapes de la reproduction. Un certain nombre d'études sur les espèces socialement monogames suggèrent un fort investissement des femelles dans la ponte (Monaghan et al. 1998) et un investissement plus prononcé des mâles dans la compétition pour le partenaire sexuel et pour l'acquisition des territoires de reproduction (Bonduriansky et al. 2008). Le cas du flamant rose paraît rentrer dans ce cadre, ce qui laisse penser que les rôles sexuels diffèrent probablement plus qu'on ne le pense chez cette espèce.

Les sexes affichent aussi des différences dans les conséquences entraînées par la condition corporelle juvénile (chapitre 3). Seule la condition juvénile des pères affecte celle du poussin. L'investissement plus élevé des mâles durant la période d'élevage des poussins peut expliquer ce résultat. Ces résultats corroborent l'hypothèse d'une différence dans les rôles reproducteurs entre les sexes. Bien que la condition corporelle juvénile des femelles ne semble avoir aucun impact sur la condition corporelle de leur poussin, il est probable qu'elle influence d'autres traits reproducteurs. Si l'on part du postulat que la condition corporelle juvénile influence positivement les futures capacités reproductrices de l'individu, il serait

judicieux de se focaliser sur les étapes de la reproduction où la performance de la femelle est déterminante. Par exemple, la production de l'œuf étant fortement pensée comme coûteuse pour la femelle flamant rose (Cézilly 1993; Tavecchia et al. 2001), nous pouvons nous attendre à ce que la condition juvénile de la femelle influence positivement la probabilité d'éclosion.

Enfin, notre étude sur l'âge au recrutement montre que même si mâles et femelles expriment une même valeur dans un traits d'histoires de vie, les mécanismes sous-jacents peuvent être différents (chapitre 4). Chez les femelles, retarder l'âge de première reproduction à un âge moyen de 6-7 serait la conséquence des faibles capacités des jeunes femelles à faire face au coût de la reproduction. Les mâles ne semblent pas faire face à ce coût et pourtant ils retardent aussi leur reproduction à un âge comparable. Ce décalage entre âge optimal et âge observé de première reproduction chez les mâles suggère que les jeunes mâles rencontrent des difficultés à accéder à la reproduction. Cela peut-être dû à leur manque de compétitivité qui les obligerait à repousser leur première reproduction. Par exemple, la compétition pour l'accès au site de nidification a été proposée comme facteur possible expliquant le report de première reproduction (Francesiaz 2016). De même, les jeunes mâles pourraient avoir des difficultés à trouver un partenaire sexuel du fait des comportements agonistiques de leurs aînés pouvant entraîner leur exclusion des groupes de parades. Enfin, les jeunes flamants roses présentant une homogamie pour l'âge dans leur appariement, il est probable que les jeunes mâles ne trouvent pas de jeunes femelles prêtes à se reproduire.

Hétérogénéité individuelle

L'absence de compromis entre âge de première reproduction et survie chez les mâles ainsi que la covariation de l'âge au recrutement et du succès reproducteur global suggère la présence d'hétérogénéité individuelle au sein de la population. C'est-à-dire des différences entre individus dans leur niveau de performance supposées constante tout au long de la vie (composante stable de l'hétérogénéité individuelle).

Les corrélations positives entre les traits d'histoire de vie ont été fréquemment observées en milieu naturel (Cam et al. 2002; Hamel et al. 2009; Fay et al. 2016). Ainsi, chez la mouette tridactyle, *Rissa tridactyla*, les oiseaux qui étaient les plus susceptibles de survivre étaient également les plus susceptibles de se reproduire. Ces corrélations positives entre traits d'histoire de vie ne font sens que si les individus varient dans leurs capacités à acquérir des

ressources (Van Noordwijk and de Jong 1986). Suivant cette capacité les individus ne seront pas soumis aux mêmes compromis ou à leur même intensité. Un individu ayant de faibles capacités d'acquisition des ressources devra faire des compromis dans l'allocation de son énergie entre ses différents traits d'histoire de vie. A l'inverse, un individu ayant une haute capacité d'acquisition de ressource pourra allouer d'avantage à la reproduction sans que cela n'induisse de surcoûts. La présence de ces derniers au sein de la population peut masquer les possibles compromis évolutifs présents chez les autres individus.

Le récent essor des méthodes de capture-marquage-recapture permet de prendre en compte cette hétérogénéité individuelle. En particulier il nous est possible de contrôler pour les variations interindividuelles non observables dans les paramètres démographiques (traits liés à l'aptitude phénotypique), ces différences entre individus étant supposées stables au cours de la vie ("Hidden Persistent Demographic Heterogeneity HPHD ", Cam et al. 2016). Ainsi il nous est possible aujourd'hui de dépendre plus précisément les patrons entre traits d'histoire de vie et les compromis évolutifs en jeu.

Conclusion

Chez le flamant rose, l'âge, le sexe et la condition corporelle juvénile sont des attributs individuels responsables du moins en partie, de la variabilité dans la réussite de la reproduction. Cette thèse montre l'intérêt d'étudier la reproduction dans sa totalité, avec un accent sur l'accès à la reproduction. Elle encourage aussi de prendre en compte le sexe des individus dans l'étude des comportements reproducteurs et des compromis évolutifs impliqués chez les espèces monogames même si *a priori* les rôles sexuels apparaissent similaires.

Le programme de suivi à long-terme des flamants roses offre une grande richesse de données capable de répondre à de nombreuses questions que ce soit au niveau de la population ou au niveau individuel. De tels suivis sont indispensables tant dans le domaine de la conservation que pour la recherche fondamentale. La continuité de ce programme semble prometteuse dans l'étude des processus de sénescence chez une espèce longévive, et dans l'étude de l'hétérogénéité individuelle au sein des traits d'histoire de vie.

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ANNEXES



Assessing plastic band degradation and loss in a long lived bird and its effect on survival estimation

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Abstract. Methods used to estimate demographic parameters of bird populations from ringing data rely on the tacit assumption that once an individual has been ringed, it will remain identifiable throughout its life. However, this is not true if rings are subject to wear and tear or can be lost. Degradation and loss of rings will directly affect estimation of demographic parameters, although the magnitude of the effect may vary according to how frequently ring degradation and loss occur in the wild. Here we use observation histories of 18 686 Greater flamingos (*Phoenicopterus roseus*) banded as chicks with two bands (PVC and metal) in Camargue (South of France) between 1986 and 2013 to assess PVC band degradation and loss in this long-lived species. We detected only a low proportion (0.92% of all resighted individuals) of broken or lost bands. The annual probability that a band remained intact slowly decreased with band age but generally remained high for the duration of the survey (>0.98 after 20 years), except for 5 cohorts (1993, 1994, 1996, 1997 and 1998) that were more affected than others. The difference in tarsus diameter between sexes in Greater Flamingo did not appear to influence band loss as we found contrasted effect of sex on retention rates before and after 15 years following banding. Multievent modeling suggests that bands progressively deteriorate with a marked increase in the annual probability of being lost after a break, 1.2% (95% CI [0.41; 3.7]), or after having slipped down the tarsus, 7.1% (95% CI [2.8; 16.6]) instead of 0.05% (95% CI [0.035;0.072]) directly. However, band loss appeared to have only a marginal effect on the estimation of adult survival (underestimation of at most 0.007 for birds aged 16-28 year). Monitoring of individuals with lost bands should be pursued to ensure that this conclusion remains valid in the longer term.

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Key words: *Phoenicopterus roseus*, PVC band, retention rate, survival rate, bias, multievent.

[†]*Alan Johnson deceased on 25 December 2014. Alan started ringing Greater flamingos with PVC rings in 1977 contributing to set up one of the longest ring-resighting dataset on a waterbird species in the world. In 2013, while he has been retired for 10 years, he developed concern for the fate of the old PVC bands. He thus initiated this work by checking metal rings of Greater flamingos having lost their PVC bands. Alan thus deserves full credit for having initiated the study which results are presented in this paper.*

Marking is central to the study of bird population dynamics. Marks allow tracking individuals in time and space so as to estimate movements, home range and habitat use. The analysis of the subsequent recaptures or resightings of marked individuals at discrete time intervals also provides estimates of essential demographic parameters such as population size (Pollock 1982), population growth rate (Pradel 1996) or survival (Lebreton et al. 1992). As marked animals may not be detected perfectly after marking, capture-mark-recapture (CMR) models have been developed to take into account that probabilities of recapture (or resighting) are most of the time below 1 (Lebreton et al. 1992, Pradel 2005).

A key assumption of CMR models is that individuals do not lose their marks during the study (Pollock and Raveling 1982). However, most marks may deteriorate with time and eventually be lost so that the marked individual cannot be identified anymore (Schwarz et al. 2012). The loss of marks could bias demographic parameter estimation. For example, estimate of survival may be biased negatively when individuals that lost their marks are considered as dead (Nelson et al. 1980). In simple cases, i.e. when retention rate is known and independent of the age of the band, the correction of the survival estimate and its variance are straightforward (see Arnason and Mills 1981, Nichols and Hines 1993). Yet, when retention rate varies with time, or other factors such as sex or cohort, the effect on survival estimate may be more difficult to assess. In long-lived species, marks may wear out faster than the lifespan of most individuals (Nelson et al. 1980). Assessing mark loss rate in long-lived species is critical because it may hamper the study of key phenomena such as senescence.

Double-marking, associating a permanent mark (e.g. metal legband or pit tag) to the observable but non-permanent mark (e.g. plastic band or neck collar), provides a way to detect mark loss and estimate mark loss rate (Juillet et al. 2011). Recaptures (or recoveries) enabling control of the permanent mark provide a way to assess to what extent estimates based only on resightings of non-permanent marks are biased. Most quantitative estimates of mark loss have been obtained in double-marked (e.g. metal legband and plastic neckband) waterfowl species using recapture and/or recovery data. For example, plastic neckband loss were found to increase over time and males to have higher mark loss than females in both Canada geese, *Branta canadensis* (Samuel et al. 1990, Campbell and Becker 1991) and lesser snow geese, *Chen caerulescens* (Johnson et al. 1995, Samuel et al. 2001).

