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Impacts des activités anthropiques sur le comportement et les traits d'histoire de vie d'une espèce patrimoniale

Juliette Rabdeau

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THESE

Présentée par :

Juliette RABDEAU

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**Impacts des activités anthropiques sur le comportement et les traits
d'histoire de vie d'une espèce patrimoniale**



Réalisée au Centre d'Etudes Biologiques de Chizé UMR 7372 CNRS - La Rochelle Université

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Introduction générale



Depuis un siècle, près de 200 espèces de vertébrés ont disparu de la planète et presque autant n'ont plus été observées *in natura* (Ceballos et al. 2015 ; Figure 1). Entre 1970 et 2012, l'abondance des populations de vertébrés a chuté de 58% (WWF 2016). Actuellement, l'Union Internationale pour la Conservation de la Nature (UICN) répertorie en danger d'extinction près de 8700 espèces de vertébrés sur 50 000 évaluées et 5100 espèces d'invertébrés sur 22 000 évaluées (UICN 2019). Ces déclinés et extinctions n'ont jamais été aussi rapides dans le passé puisque le taux d'extinction des espèces au cours du siècle dernier serait cent fois plus important que celui des cinq dernières extinctions de masse, conduisant à qualifier la période actuelle de sixième extinction (Ceballos et al. 2015). Or, contrairement aux autres grandes extinctions liées à des causes naturelles, les causes de cette sixième extinction sont liées à l'augmentation conjointe de la population humaine mondiale (7,7 milliards en 2019, United Nations 2019) et de ses activités (Ehrlich & Ehrlich 2013, Chiron et al. 2014, Spooner et al. 2018), marquant le début d'une nouvelle ère, l'Anthropocène (Crutzen & Stoermer 2000, Gibbon et al. 2000, Newton 2004, Dirzo et al. 2014, Murphy & Romanuk 2014, Ceballos et al. 2015, Ripple et al. 2019).

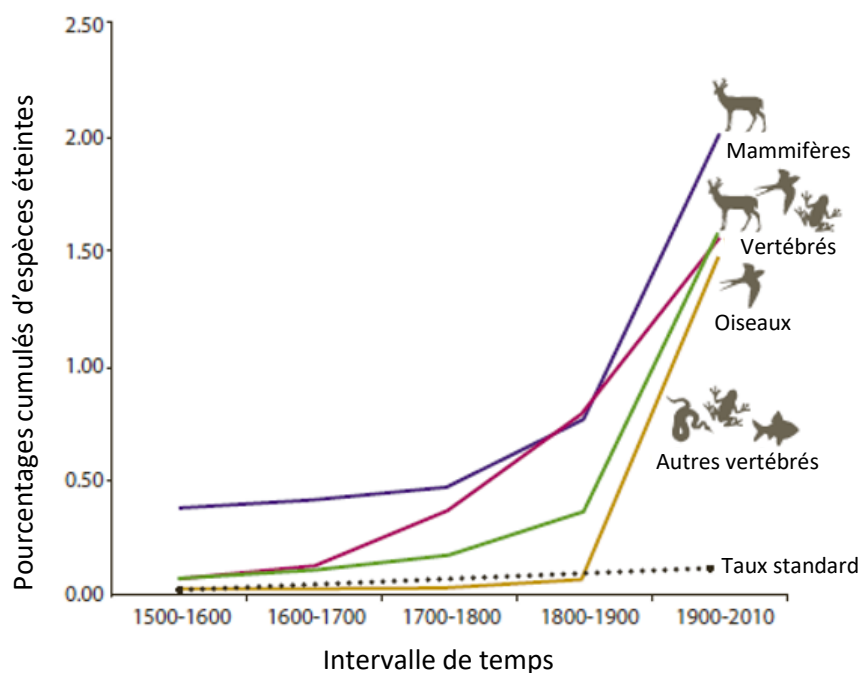


Figure 1 : Pourcentages cumulés d'espèces de vertébrés déclarées éteintes ou éteintes dans la nature par l'UICN (2012) en fonction des siècles depuis 1500 (d'après Ceballos et al. 2015). La courbe noire en pointillé représente le pourcentage cumulé d'extinction calculé à partir d'un taux standard d'extinction d'espèces soit deux extinctions pour 10 000 espèces de vertébrés sur 100 ans.

1. Impacts des activités anthropiques

En dehors de la pollution et du réchauffement climatique, les principales activités humaines responsables de la crise de la biodiversité recouvrent la surexploitation des espèces, les invasions biologiques et la perte ou la fragmentation des habitats et touchent tous les écosystèmes de la planète (Dirzo et al. 2014, Murphy & Romanuk 2014, Maxwell et al. 2016, Ripple et al. 2019). Ainsi, sur 8700 espèces menacées ou quasi-menacées évaluées par Maxwell et al. (2016), plus de 6200 sont affectées par la surexploitation à travers la pêche, la chasse, le braconnage et la commercialisation d'animaux vivants ou de parties de corps (e.g. défenses d'éléphants et cornes de rhinocéros) (Cawthorn & Hoffman 2015, Ripple et al. 2019). Les invasions biologiques impactent plus de 2000 espèces sur les 8700 espèces menacées ou quasi-menacées (Clavero et al. 2009, Maxwell et al. 2016, Ripple et al. 2019). Généralement involontaires, elles sont liées à l'augmentation des échanges commerciaux et du tourisme à l'échelle mondiale. Elles peuvent affecter directement des espèces natives par prédation mais aussi indirectement par compétition pour des ressources alimentaires et d'autres ressources de l'habitat (Simberloff 2010). La perte et la fragmentation des habitats sont également une cause majeure du déclin des populations et sont liées notamment à la croissance des zones urbanisées, au développement des infrastructures routières et à l'intensification de l'agriculture (Tilman et al. 2001, McKinney 2002, Cadenasso et al. 2007). Elles ont des effets directs sur les espèces en augmentant les risques de collision liés aux réseaux d'infrastructures et des effets indirects en limitant leurs déplacements et en réduisant leurs ressources (Fahrig 2003, Hanski 2005, Chace & Walsh 2006, Coffin 2007, Lesiński 2007, Fahrig & Rytwinski 2009, Gaisler et al. 2009, Hager 2009, Boulanger & Stenhouse 2014). L'intensification agricole, quant à elle, impacterait environ 5400 espèces sur les 8700 menacées (Maxwell et al. 2016). L'intensification de l'agriculture implique, entre autres, la diminution des habitats naturels et semi-naturels, la simplification des configurations paysagères (disparition des haies, diminution du nombre de parcelles et augmentation de leur superficie), la réduction de la diversité de cultures, la mécanisation des activités agricoles et l'utilisation accrue de produits phytosanitaires (Ghilain & Bélisle 2008, Geiger et al. 2010). Elle peut avoir des effets directs létaux et sub-létaux sur les organismes, mais également des effets indirects sur la survie via une réduction des ressources alimentaires, de refuge et de site de nidification (Donald et al. 2001, Inchausti & Bretagnolle 2005, Gibbons et al. 2015, Gaget et al. 2019). Par exemple, les

néonicotinoïdes auraient des effets létaux sur un grand nombre d'espèces de vertébrés par ingestion de graines enrobées de ces molécules et également des effets sub-létaux en réduisant les réponses immunitaires et la fécondité, et en augmentant les anomalies de développement (Lopez-Antia et al. 2013, Gibbons et al. 2015).

Au-delà de ces activités humaines ayant un impact majeur sur les espèces animales, d'autres activités sont à l'origine d'effets plus subtils sur les espèces. Ainsi, le dérangement est défini comme une perturbation générée par la présence humaine, des objets (e.g. voitures, hélicoptères) ou des bruits anthropiques (e.g. trafic routier, bruit industriel), qui engendre un stress chez l'animal et des changements au niveau de ses réponses physiologiques et comportementales (Frid & Dill 2002, Steven et al 2011, Blumstein et al. 2017). Plus de 1200 espèces sur les 8700 menacées seraient affectées par le dérangement anthropique (Maxwell et al. 2016). Le tourisme, les activités sportives, les activités de recherches, le bruit lié au trafic routier peuvent créer un dérangement et avoir des conséquences à moyen et long termes sur la dynamique des populations animales. Le dérangement anthropique affecte alors indirectement la survie et la reproduction des animaux à travers ses effets directs sur leurs réponses physiologiques et comportementales.