Flamingos are long-lived species which are studied worldwide thanks to PVC banding of chicks before fledging and their subsequent resightings: James Flamingo *Phoenicoparrus Jamesi* and Andean flamingos *Phoenicoparrus andinus* in Argentina, Caribbean flamingos *Phoenicopterus ruber* in Mexico (Childress et al. 2009, Lee et al. 2011) and Greater flamingos *Phoenicopterus roseus* in the Mediterranean (Johnson and Cézilly 2007). In Greater flamingos, there is circumstantial evidence for Laminated Polyvinyl Chloride (PVC) band loss. Indeed, several PVC bands have been found broken in the field and some individuals have been observed with only their stainless-steel band. The loss of plastic band could thus underestimate survival and lead to erroneous biological interpretations (Arnason and Mills 1981, Kremers 1988), particularly for this long-lived species (Johnson and Cézilly 2007). However, in this species, recaptures of adults are difficult and recoveries are rare so that band loss may only be recorded when metal rings are readable at short distance.

In this paper, we first aimed at estimating the retention rate of PVC bands in the Greater flamingo and the possible effect of three factors: cohort (i.e. year of banding), age of the band (i.e. time elapsed since banding) and sex of the individuals. As PVC band manufacturers differed among years, we expected band retention rate to vary with cohort with some cohorts being more affected than others. Moreover, as females are smaller and have a thinner tarsus than males (Johnson et al. 1993, Boucheker 2012), we expected that females may lose their PVC band earlier because bands could possibly more easily slip down the leg.

Our second objective was to model the temporal dynamics of band degradation and loss and to estimate the potential bias generated by PVC band loss on the estimation of survival, a key demographic parameter. We expected that band loss causes negative bias in survival because flamingos having lost their PVC band may be considered as dead.

METHODS

Each year since 1947, a sample of Greater Flamingo chicks has been caught and marked in the Camargue (southern France). During this long-term marking program, different types of marks have been used (wing tags, leg bands; Johnson 2000). However, since 1977, chicks have been marked on their right tibia with a PVC leg band engraved with a unique alpha-numeric code of 3 to 4 characters. From 1986 onward, each flamingo banded has additionally received a 2.4 cm wide stainless-steel band engraved with a unique code on

its left tibia. Codes of plastic bands can be read at up to 300 m while those of metal bands are only readable at less than 100 m with a 60 × telescope (review in Johnson 2000). If a flamingo has lost its PVC band, efforts are made to read the metal band. Since 1995, the sex of a subsample or of all individuals banded has been determined molecularly. However, for older individuals, sex was assessed from size dimorphism and behaviour at adult age (Balkiz et al. 2007). From 1986 to 2013 (except in 2002 and 2007 because no banding occurred during those years), 18 686 Greater Flamingos have been double-banded in France (5 344 females, 4 906 males and 8 436 birds of unknown sex).

As we suspected that band loss may be under appreciated, we aimed at increasing effort to detect flamingos with band loss by dedicated band-resighting sessions at the Ornithological Park of Pont de Gau (43°29'20.09"N, 4°24'14.67"E). This lagoon provides an exceptional proximity to flamingos (often less to 30 meters) and allows reading the metal bands of most individuals present. Resightings sessions were thus made there every winter from 2012 to 2014 and in spring 2014 to complete the existing band-resighting dataset.

We first analyzed the resightings of 18 686 flamingos double-banded in the Camargue from 1986 to 2013 using logistic models to determine the probability that PVC bands remain intact and factors influencing this probability. In each year we examined the proportion of individuals of each cohort and sex observed with an intact PVC band relative to those observed with a defective band (broken, slipped or unseen). From this proportion, we estimated θ_i , as in Nelson et al. (1980), the probability that a PVC band remains intact to the end of the i^{th} year after banding. The response variable was modeled using the logit link and a binomial error term. We evaluated the effect of sex, age of band and cohort through model selection. Individuals of unknown sex were discarded from the analysis. To determine factors influencing θ , we first reduced the dataset to a constant time-period corresponding to the 16 first years after banding for cohorts banded from 1986 to 1998. To get an overview of the temporal variation of θ , we also performed the analysis for the entire time-period by following cohorts banded from 1986 to 2001 and resighted until 2014. The most recent cohorts (after 2003) were not included in the last analysis because they were not followed during a long enough time period to assess band loss.

In a second step, we used multievent models (Pradel 2005) and software E-Surge (v 1.8; Choquet and Nogue 2011) to assess the process conducting to band loss. We analyzed the resightings made over each year of the same 18 686 double-banded flamingos. At each

observation of a banded bird, the PVC band may be in a finite set of states $E = \{A, B, C, D, \text{dead}\}$ with A : bird alive wearing an intact PVC band, B : bird alive wearing a broken PVC band (here the band is only partly broken and remains on tibia), C : bird alive wearing a slipped PVC band and D : bird alive having lost its PVC band (Fig. 1). However, the states may not be observed directly; rather, at each resighting occasion t (here each year from January to December, $t = \{1986, 1987, \dots, 2014\}$), several events may be recorded: 0 bird not observed, 1 bird observed wearing both its metal and intact PVC band, 2 bird observed wearing a broken PVC band, 3 bird observed with its PVC band slipped down the tarsus and 4: bird observed and identified by its metal band with its PVC band unseen (it could have slipped down and be hidden underwater or be lost) (set of events $\Omega = \{0, 1, 2, 3, 4\}$). Multievent models can then be used to estimate transition rates among states (Ψ), taking into account both survival probability (Φ) and the probability of each event (β) given the underlying state of the individuals (see *supplementary material*). Uncertain observations and observation mistakes (i.e. when a broken band was recorded as being intact) were kept as such in the dataset. Because of the few numbers of observations of bands with problems, the transition rates among states Ψ had to be set constant over time and cohorts. As in previous studies of the species (e.g. Pradel et al. 2012), we let juvenile survival (less than 1 year-old) differ from adult survival (more than 1 year-old), and we split adult survival into two age classes (2 to 15 years, 16 to 28 years). Detection probability was set to be time-dependent for birds wearing an intact band and constant for other states because of the few observations per year in these states. Detection rates were set equal for birds wearing a band slipped down the tarsus and lost. This general model (Model $1\Phi_o$) allowed estimating transition rates among different states of PVC bands.

Resightings of flamingos with only a metal ring were too scarce to produce an independent estimate of survival which could be used as a reference to estimate the extent of bias that may be caused by PVC band loss. To overcome this difficulty, we used two different approaches. First, we assessed the bias that may be generated by ignoring observations of birds with degraded bands and lost bands. We thus compared survival estimates of model $1\Phi_o$ to those of a model considering individuals with broken PVC band as birds with intact bands (event 2 similar to event 1) and individuals with slipped or unseen PVC band as not observed (event 3 and 4 similar to event 0; Model $2\Phi_o$). In a second step,

we evaluated if individuals from cohorts with higher band loss rate had lower survival estimate by including a cohort effect (c) on survival (Model $3\Phi_{a,c}$).

In all analyses, the Akaike's Information Criterion (AIC) was used to select the best model that adequately describes the data. We considered models as equally plausible if their difference in AIC was ≤ 2 (Burnham and Anderson 2002).

RESULTS

A total of 55 visits were made at Pont de Gau (A.J. did 20 visits during the winters 2011-2012, 2012-2013 and 2013-2014 and L.T. did 35 visits from February to April 2014), during which time 280 Greater flamingos double-banded as chicks were identified. This number includes 135 males (48.2%), 114 females (40.7%) and 31 individuals of unknown sex (11.1%). Among them, 12 (4.3% - all females) had lost their PVC band.

From august 1986 to march 2014, 16 361 individuals were resighted at least once among the 18 686 flamingos double-banded in France since 1986. Only 150 individuals (0.92%) have been observed, at least once, with a defective PVC band (52 males (34.7%), 68 females (45.3%) and 30 individuals of unknown sex (20%)). 75 (50%) individuals have been observed with a broken band, 60 (40%) with an unseen band, 8 (5.3%) with a slipped band, 6 (4%) with a slipped and then an unseen band and 1 (0.7%) with a broken and then an unseen band. These birds came from 20 different cohorts. The most affected cohorts are: 1986 (2.9% of the individuals observed from this cohort had a defective band), 1993 (2.2%), 1996 (2.9%) and 1997 (3.4%). Defaults are first recorded on average when the band is 14.62 years-old (95% CI = [13.83; 15.42]), and is more often observed as a breakage (50.7%).

Logistic approach

We built 12 biologically plausible models for both logistic analyses (Table 1 & 2). For the 16 year-period dataset, we retained the 3 best models that were equally good at describing the data. Cohort, band age and sex were retained, in interaction or not, in either one or the other of the 3 best models ($\Delta AIC < 2$; Table 1). For the 20 years dataset, the most parsimonious model retains the interactions cohort \times band age and sex \times band age. Thus, over time, the probability that the PVC band remains intact differs among cohorts and sexes. Based on model cohort \times band age of the full dataset (Table 2), five cohorts had a probability that the PVC band remains intact below 0.98 either at 16 or 20 years after banding (Fig. 2). Below this threshold, the probability that the PVC band remains intact approximated the last

documented annual survival rate in this species (0.97 ± 0.001 ; Pradel et al. 2012), i.e. PVC bands would deteriorate (or be lost) at the same rate as flamingos die. These most affected cohorts were: 1993 ($\theta_{16} = 0.989$ CI95=[0.983; 0.994] $\theta_{20} = 0.957$, CI95 = [0.934; 0.972]), 1994 ($\theta_{16} = 0.997$ CI95=[0.992; 0.998], $\theta_{20} = 0.978$, CI95 = [0.951; 0.991]), 1996 ($\theta_{16} = 0.977$, CI95 = [0.965; 0.985]), 1997 ($\theta_{16} = 0.968$, CI95 = [0.952; 0.978]) and 1998 ($\theta_{16} = 0.978$ CI95=[0.947; 0.990] (Fig. 2).

The effect of sex changed with the age of the band. During the first 15 years after banding, males have a lower probability that their PVC band remains intact than females. However, after 15 years, an opposite trend is observed with females having lower probability than males to keep their PVC band intact (Fig. 3 a, b and c). For instance, θ_{26} of males banded in 1987 was 1.25% higher than θ_{26} of females; θ_{20} of males banded in 1993 was 3.63% higher than θ_{20} of females and θ_{16} of males banded in 1997 was 5.37% higher than θ_{16} of females (Fig. 3 a, b and c).

Multi-event approach

Model $1\Phi_a$ suggests that the probability that an intact PVC band is lost directly is very low (0.0005). Results rather suggest a progressive degradation of the band either through breakage or through slipping down the tarsus. Once one of these states is reached, the probability that the band became lost increases markedly (0.012 to 0.071; Fig. 4). Overall, the probability that intact bands remain intact is high ($1-(a+b+c)=0.998$), and coherent with the probability derived from the logistic approach.