2. Réponses des animaux sauvages au dérangement anthropique

Les animaux perçoivent souvent les humains et leurs activités comme des risques de prédation (Frid & Dill 2002, Blumstein 2006). Ces dérangements anthropiques peuvent ainsi entraîner un stress ponctuel ou chronique pour un grand nombre d'espèces (Blanc et al. 2006, Barber et al. 2010, Steven et al. 2011, Tarjuelo et al. 2015). Une exposition ponctuelle à un stimulus stressant provoque des changements physiologiques et comportementaux qui induisent des réponses adaptatives pour que l'organisme retourne à un état d'équilibre, appelé homéostasie (Wingfield et al. 1998). A l'inverse, une exposition prolongée ou répétée sur plusieurs jours ou semaines induit des changements physiologiques et comportementaux qui persistent sans retour à un état d'équilibre, ce qui provoque des conséquences délétères pour l'organisme et qualifie un stress chronique (Wingfield et al. 1998).

a. Physiologie du stress

Chez les vertébrés exposés à un stimulus stressant comme la présence humaine ou ses activités, l'axe hypothalamo-hypophyso-surrénalien (HPA pour « hypothalamic-pituitary-adrenal ») de l'animal est activé avec une cascade de sécrétions d'hormones (Figure 2). Le système nerveux central envoie des informations nerveuses vers les neurones de l'hypothalamus qui synthétisent la corticolibérine (CRH pour « corticotropin releasing hormon » ; Wingfield & Kitaysky 2002 ; Figure 2). Ce peptide se fixe ensuite sur des récepteurs au niveau de l'hypophyse antérieure, activant ainsi la production de l'hormone corticotrope libérée dans le sang (ACTH pour « adenocorticotropin hormon » ; Wingfield & Kitaysky 2002, Ellis et al. 2012 ; Figure 2). Cette hormone atteint les glandes surrénales et active la synthèse de glucocorticoïdes (corticostérone ou cortisol), également libérés dans le sang (Wingfield et al. 1998, Romero & Romero 2002, Ellis et al. 2012 ; Figure 2). Cette réponse physiologique permet la mobilisation des ressources énergétiques de l'organisme à travers la gluconéogenèse (Wingfield et al. 1998, Wingfield & Romero 2001, Wingfield & Kitaysky 2002, Ellis et al. 2012).

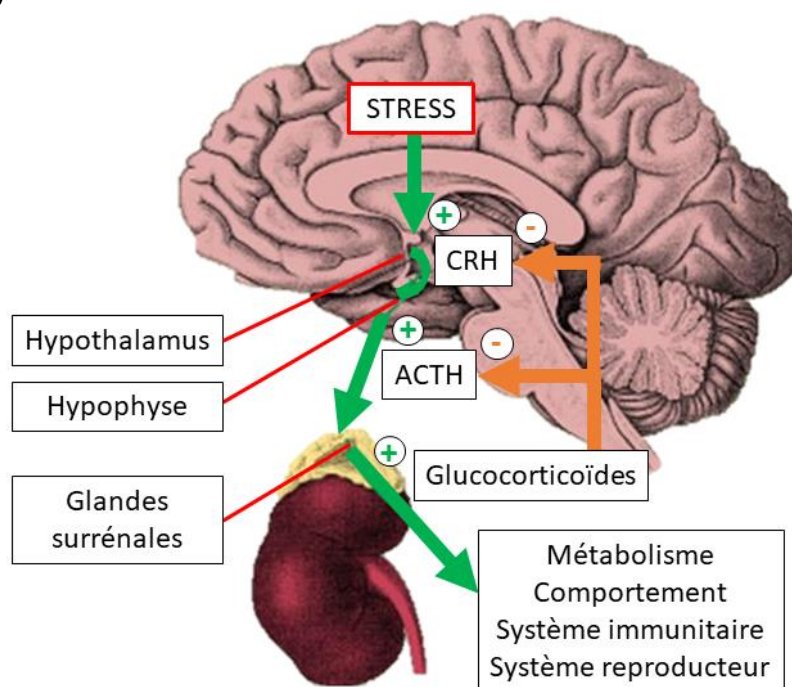


Figure 2 : Activation de l'axe hypothalamo-hypophyso-surrénalien (HPA) lors d'une exposition à un stimulus stressant, avec sécrétion des hormones corticolibérine (CRH) et corticotrope (ACTH) et des glucocorticoïdes (en vert). Rétrocontrôles négatifs des glucocorticoïdes sur les sécrétions de CRH et ACTH diminuant la sécrétion de glucocorticoïdes (en orange).