Survival estimates derived from Model $2\Phi_a$ are lower than estimates from Model $1\Phi_a$, underestimating survival from 0.01 to 0.001 for the adult age-classes 2-15 and 16-28, respectively (Table 3).

Model $3\Phi_{a,c}$ has a slightly lower AIC than Model $2\Phi_a$ ($\Delta AIC = 2.02$), thus supporting a cohort effect on survival which may result from these cohorts having higher rates of band loss. Annual adult survival estimates for the cohorts most affected by band loss is however only 0.002 to 0.007 lower than the survival estimate of the less affected cohorts (Table 3). Hence, both approaches suggest that the survival estimates derived from band resightings in this species are slightly underestimated when not taking into account known band loss.

DISCUSSION AND CONCLUSION

Mark loss may have important consequence for the statistical estimation of the demographic parameters of bird populations. In exploited avian populations, metal ring recoveries may provide an independent assessment of survival given reporting rates by hunters are reliable (Nichols et al. 1991). Other estimates of band loss have been most often obtained on waterfowl species which could be regularly recaptured (i.e. during moult or by canon nets), so that the metal rings may be used as an independent assessment of PVC mark loss (e.g. Reed et al. 2005). In many species however, individuals may only be captured before fledging and observation of marks at a distance are the only way to track them along their life. This is the case for flamingos which cannot be easily recaptured after fledging.

Our results show that only few Greater flamingos double-marked since 1986 in Camargue have been observed with a defective PVC band (0.92%). The annual probability that an intact PVC band remains intact estimated from the multievent modeling was high (99.8%). Interestingly, the multievent modeling suggests that band loss is progressive and often preceded by the break of the band or its slipping down the tarsus. Yet, the proportion of Greater Flamingos with a slipped PVC band is probably underestimated because high water levels often prevent observers from checking whether a bird is ringed or not. The low number of birds observed with only a metal ring did not allow us to model the temporal variations of the transitions among states, such that the probability that a PVC band remains intact is averaged over band age. In contrast, the logistic approach suggests that the proportion of intact PVC rings remains high (≥ 0.99) but starts decreasing slowly when the bands are about 10 year-old. The decrease tended to be more pronounced for five cohorts (1993, 1994, 1996, 1997 and 1998) that were more affected by PVC band degradation and loss than the other ones.

The decrease in the probability that a PVC band remains intact with band age indicates a threshold in the wear and tear of the plastic material which then starts to cause band degradation and loss. Over time, PVC bands may become brittle and crack (Coulson 1963). Material wear and tear appears heterogeneous as degradation rate varies much among cohorts. Indeed, some old bands (of older cohorts) had higher probabilities to remain intact than younger ones. We can hypothesize that the most affected cohorts were characterized by bands made with less resistant material. Unfortunately, we could not retrace material brands (e.g. Darvic or Vynalast) used to make PVC bands in France since

1986. PVC brands differ in the composition and additives used to make the PVC bands. Vynalast is rigid and light and has been developed to replace Darvic but appears to be breakable in wet environment (Rees et al. 1990). Band degradation may also depend on the method used to mould them, on the depth of engraving and the use of some specific letters which may weaken band rigidity.

Our hypothesis that the difference in tarsus diameter between sexes in Greater Flamingo influenced band loss was not clearly supported as results showed contrasted degradation rates for males and females before and after 15 years since banding.

In all models survival estimates for both adults and juveniles were on par with those of previous studies (Cézilly et al. 1996, Tavecchia et al. 2001, Balkız et al. 2010, Pradel et al. 2012). The two approaches used to assess survival variation with or without taking into account band loss indicated that survival rates of adults aged >16 year-old may be negatively biased of up to 0.7 % only for the most affected cohorts. This would indicate that flamingo's average life expectancy is 23.8 year-old instead of 20.3 year-old.

Our results highlight the need to carefully choose PVC material to use when launching a banding resighting study on long-lived species such as flamingos. We also recommend improving as possible the design of marks, in order to increase their retention rate. Finally, proper estimates of band loss were possible only because metal rings were also present and remained readable from a distance on the long term. Hence we encourage maintaining a constant resighting effort on birds wearing only a metal ring to ensure the long term monitoring of band loss rate.

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Figures captions:

Figure 1. Pathways of possible transitions (lower case letters) between the different states (capital letters) for a Greater Flamingo regarding its PVC band (see text for details).

Figure 2. Probability that a PVC band remains intact as a function of band age for the cohorts from 1986 to 2003 based on estimation of model 2 (age x cohort) of the full dataset.

Figure 3. Probability that a PVC band remains intact for males and females greater flamingos banded in (a) 1987, (b) 1993 and (c) 1997 determined from Model 1 (sex*band age + cohort*band age) of the full dataset (see text for details). Broken curves represent confident intervals (95% CI). The cohorts were chosen as examples to illustrate the output of the model.

Figure 4. Transition probabilities (lower case letters) for the different states (capital letters) of a PVC band in Greater flamingos.

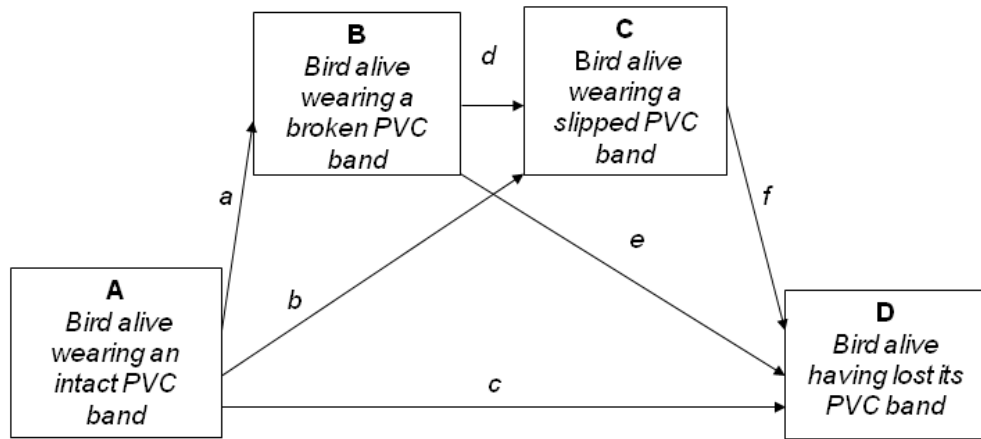


Fig.1

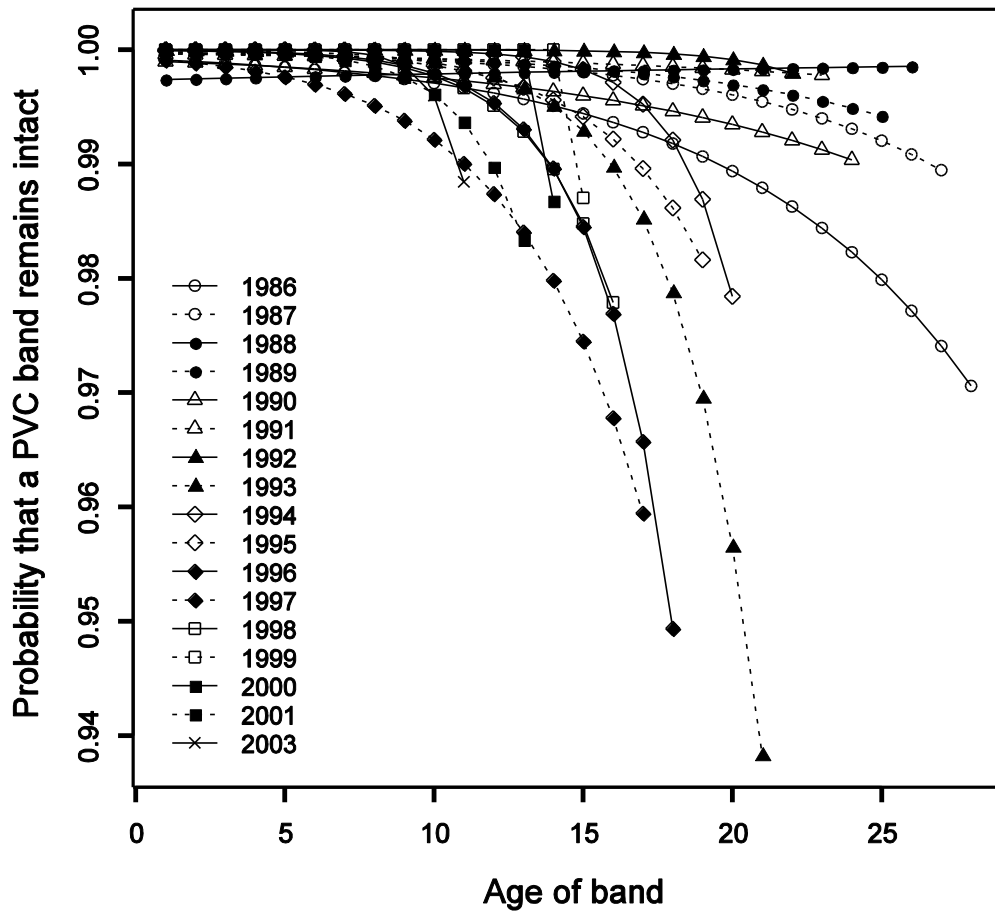


Fig. 2

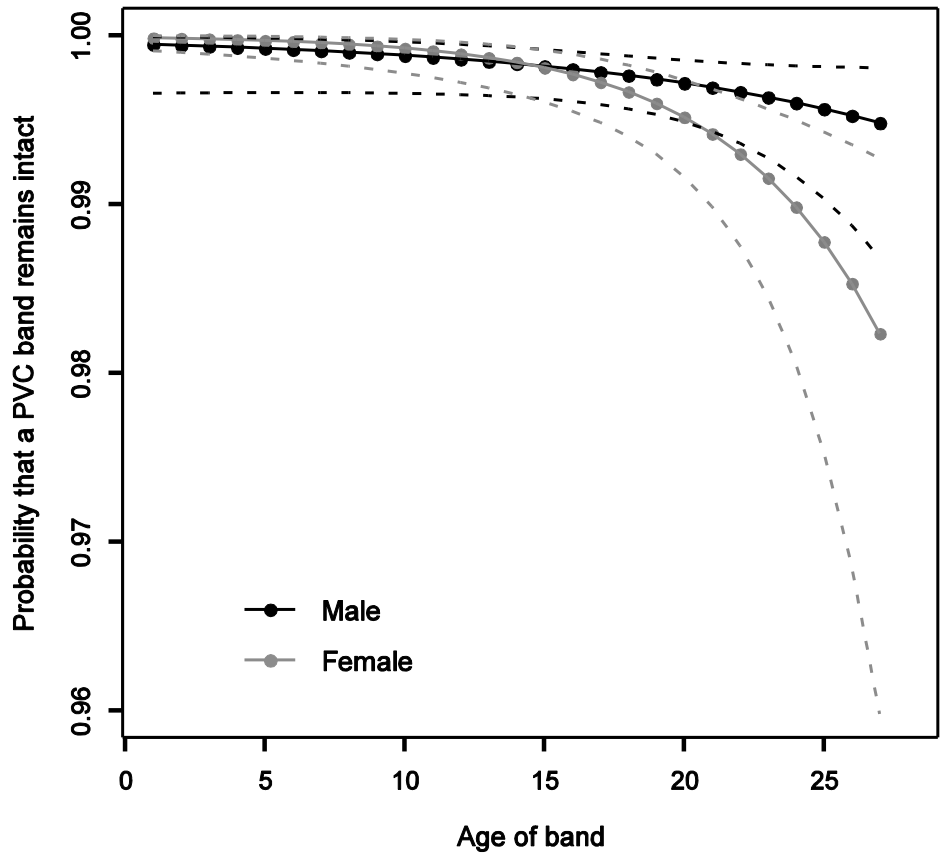


Fig 3a.

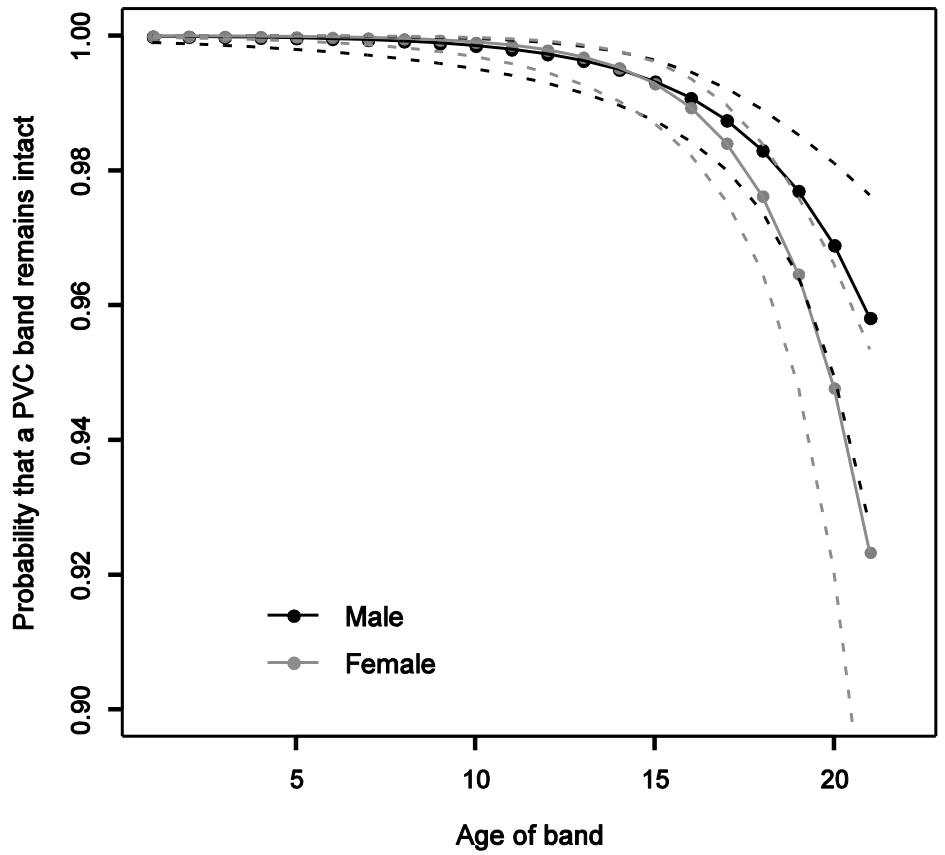


Fig 3b

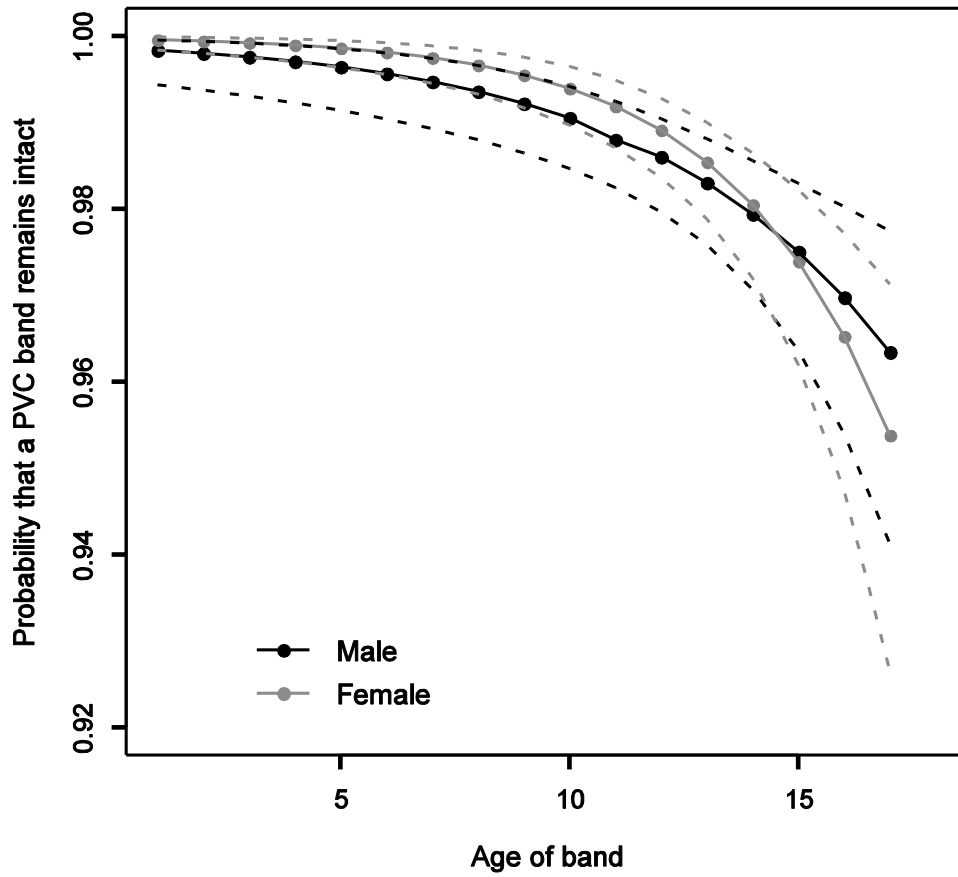


Fig. 3c

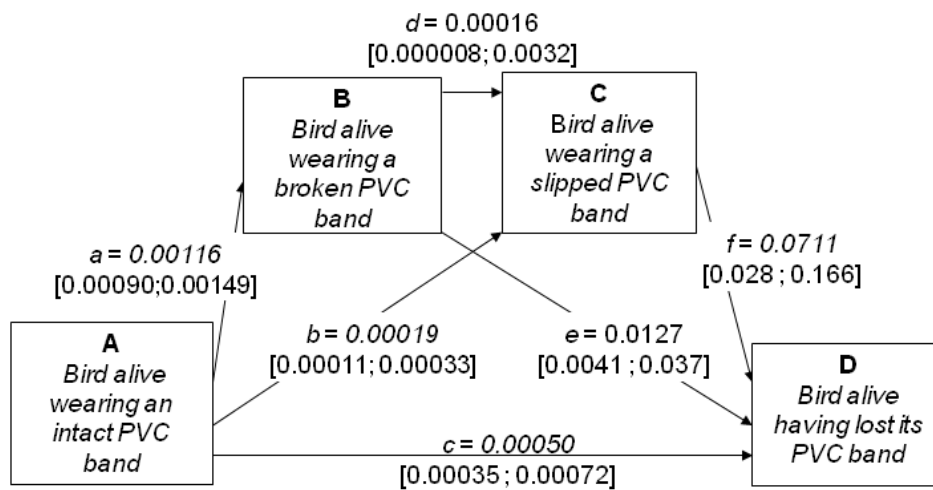


Fig. 4

Table 1. Candidate models for the probability that PVC band remains intact of Greater Flamingo banded in the Camargue (France) from 1986 to 1998 and resighted until 2014. For each model, we provide the number of estimable parameters (np), the residual deviance (rDev), the Akaike's Information Criterion (AIC) and the difference in AIC compared to lowest AIC of the model set (Δ AIC).

Models	np	rDev	AIC	Δ AIC
Sex×Age + Cohort×Age	29	153.8	342.9	0
Age×Cohort	26	159.3	344.4	1.5
Age×Cohort + Sex	27	157.3	344.4	1.5
Sex×Age + Cohort	17	186.8	351.9	8.9
Sex + Cohort + Age	28	191.6	354.7	11.8
Cohort + Age	15	193.8	354.9	11.9
Sex×Age	5	259.5	400.6	57.7
Age	3	265.7	402.8	59.9
Sex + Age	4	263.9	403.0	60.1
Cohort	14	261.0	420.1	77.1
Sex + Cohort	15	259.0	420.1	77.1
Sex	3	332.1	469.2	126.3

Table 2. Candidate models for the probability that PVC band remains intact of Greater Flamingo banded in the Camargue (France) from 1986 to 2003 and resighted until 2014. For each model, we provide the number of estimable parameters (np), the residual deviance (rDev), the Akaike's Information Criterion (AIC) and the difference in AIC compared to lowest AIC of the model set (Δ AIC).

Models	np	rDev	AIC	Δ AIC
Sex×Age + Cohort×Age	49	310.5	697.6	0
Age×Cohort	49	324.5	707.7	10.0
Age×Cohort + Sex	50	323.2	708.4	10.8
Sex×Age + Cohort	28	388.7	729.9	32.2
Cohort + Age	26	401.1	738.2	40.6
Sex + Cohort + Age	27	399.9	739.0	41.4
Sex×Age	5	580.4	875.6	178.0
Age	3	590.5	881.6	184.0
Sex + Age	4	588.5	881.7	184.1
Cohort	25	593.7	928.9	231.2
Sex + Cohort	26	592.2	929.4	231.7
Sex	3	750.2	1 041.4	343.8

Table 3. Estimates and 95% confidence intervals for survival for juveniles (Φ_{juvenile}) and adults (Φ_{adult} ; > 1 year-old) derived from model 1 Φ_a , 2 Φ_a and 3 $\Phi_{a,c}$ (see text for details of the models). Model 3 $\Phi_{a,c}$ includes a cohort effect allowing separate survival estimates for cohorts with high band loss (1993, 1994, 1996, 1997 and 1998) and the other cohorts that appeared less affected by band loss.