En parallèle, les catécholamines comme l'adrénaline sont synthétisées par les cellules de la glande médullosurrénale et augmentent la production de glucose (Wingfield et al. 1998, Ellis et al. 2012). Elles augmentent également la fréquence cardiaque des animaux comme montré chez les ours noirs (*Ursus americanus*) lors de la traversée d'une infrastructure routière (Ditmer et al. 2018). Ces réponses physiologiques à un stress ponctuel permettent d'augmenter l'activité locomotrice et de fuir rapidement la perturbation (Wingfield et al. 1998). L'organisme retrouve ainsi un état d'équilibre grâce à un rétrocontrôle négatif des glucocorticoïdes sur les sécrétions de CRH et ACTH (Wingfield et al. 1998, Wingfield & Romero 2001 ; Figure 2).

Cependant, lors d'une exposition prolongée ou répétée à un dérangement anthropique, l'axe HPA de l'animal est continuellement activé et produit des niveaux élevés et continus de glucocorticoïdes générant un stress chronique (Wingfield et al. 1998, Wingfield & Romero 2001, Ellenberg et al. 2007, Busch & Hayward 2009). Par exemple, chez la crécerelle d'Amérique (*Falco sparverius*), les femelles qui nichent sur des sites avec plus de dérangement anthropique lié au trafic routier, ont des concentrations basales en corticostérone élevées, contrairement aux femelles nichant sur des sites avec moins de trafic routier (Strasser & Heath 2013). Ce stress chronique inhibe le développement, la croissance et le système immunitaire de l'animal en diminuant la différenciation et la prolifération des cellules immunitaires (Wingfield et al. 1998, Martin 2009, Ellis et al. 2012). Il inhibe également le système reproducteur à travers différents mécanismes (Wingfield et al. 1998). Des sécrétions prolongées de glucocorticoïdes provoquent une mobilisation continue des ressources énergétiques qui pourraient être allouées aux processus de reproduction lors de la production d'œufs, la gestation ou l'élevage des jeunes (Ellis et al. 2012). Elles affectent également le système endocrinien reproducteur en diminuant la production de l'hormone de libération des gonadotrophines hypophysaires (GnRH), l'hormone folliculostimulante (FSH) et l'hormone lutéinisante (LH) qui stimulent la production des gamètes (Romero & Butler 2007). Par exemple, les mésanges charbonnières (*Parus major*) et les merles bleus de l'Est (*Sialia sialis*) qui nichent dans des habitats avec une nuisance sonore anthropique (trafic routier et autres activités humaines) plus élevée, pondent moins d'œufs que les femelles qui nichent dans des habitats moins exposés aux bruits anthropiques (Halfwerk et al. 2011, Kight et al. 2012). Des concentrations élevées en glucocorticoïdes peuvent également inhiber la sécrétion de la

prolactine, une hormone intervenant dans le maintien des comportements parentaux (Spée et al. 2010).

b. Réponses comportementales

Les réponses physiologiques provoquées par le dérangement anthropique comme l'augmentation de la fréquence cardiaque et de la gluconéogenèse, engendrent des réponses comportementales chez l'animal (Wingfield et al. 1998, Blanc et al. 2006, Gill 2007). En cas de perturbation anthropique, les animaux se figent, sont plus vigilants et fuient plus souvent comme montré chez l'aigle ibérique (*Aquila adalberti*) dont la probabilité de fuite augmente lorsque les activités pédestres sont plus proches du nid (González et al. 2006, Jayakody et al. 2008, Casas et al. 2009, Murchison et al. 2016). En contrepartie, la proportion de temps de repos et de fourragement est diminuée ce qui augmente les coûts énergétiques pour l'animal et compromet sa condition voire sa survie (Yasué 2005, Weston & Elgar 2005, Casas et al. 2009). Par exemple, les ours bruns de Scandinavie (*Ursus arctos*) réduisent leur activité de fourragement pendant les pics d'activités cynégétiques ce qui peut affecter leur condition corporelle avant l'hivernation (Hertel et al. 2016). En période de reproduction, l'investissement parental est souvent réduit en présence d'un dérangement anthropique, pouvant entraîner de graves conséquences sur la progéniture (Arroyo & Razin 2006, González et al. 2006, Ellenberg et al. 2007, Remacha et al. 2016). Le temps alloué à l'incubation chez les oiseaux est diminué, le développement embryonnaire est ainsi affecté et le succès d'éclosion réduit (Fernández & Azkona 1993, González et al. 2006). Pendant l'élevage, les pygargues à tête blanche (*Haliaeetus leucocephalus*), par exemple, diminuent leur temps de présence au nid, le temps consacré à entretenir le nid et à nourrir les jeunes, en présence de campeurs à proximité de leur nid (Steidl & Anthony 2000). Par conséquent, la condition corporelle et physiologique de la progéniture est souvent réduite et les risques de famine, d'hypothermie et de prédation sont augmentés (Fernández & Azkona 1993, Gill 2007, Remacha et al. 2016). En affectant directement le comportement des parents dans leurs soins à la progéniture, le dérangement anthropique réduit indirectement le succès reproducteur des animaux (Fernández & Azkona 1993, Arroyo & Razin 2006, Ellenberg et al. 2007, Halfwerk et al. 2011, Remacha et al. 2016). Cependant, lors d'une exposition répétée à un dérangement