	Parameters	Type of cohort	Estimates	95% CI
Model 1 Φ_a	$\Phi_{\text{juveniles}}$		0.7439	[0.7367 ; 0.7510]
	Φ_{a2_15}		0.9650	[0.9638 ; 0.9662]
	Φ_{a16_28}		0.9592	[0.9557 ; 0.9625]
Model 2 Φ_a	$\Phi_{\text{juveniles}}$		0.7435	[0.7362; 0.7506]
	Φ_{a2_15}		0.9643	[0.9631; 0.9655]
	Φ_{a16_28}		0.9582	[0.9546 ; 0.9615]
Model 3 $\Phi_{a,c}$	$\Phi_{\text{juveniles}}$	Low band loss	0.7386	[0.7301 ; 0.7468]
	$\Phi_{\text{juveniles}}$	High band loss	0.7571	[0.7428 ; 0.7708]
	Φ_{a2_15}	Low band loss	0.9649	[0.9634 ; 0.9663]
	Φ_{a2_15}	High band loss	0.9632	[0.9610 ; 0.9653]
	Φ_{a16_28}	Low band loss	0.9589	[0.9551 ; 0.9623]
	Φ_{a16_28}	High band loss	0.9520	[0.9408 ; 0.9611]

Electronic Supplementary Material

At each observation of a banded bird, we considered that the PVC band was in a finite set of states $E = \{A, B, C, D, dead\}$ with A : bird alive wearing an intact PVC band, B : bird alive wearing a broken PVC band, C : bird alive wearing a slipped PVC band and D : bird alive having lost its PVC band.

In the field, observations corresponded to the following set of events $\Omega = \{0, 1, 2, 3, 4\}$ with 0: bird not observed, 1: bird observed wearing both its metal and intact PVC band, 2: bird observed wearing a broken PVC band, 3: bird observed with its PVC band slipped down the tarsus and 4: bird observed and identified by its metal band with its PVC band unseen (it could have slipped down and be hidden underwater or be lost).

The initial state corresponds to the ringing states so that all birds are in state A , i.e. “alive wearing an intact PVC band”:

$$\text{Initial state} = A \begin{bmatrix} A & B & C & D \\ 1 & 0 & 0 & 0 \end{bmatrix}$$

Transition matrices between states were split into two steps: the first step corresponded to *Survival* Φ between two resighting occasions and the second step to *Transitions* between states between resighting occasions.

$$\begin{matrix} & A & B & C & D & dead \\ A & \Phi & 0 & 0 & 0 & 1-\Phi \\ B & 0 & \Phi & 0 & 0 & 1-\Phi \\ C & 0 & 0 & \Phi & 0 & 1-\Phi \\ D & 0 & 0 & 0 & \Phi & 1-\Phi \\ dead & 0 & 0 & 0 & 0 & 1 \end{matrix}$$

$$\begin{matrix} & A & B & C & D & dead \\ A & 1-(a+b+c) & a & b & c & 0 \\ B & 0 & 1-(d+e) & d & e & 0 \\ C & 0 & 0 & 1-f & f & 0 \\ D & 0 & 0 & 0 & 1 & 0 \\ dead & 0 & 0 & 0 & 0 & 0 \end{matrix}$$

The probabilities of the events given the birds are in a given state have also been split in two steps. The first step describes the detection probability conditional on the state. The second step is the probability of the different events given the bird in different state is seen. Birds in state B (wearing a broken PVC band) may be either observed as such (event 2) or the fact that the band is broken may remain unnoticed (event 1).

$$\begin{array}{l}
 \text{A} \\
 \text{B} \\
 \text{C} \\
 \text{D} \\
 \text{dead}
 \end{array}
 \begin{array}{c}
 \text{Not seen} \quad \text{Seen|A} \quad \text{Seen|B} \quad \text{Seen|C} \quad \text{Seen|D} \\
 \left[\begin{array}{ccccc}
 1-\beta & \beta & 0 & 0 & 0 \\
 1-\beta & 0 & \beta & 0 & 0 \\
 1-\beta & 0 & 0 & \beta & 0 \\
 1-\beta & 0 & 0 & 0 & \beta \\
 1 & 0 & 0 & 0 & 0
 \end{array} \right]
 \end{array}$$

$$\begin{array}{l}
 \text{Not seen} \\
 \text{Seen|A} \\
 \text{Seen|B} \\
 \text{Seen|C} \\
 \text{Seen|D}
 \end{array}
 \begin{array}{c}
 \begin{array}{ccccc}
 0 & 1 & 2 & 3 & 4 \\
 \left[\begin{array}{ccccc}
 1 & 0 & 0 & 0 & 0 \\
 0 & 1 & 0 & 0 & 0 \\
 0 & \delta & 1-\delta & 0 & 0 \\
 0 & 0 & 0 & 1 & 0 \\
 0 & 0 & 0 & 0 & 1
 \end{array} \right]
 \end{array}
 \end{array}$$

Greater Flamingos show laterality in complex behaviours in the wild

In preparation for Laterality

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ABSTRACT

Laterality refers to the preference of individuals to use one of the two sides of the body to perform certain tasks as a consequence of the specialization of cerebral hemispheres in different tasks. Individuals may benefit from lateralization through being better able at allocating attention to simultaneous and complex tasks. Studies on behavioural laterality further suggest that sex and age can influence the degree of the lateralization within a population, and that lateralization can be expressed at the individual-level or at the population-level. However, most of those studies have been performed on captive subjects, thus reducing the effects of the natural environment on behaviour. In the present study, we tested for the existence of behavioural laterality in the Greater Flamingo, *Phoenicopterus roseus* through observations of the behaviour of focal individuals aged 5-37 years old in the wild. We particularly examined laterality during resting, a simple behaviour, and during foraging and courtship, two more complex behaviours in terms of motor coordination and attention. We found that 63% and 57% of the sampled population presented a left-side bias when foraging and when performing one particular courtship behaviour (twist-preen), respectively. We also found evidence for laterality in a second courtship behaviour (walk), but only at the individual-level. On the contrary, there was no evidence of lateralization in resting behaviour. Sex or age had no statistical effect on courtship behaviour lateralization, although there was a slight positive effect of age on individual lateralization in one courtship behaviour (twist-preen). Our results suggest that the complexity of behaviour may influence the degree of laterality, at least in the Greater Flamingo.

KEYWORDS

Laterality; complex behaviour lateralization; resting posture; foraging behaviour; courtship display; wildlife flamingo; *Phoenicoterus roseus*

INTRODUCTION

Laterality refers to the preference of individuals to use one of the two sides of the body to perform certain tasks (Rogers & Andrew, 2002; Rogers, Vallortigara & Andrew, 2013). Described in several animal groups (review in: Vallortigara & Rogers, 2005; Frasnelli, Vallortigara & Rogers, 2012), this phenomenon is linked to the specialization of cerebral hemispheres in the processing of different tasks.

The concentration of neural connections to processing a specific task in one of the brain sides lets free neural connections of the other hemisphere for other functions (Levy, 1977) and can bring benefits to the individual in terms of survival and reproduction (Magat & Brown, 2009; Rogers, Zucca & Vallortigara, 2004; Vallortigara & Rogers, 2005). For instance, it has been shown that chickens with strong-lateralized view better discriminate grains from gravel than chickens with weak-lateralized view (Rogers, 1996). In addition, Rogers et al. (2004) found that, when exposed to a risk of predation, lateralized chickens learned to discriminate between grains and gravel whereas non-lateralized chickens did not, suggesting that the processing of different functions in different hemispheres also enhances the performance of simultaneous tasks. Moreover, lateralized individuals seem to be more able to allocate attention in complex behaviours requiring cognitive skills (Dadda & Bisazza, 2006; Rogers et al., 2004; Vallortigara & Regolin, 2006). On the other hand, the degree of hemispheric specialization and, hence, that of lateralization, might be limited by the ecological cost of making suboptimal decisions whenever relevant information is located on both sides of the body (Dadda, Zandonà, Agrillo & Bisazza, 2009)

Individual characteristics can influence the degree of lateralization within a population. For instance, age seems to be important for the maturation of neural control of the hand in primates, since young individuals are less strongly lateralized than older ones in tool manipulation (Humble & Matsuzawa, 2009; Teixeira, 2008; Ward, Milliken, Dodson, Stafford & Wallace, 1990). The degree of lateralization can also be sex-dependent (Adret & Rogers, 1989; Camp, Robinson & Becker, 1984; Romano et al., 2015), due to variation in sexual selection between sexes (Vallortigara & Rogers, 2005) or to differences in the regulation of brain ontogeny by steroids between males and females (Pfannkuche, Bouma & Groothuis, 2009).

Within a population, the lateralization can be expressed at the individual-level (each individual has its own preference for one side of the body sides) or at the population-level (a

majority of individuals shows a preference for the same side of the body). Tasks that tend to be carried out solitarily are expected to generate individual-level laterality (Vallortigara & Rogers, 2005). On the contrary, population-level lateralization is often found in social tasks that require the individual to align its preference with the rest of the population in order to improve its fitness (Vallortigara & Rogers, 2005), as in synchronized movements of fish shoals. As a consequence, population-level lateralization by social constraints is often predicted in gregarious or social species (Bisazza, Cantalupo, Capocchiano & Vallortigara, 2000; Ghirlanda & Vallortigara, 2004; Vallortigara & Rogers, 2005).

Despite an accumulation of laterality studies in vertebrates, predicting the strength or the level of lateralization of specific behaviours in a given species remains difficult. A possible reason for this is that most studies have been carried in artificial environment (Rogers, 2002), which may alter behaviour compared to the situation in the wild, where different stimuli coming from all possible directions simultaneously can negatively influence individual's predisposition to always respond toward the same side (Krakauer et al., 2016; Vallortigara & Rogers, 2005; Ventolini et al., 2005). Hence, it is critical to assess how laterality is expressed *in situ* to understand its evolution.

In the present study, we assessed the existence of lateralization in the Greater Flamingo, *Phoenicopterus roseus*, in the wild. Laterality of Caribbean and Lesser flamingo has been studied in captivity with no consistent pattern in lateralization regarding the type of behaviour. Neck position during resting was found to be lateralized at the population-level (Anderson, 2009; Anderson, Williams & O'Brien, 2009) whereas there was not lateralization of the leg on which the individual rests or in foraging behaviour (Anderson, 2009; Peluso & Anderson, 2014). Here, we examined laterality during resting, a simple behaviour, and during foraging and courtship, two more complex behaviours in terms of motor coordination and attention. In addition, we evaluate the effect of age and sex in those behaviours that were found to be lateralized.

METHODS

Species and Study Site

The Greater Flamingo is a long-lived colonial species, with a seasonal monogamous mating system. It is distributed across wetlands of Africa, Asia and throughout the southern part of Europe (Johnson & Cézilly, 2007).

Observations were made in the Camargue, South of France, one of the most important breeding sites of the Greater Flamingo in the Mediterranean region. Since 1977 on average, 12% (7 to 30%) of the chicks fledged in the Camargue have been marked annually with a PVC plastic ring engraved with a unique three or four digit alphanumeric. Sex of the individuals was determined either from molecular data (Balkız et al., 2008) or from repeated sex assignment from observations based on sexual dimorphism (males being on average taller than females).

Observations of both ringed and non-ringed adults were made at several saltpans and marshes from January to April in 2011, 2014 and 2015.

Resting behaviour observations

Flamingos usually rest with the head lying along their back and the neck curved making a loop toward one side of the body (Johnson & Cézilly, 2007). In this position, sleeping individuals can remain vigilant and monitor the environment through regular eye opening. We recorded the side position of the neck in resting ringed individuals using scan sampling (Altmann, 1974). We considered flamingo's neck to be on the right side when the neck was curved to the right of the flamingo's middle line and on the left side otherwise (Anderson et al., 2009).

We also noted on which leg the observed individual stood on (right or left leg) during resting. Because individuals are marked with an engraved plastic ring on the right leg and also an engraved metal ring on the left leg, it was possible to identify the focal individual and to avoid resampling, even if the ring was hidden in the plumage because the leg was bent. However, metal rings are more difficult to read. Therefore, we only observed individuals with both metal and plastic rings close enough so that both rings' codes could be read.

Observations on the same individual were made on different days. Only data from resting individuals observed in at least three occasions were retained.

Foraging behaviour observations

Individuals foraging by *stamping* were observed using focal sampling (Altmann, 1974) with binoculars or a telescope. In this foraging method, flamingos stamp the soil to get invertebrates from the mud and trap them in the water with their beak, turning at the same time around their bill in a circle motion (Johnson & Cézilly, 2007). We scored the direction of rotation during the stamping (“right” if clockwise and “left” if anti-clockwise) in at least three bouts per individual. A bout started with a series of rotations (>2) in the same direction and stopped when the individual changed the rotation direction, or when the individual walked to another patch before starting again to stamp. We retained focal bouts which lasted for at least five minutes. We waited a week before resampling at the same site to avoid resampling the same individuals by chance.

Courtship behaviour observations

During the wintering season males and females Greater flamingos perform communal courtship displays in groups of a few to several hundred individuals. We recorded five-minute videos of ringed males and females during courtship. We then analysed individual sequences of courtship behaviours (Tab. 1) of focal individuals (Altmann, 1974) and, for each asymmetrical behaviour, recorded the side on which the behaviour was performed.

Because during walking, the side of the step directly depends on the previous side step, we took into account the first step to define the side of behaviour *walk* as the first leg used to walk could result from behavioural lateralization (Tomkins, Thomson & McGreevy, 2010). We made similar assumption regarding *head-flag* by taking the first head-flagging side into account. As consecutive *twist-preens* (and *preens*) alternate sides and thus depend on the first *twist-preens* (or *preens*) side (see Appendix), we decided to retain the first *twist-preen* side of a sequence of successive *twist-preens* (or *preens*).

Only data from displaying individuals who had at least three repetitions of asymmetrical behaviours were retained.

Statistical analysis

Laterality index

For each individual, we computed the laterality index, LI, as the difference between the number of times the individual performed the behaviour on its right and the number of times he did so its left side, with $LI = [(Right - Left) / (Right + Left)]$ (Anderson et al., 2009). Absolute Laterality Index (ALI; scores from 0 to 1) is a measure of the extent of individual behavioural lateralization, without taking into account lateralization side. Scores close to zero support a tendency to symmetry, while scores close to one points to strong lateralization. On the other hand, Relative Laterality Index (RLI; scores from -1 to 1) measures the direction of lateralization of the individual. A positive score means that the individual tends to be right-sided, while a negative score provides evidence for a left lateralization.

Individual-level lateralization analysis

In order to assess if the observed individuals were lateralized for each behaviour, we examined whether the mean ALI of the observed population was included in the confidence intervals of the mean generated from simulations of populations of non-lateralized individuals. For each lateralized behaviour, we simulated an individual with an equal amount of observation bouts than the observed individuals, but with a probability that the posture was on the left or right hand followed a Bernoulli distribution with $p=0.5$ at each bout. Then, we simulated as many individuals as there were in the original population sample and calculated the mean laterality index of the simulated population. The procedure was repeated for 10 000 simulated populations. We then built the frequency distribution of the laterality index means for the 10 000 simulated populations. Finally, 5% and 95% quartiles of the simulated populations were estimated, corresponding to the confidence intervals for the simulated distribution. If the observed laterality mean was outside the simulated distribution confidence intervals, then we concluded that the observed individuals were lateralized for the considered behaviour. This approach allows dealing with the limits of parametric test given the small number of observed individuals and the small number of repetitions of behaviour per individual.

Population-level lateralization analysis

In order to verify if the mean of the observed RLI was included in the confidence intervals for a distribution built from simulations of non-lateralized individuals' populations, we followed the same procedure as when assessing the individual-level behaviour lateralization. Distributions frequency of individual's RLI for each lateralized behaviour are also given, as well as the mean lateralization index and its confidence interval with 95% probabilities.

Sex and age effect on behavioural lateralization

To evaluate the effect of sex and age on individual lateralization we used generalized linear model with individual's ALI as the response variable, and sex and age as explanatory factors. Model selection was performed from the Akaike Information Criterion (AIC; Akaike, 1973), corrected for a small sample size (AICc; Burnham & Anderson, 2002). Additionally, we compute confidence intervals with 95% probabilities for sex and age estimators to assess effect signification.

All analyses were conducted using the R programming language, version 3.03 (R Development Core Team, 2014).

RESULTS

We recorded the neck position of 97 ringed individuals when resting (26 females, 43 males and 28 of unknown sex) in at least three occasions. Of those individuals, 91 were observed standing on one leg (25 females, 42 males and 24 of unknown sex). Rotation direction used during stamping was recorded on 77 non-ringed individuals, three to eight bouts per individual. Finally, 80 ringed individuals were recorded performing courtship displays (40 females 8-37 years old and 40 males 5-34 years old). Behaviours *wing-leg stretched* and *scratch* were discarded from the analyses because of their too weak occurrence in the data set.

Individual-level lateralization

The ALI means of the sampled individuals for both, neck position and leg-supporting posture fell into the confidence interval of the ALI means of the non-lateralized simulated populations (Fig. 1a-b) suggesting that resting postures were not lateralized.

For stamping behaviour, observed mean ALI was high and outside of the confident interval of the mean ALI of the simulated populations (Fig. 1c), indicating that stamping behaviour was lateralized.

Regarding courtship, *twist-preen* observed mean ALI was greater than the upper confidence limit of mean ALI of the simulated populations (Fig. 1d). Likewise, the mean ALI of *walk* was greater than the upper confidence limit for the mean ALI of the simulated populations (Fig. 1h). In contrast, the observed mean ALI of preening, alert and head-flagging behaviours fell into the confidence interval of the ALI means of the non-lateralized simulated populations (Fig. 1e-g). These results suggest that *twist-preen* and *walk* were the only behaviours lateralized in the courtship sequence.

Population-level lateralization

During stamping, 63% of the individuals turned to the left side in all repetitions of this movement (Fig. 2a). Apart of one single individual exception, the remaining individuals turned to the right side in all repetitions. A population-level tendency to turn to the left side was confirmed by both the negative value of the mean RLI of the sampled population ($\bar{X}_{observed} \pm SE = -0.269 \pm 0.966$) and by the fact that this value fell below the lower confidence limit of the simulated population RLI means [$\bar{X}_{simulated} CI = (-0.101 \text{ to } 0.102)$] (Fig. 2b).

During courtship, 57% of the observed flamingos tended to perform a single *twist-preen* or to initiate a *twist-preen* sequence towards the left side rather than toward the right side (Fig. 2c). Furthermore, the mean RLI of the sampled population was negative and inferior to the lower confidence interval range for RLI means of the simulated populations for *twist-preens* (Fig. 2d), suggesting a lateralization of this behaviour at the population-level.

In contrast, when walking during courtship, only 53% of the individuals did the first step with the left leg more frequently than with the right one (Fig. 2e). Furthermore, the mean RLI of the sampled population fell into the confidence interval range of the simulated population (Fig. 2f), suggesting an absence of lateralization at the population-level.

Sex and age effect on behaviour lateralization

Regarding *twist-preen*, a model including sex and age and a model including only the age effect did not differ by more than two points of AICc (Tab. 2) and, therefore, performed equally well in explaining the observed variation in ALI from the sampled individuals. In addition, *twist-preen* lateralization increased slightly with age ($slope = 0.022$, CI_{slope} : 0.002 to 0.041) (Fig. 3).

Concerning variation in walking behaviour, the null model, a model including only a sex effect and a model including only an age effect did not differ by more than two points of AICc (Tab. 2). Hence, none of the factors had an effect on the observed variation in ALI in walking behaviour.

DISCUSSION

Our results indicate that, in the wild, Greater flamingos exhibit population-level lateralization of their foraging behaviour when stamping and of their courtship behaviour when performing *twist-preen*. Additionally, we found that when walking during courtship, Greater flamingos exhibited individual-level lateralization. On the contrary, we found no evidence of lateralization during resting behaviour or in the use of other courtship postures.