anthropique, des mécanismes peuvent permettre de diminuer l'amplitude des réponses comportementales et physiologiques ou à l'inverse l'augmenter, en fonction de la durée et de l'intensité du stimulus stressant (Chace & Walsh 2006, Baudains & Lloyd 2007, Viblanc et al. 2012, Lee et al. 2017).

c. Habituation versus sensibilisation

Le phénomène d'habituation représente une diminution dans l'intensité des réponses comportementales et physiologiques des animaux exposés de façon répétée au même stimulus (Nisbet 2000, Cyr & Romero 2009, Blumstein 2016). A l'inverse, la sensibilisation au stimulus se produit lorsque l'intensité des réponses des animaux est augmentée (Blumstein 2016). Ces deux phénomènes ont été particulièrement étudiés dans des contextes d'activités récréatives ou d'urbanisation mais très peu à travers les activités de recherche et de conservation alors qu'elles impliquent souvent la manipulation et/ou l'exposition répétée du même animal (voir cependant van Oers & Carere 2007). L'habituation au dérangement anthropique ou à l'inverse la sensibilisation dépend de l'espèce/population exposée, de la durée, de l'intensité et de la nature du dérangement (Blanc et al. 2006, Blumstein 2016). Par exemple, deux espèces de manchots appartenant à la même famille des Sphéniscidés ne répondent pas de la même façon au dérangement. Lors d'une exposition à un facteur anthropique stressant (bruit métallique, approche humaine), les manchots royaux (*Aptenodytes patagonicus*) se reproduisant à proximité d'un camp de base de recherches scientifiques, ont une fréquence cardiaque plus faible que ceux nichant sur des sites isolés du camp (Viblanc et al. 2012). En revanche, les manchots antipodes (*Megadyptes antipodes*) qui se reproduisent sur des sites exposés au tourisme ont des concentrations de corticostérone plus élevées que ceux nichant sur des sites non exposés (Ellenberg et al. 2007). Les manchots antipodes sensibilisés sur les sites touristiques ont également un succès reproducteur réduit en raison d'une plus forte probabilité d'abandon du nid comparé aux manchots nichant sur les sites non exposés (Ellenberg et al. 2007). Par conséquent, dans le cas où les animaux ne s'habituent pas au dérangement anthropique, ils peuvent chercher à éviter les perturbations humaines en sélectionnant des habitats moins anthropisés.

d. Evitement du dérangement anthropique à travers la sélection d'habitat

Les animaux sélectionnent leur habitat en fonction de sa capacité à fournir des conditions abiotiques favorables, des ressources alimentaires pour eux-mêmes et leur progéniture, et de façon à éviter la prédation (Hall et al. 1997, Clark & Shutler 1999, Jones 2001, Pärt & Doligez 2003, Barbaro et al. 2008). Les infrastructures et activités humaines sont très présentes dans un grand nombre d'écosystèmes et peuvent être intégrées dans le processus de sélection d'habitat des animaux (Remacha & Delgadoa 2009, Hanane 2014, Pedrana et al. 2014, Cristescu et al. 2016). Les animaux évitent ainsi les perturbations anthropiques produites par les habitations, les axes routiers, les sentiers de randonnées ou autres activités sportives ou encore les sites touristiques (Yasué & Dearden 2006, Martínez-Abraín et al. 2010, Hanane 2014, Pedrana et al. 2014) comme montré chez les belugas (*Delphinapterus leucas*) qui évitent les bateaux touristiques (Blane & Jaakson 1994). Les animaux utilisent également plus les zones de refuge moins ou non anthropisées (Blanc et al. 2006). Par exemple, pendant les jours de chasse, les vanneaux huppés (*Vanellus vanellus*), les pluviers dorés (*Pluvialis apricaria*) et les outardes canepetières (*Tetrax tetrax*) utilisent plus fréquemment les zones de réserves sans chasse (Casas et al. 2009). De la même façon, en période de reproduction, les animaux choisissent un habitat peu anthropisé, ce qui limite les effets négatifs du dérangement anthropique sur leur succès reproducteur (Kolbe & Janzen 2002, Lowe et al. 2014). Par exemple, les gravelots de Péron (*Charadrius peronii*) sélectionnent des plages peu fréquentées par les touristes pour nicher, ce qui augmente leur succès d'éclosion et d'envol (Yasué & Dearden 2006). Bien que globalement, le dérangement anthropique impacte à la fois la physiologie et le comportement des individus, au sein d'une même population, les individus peuvent répondre de façon contrastée.