Contrary to previous studies on the Caribbean Flamingo (*Phoenicopterus ruber*) (Anderson, 2009) and the Lesser Flamingo (*Phoeniconaias minor*) (Anderson et al., 2009) in zoos, we found no evidence for population-level lateralization of neck-position in Greater flamingos observed in the wild. This lack of lateralization observed during resting could be linked to the need to change the position of the head on the back in order to alternate the side monitored, yet it is unclear if the eye that was used to scan the environment was correlated with the side toward which the neck was curved. More detailed observations must be carried out to clarify this issue. Another explanation could be that individuals require changing neck-position frequently to avoid muscular fatigue caused by maintaining a static position for a long time. Avoiding muscular fatigue could also explain why, similar to what has been observed in captivity (Anderson, 2009), flamingos did not show a preference for resting on either one of their legs in the wild. It is also possible that individuals also require alternating the leg directly exposed to the air to maintain their body temperature (Anderson & Williams, 2010; Bouchard & Anderson, 2011).

The lateralization of the stamping foraging behaviour and of the *walk* in courtship displays can be explained by the fact that cerebral hemisphere specialization may facilitate the control of the body balance during this elaborated type of displacement. Footedness has also been observed in other higher vertebrates using the first-step test (McGreevy & Thomson, 2006; Tommasi & Vallortigara, 1999; Tomkins et al., 2010). Peluso and Anderson (2014) did not find evidence of lateralization of stamping in a Caribbean flamingo's zoo population, but it should be noted that they relied on a much smaller sample ($N = 13$) and fewer stamping bouts per individual (1 to 7) than us ($N = 77$; bouts: 3 to 8). Another difference is that previous authors recorded bouts on the same individual over different days whereas we recorded stamping side in following bouts. Those differences on the sampling design could influence the results of both stamping behaviour studies.

Stamping behaviour appears also to be lateralized at the population-level. The tendency to turn anti-clockwise implies that individuals use the right leg to make the first step when turning, whereupon the left leg is the one that holds the weight of the body and therefore ensure body balance. Population-level laterality in motor behaviour cannot be explained by social facilitation, since flamingos forage with the head under water, making them incapable of seeing other individuals. A more parsimonious explanation would be that this population-level lateralization is a by-product of the dominance of one cerebral hemisphere for several tasks (Mascetti & Vallortigara, 2001). If footedness population bias in motor behaviour seems to be common in mammals (McGreevy & Thomson, 2006; Tomkins et al., 2010), we only found a study in chicken chicks that reveal population-level lateralization of motor behaviour in birds (Tommasi & Vallortigara, 1999) and the side bias matches with ours.

Regarding courtship, it is not surprising that behaviours *alert* and *head-flag* were not lateralized, since both could be related to the monitoring of the social environment during courtship interactions. The same was true of the *preen* posture, which is a derived activity (Bradbury & Vehrencamp, 1998; Tinbergen, 1952) whose current function could still relate to the original function of preening both wings.

Population preference for the left side in the first twist-preen displayed could be explained by social constraints during mate choice. Tommasi (2005) argue that asymmetric courtship behaviours are good candidates to cause lateralization in species with lateral eyes, such as flamingos. Moreover, in species where there is population-level lateralization of the vision, individuals may tend to align their courtship behaviour towards the eye that would be

the most efficient one for mate detection (Ventolini et al., 2005). However, because flamingos perform communal courtship, it seems unlikely that an individual in the group could display toward the most efficient eye of several individuals. It is therefore more likely that the observed left side display lateralization stems from a right brain hemisphere specialization in the processing of tasks related to sexual behaviour (since the right hemisphere controls the left side of the body and the left hemisphere controls the right one). Rogers (2012) found that, in most of bird species, the left eye is specialized in the detection of social stimuli and social behaviours are left-side lateralized. She then argues that this pattern is probably due to right hemisphere dominance in those related tasks. Greater flamingos could then follow this general pattern.

It is interesting to note that the lateralized behaviours are those that included movements and cognitive tasks simultaneously: stamping behaviour implies displacement and foraging; walking during courtship implies displacement and environment monitoring, and twist-preen implies courtship and environment monitoring. This complexity could explain the lateralization of these behaviours (Vallortigara & Regolin, 2006) compared to simple, less cognitively demanding resting behaviours.

Sex and age effect on lateralization

Sex or age had no effect on courtship behaviour lateralization, although there was a slight positive effect of age on individual lateralization in twist-preen behaviour. Similarly, Peluso and Anderson (2014) found that the laterality index of foot scratch behaviour increases with age in a sample of captive Caribbean flamingos. This trend may result from a reinforcement of laterality with age. There is evidence that lateralization is stronger in older individuals in some non-primate species (Canning et al., 2011; McGreevy & Thomson, 2006), which implies that individuals who show a side bias behaviour from an early age subsequently develop brain connections that make this bias more pronounced with age. The same explanation could be proposed for long-lived birds, such as flamingos.

The fact that there was no evidence of sex effect on courtship lateralization could be explained by a similar sexual selection pressure for both sexes. In fact, flamingos have low sexual dimorphism linked to their monogamous mate system and a mutual mate choice taking place during group displays (Johnson & Cézilly, 2007). This does not imply that there is no

information exchange about the sex of the individual during courtship, but that lateralization of the first twist-preen and walk does not provide this kind of information in flamingos.

In conclusion, our results suggest that, in the Greater Flamingo, complex behaviour like foraging and courtship could be lateralized, whereas simpler behaviours like resting are not. Our observations of neck side-position during resting contrast with previous observations in captive flamingo populations (Anderson, 2009; Anderson et al., 2009) highlighting the importance of carrying out studies in the wild. This is the first study in flamingo where laterality is evaluated in courtship displays. Further investigations should examine the consequence of observed lateralization on individual fitness in the wild, in order to better understand the evolution of laterality in flamingos.

APPENDIX

Twist-preening sequence analysis

Because flamingos tend to alternate between their right and left sides during a sequence of successive twist-preens, successive twist-preen cannot be regarded as independent observations. Therefore, the possibility of twist-preen side alternation in a sequence was evaluated. To that end, we analysed in details the behavioural sequence from videos of focal individuals performing courtship.

A twist-preening sequence starts after a head-flagging movement or a walking behaviour. We also retain for the analysis twist-preening sequence interspersed with inverted-wing salute or alert postures. Finally, we obtained 63 sequences from 40 individuals. First twist-preening side and second twist-preening side were retained. To compute the effect of the first twist-preening side on the second twist-preening side, we performed General Linear Mixed Model analysis (GLMM). Because there was more than one sequence per individual, we took into account the identity of the individual as a random factor, in order to avoid pseudoreplication caused by repeated measures.

The model that assumes a relationship between the first and the second twist-preening side had an AICc score ($slope = -19.134$, $CI_{slope}: -29.682$ to -8.586 , $AICc = 49.013$) more than two units lower than the null model ($AICc = 88.786$). Therefore, we accepted that the second twist-preening side depends on the first one.

Preen sequence analysis

Flamingos also alternate between left and right when performing subsequent preens. Therefore, the same analysis was performed as for preening sequences: a preening sequence starts after a head-flagging movement or a walking behaviour. We also retain preening sequences interspersed with alert postures. Then, we analyse the relationship between the first and second preen of 212 sequences recorded from 66 individuals. Again, the model with an order effect had an AICc score ($slope = -1.348$, $CI_{slope}: -2.363$ to -0.333 , $AICc = 277.100$) more than two units lower than the null model ($AICc = 297.021$). Therefore we considered that the second preen side depended on the first one.

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TABLES

Table 1. Greater Flamingo courtship display behaviour definitions. Based on Johnson and Cézilly (2007).

Asymmetrical behaviours	
<i>Twist-preen</i>	The individual twists its head and neck back and drops down the wing on one side to preen behind the wing. An individual may repeat this movement several times in rapid succession changing the side towards which the movement is made.
<i>Preen</i>	The individual twists the head and scratches one body side with the beak. An individual may repeat this movement several times in rapid succession changing the side towards which the movement is made.
<i>Wing-leg stretch</i>	The individual stretches a wing and a leg from the same body side.
<i>Scratch</i>	The individual scratches the neck just behind the chin using a leg.
<i>Alert</i>	The individual stands erect with the neck stretched vertically upwards, the bill held high with the distal half nearly horizontal.
<i>Head-flag</i>	With the neck stretched the individual flags the head jerkily from side to side. This movement is often preceded by an alert posture.
<i>Walk</i>	When the individual walks, the asymmetry of this behaviour is defined by the fact that individuals begin the walk advancing one leg.
Symmetrical behaviours	
<i>Wing-salute</i>	The individual throws open their wings to their full extent, maintaining their stretched neck and the bill slightly above the horizontal. This movement is often performed by several individuals at the same time in the group display and usually precedes a twist-preen sequence.
<i>Inverted wing-salute</i>	With wings partially open, the individual bends forward from an erect position so that the cocked tail is held higher than the chest. The neck is extended straight forward and in a line with the body. Usually, this movement is performed between two twist-preens.

Table 2. Linear model results on the effect of the age and the sex on the lateralization at the individual-level of courtship behaviours twist-preen and walk. The laterality index on absolute values (ALI) is used as a measure of the individual-level lateralization. The sample size (N), the degrees of freedom (df), the effect size (R^2), the AICc scores and its difference with the lower AICc score ($\Delta AICc$) is given for each model.

Model	df	R^2	AICc	$\Delta AICc$	
<i>Twist-preen*</i>					
ALI ~ sex + age		17	0.276	11.992	0
ALI ~ sex		18	0.004	16.352	4.360
ALI ~ age		18	0.158	12.998	1.006
ALI ~ 1		19		14.722	2.730
<i>Walk**</i>					
ALI ~ sex + age		44	-0.020	37.927	3.508
ALI ~ sex		45	-0.010	36.163	1.744
ALI ~ age		45	-0.009	36.118	1.669
ALI ~ 1		46		34.419	0

* $N = 20$

** $N = 47$

CAPTIONS FOR FIGURES

Figure 1. Frequency distribution of laterality index means in absolute value (ALI) for 10000 simulations of populations of individuals randomly lateralized for behaviours: (a) neck position, (b) leg-supporting posture (c) stamping, (d) twist-preen, (e) preen, (f) alert, (g) head-flag and (h) walk. Bold lines represent the location of the mean ALI of the sampled population. Dashed lines represent lower and upper limits of the distribution, for a 5% confidence interval.

Figure 2. Frequency distributions of laterality index in relative values (RLI) for lateralized behaviours (a-b) stamping, (c-d) twist-preen and (e-f) walk. On the left side: histograms of RLI of observed individuals. Bold lines represent the location of the mean RLI and dashed lines represent lower and upper limits of the distribution, for a 5% confidence interval. On the right side: histograms of RLI means for 10000 simulations of populations of individuals randomly lateralized. Bold lines represent the location of the observed mean RLI of the sampled population and dashed lines represent lower and upper limits of the distribution, for a 5% confidence interval.

Figure 3. Relationship between age and individual's laterality index in absolute values (ALI) for twist-preen courtship behaviour.

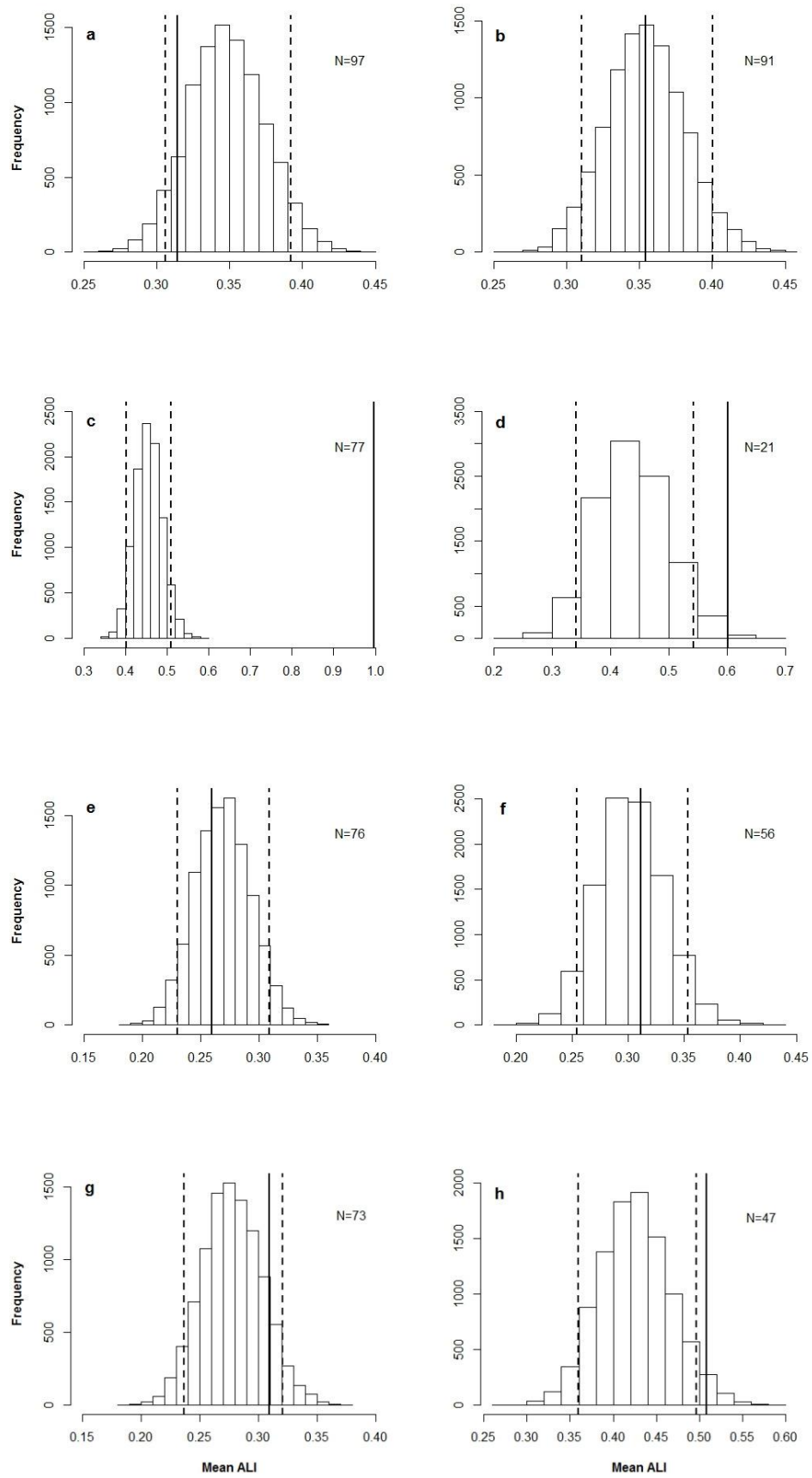


Fig.1

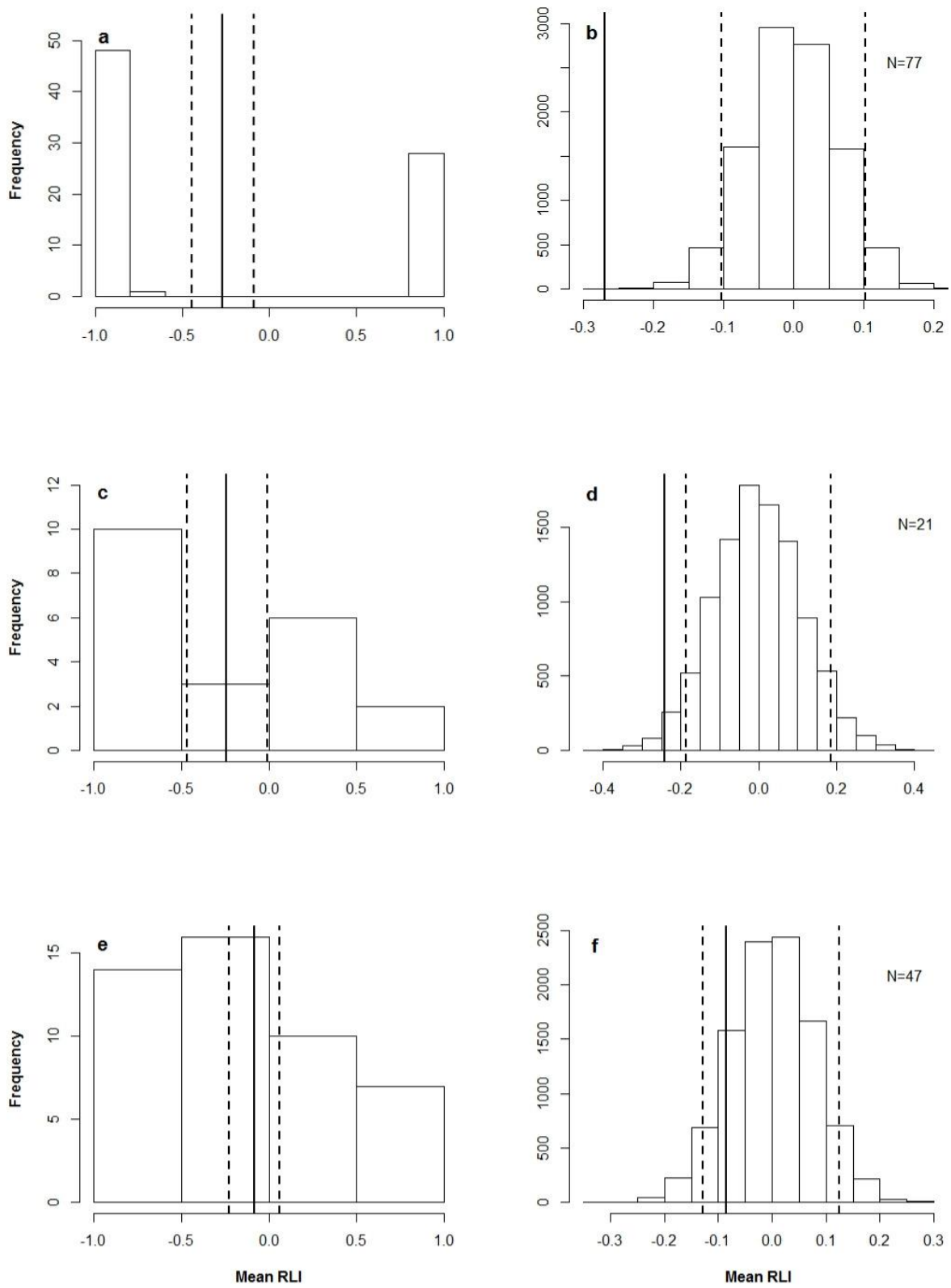


Fig.2

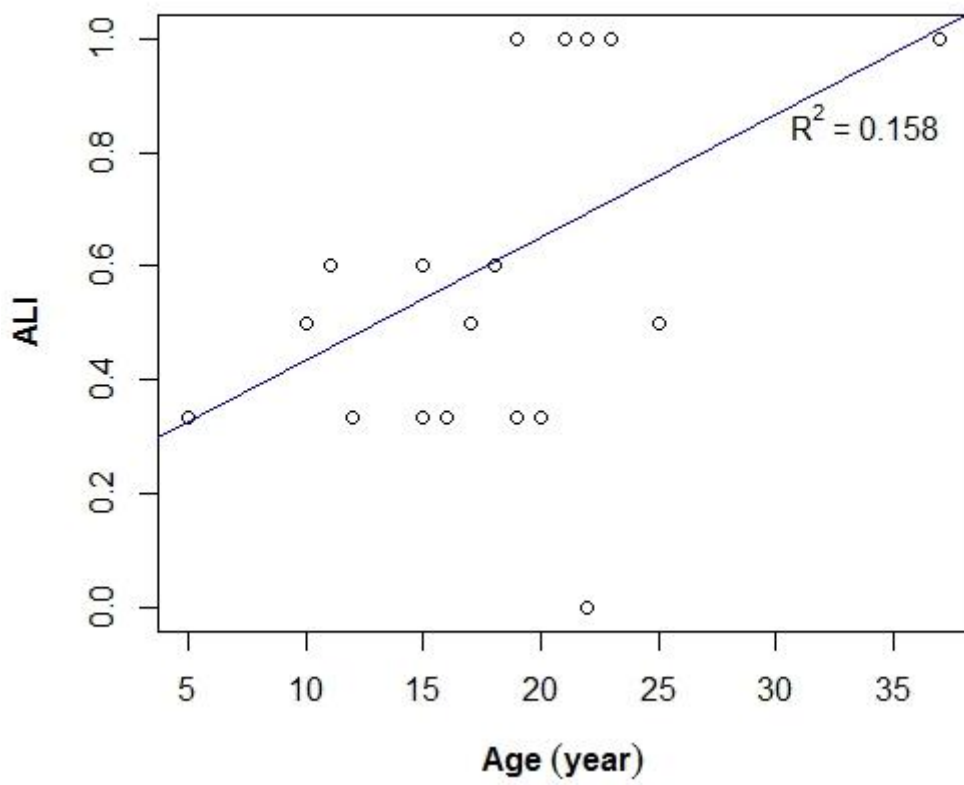


Fig. 3