3. Et la personnalité dans tout cela ?

Le comportement animal a été longtemps étudié comme un trait hautement flexible, permettant aux individus de changer rapidement leur phénotype comportemental pour réagir de manière adaptative aux changements des conditions environnementales (Piersma & Drent 2003, Briffa et al. 2008). Cependant, dans une même population, les individus peuvent différer

de façon constante les uns des autres à la fois dans leur comportement moyen et dans leur réponse comportementale face aux variations des conditions de l'environnement, à travers le temps et différents contextes (i.e., personnalité animale, plasticité comportementale, normes de réactions comportementales, Nussey et al. 2007, Réale et al. 2007, Biro & Stamps 2008, Dingemanse et al. 2010). Ces différences comportementales inter-individuelles ont été mises en évidence à travers les différents taxons du règne animal et pour différents traits comme l'activité, l'agressivité, l'exploration, la socialité ou encore la témérité (Carere & Maestripieri 2013). Ce dernier trait représente les comportements de prise de risques des individus en présence d'un potentiel prédateur par exemple, et dans une même population, certains individus peuvent être plus téméraires que d'autres dans des situations risquées. Chez la chouette de l'Oural (*Strix uralensis*), les individus d'une même population diffèrent dans leur comportement moyen et leur plasticité de défense du nid en fonction de la disponibilité alimentaire (Kontinen et al. 2009). Plusieurs traits comportementaux peuvent être corrélés entre eux et former un syndrome comportemental (Sih et al. 2004, Sih & Bell 2008, Evans et al. 2010, Bókony et al. 2012, Sih et al. 2012). Ainsi, les individus téméraires face à un prédateur seraient à la fois plus agressifs envers leurs congénères, exploreraient leur environnement plus superficiellement et auraient une plus faible plasticité comportementale que les individus timides (Koolhaas et al. 1999, 2010, Sih et al. 2004, Sih & Bell 2008, Réale et al. 2009, 2010).

a. Implications écologiques et évolutives des différences inter-individuelles

Les traits de personnalité sont impliqués dans les mouvements des individus entre les habitats mais aussi dans la distribution des individus au sein de l'habitat (Cote et al. 2010, Wolf & Weissing 2012). En fonction de leur personnalité, les individus sont distribués différemment à travers les habitats comme suggéré par l'hypothèse de choix d'habitat en fonction de la personnalité (Edelaar et al. 2008, Selmann et al. 2014, Jacob et al. 2015, Holtmann et al. 2017, Sprau & Dingemanse 2017). Les individus choisissent un habitat pour ses composantes abiotiques et biotiques appropriées à leur personnalité (Wolf & Weissing 2012, Holtmann et al. 2017). Par exemple, chez l'eider à duvet (*Somateria mollissima*), les femelles timides nichent sur des sites plus ouverts avec une végétation moins dense que les femelles téméraires, ce qui leur confère plus de visibilité sur l'approche de potentiels prédateurs et

plus d'opportunités de fuite (Seltmann et al. 2014). Les différences comportementales inter-individuelles peuvent également être liées aux traits d'histoire de vie des individus comme la mortalité et la fécondité, ce qui peut avoir des conséquences sur la croissance des populations (Biro & Stamps 2008, Smith & Blumstein 2008, Wolf & Weissing 2012). En effet, selon un concept dérivé de la théorie des traits d'histoire de vie, appelé hypothèse des rythmes de vie (*pace-of-life syndrome hypothesis*), les traits comportementaux sont associés aux traits physiologiques et aux traits d'histoire de vie (Koolhaas et al. 1999, Koolhaas 2010, Réale et al. 2010, Atwell et al. 2012, Seltmann et al. 2012, Møller 2014). Par exemple, les individus téméraires prennent souvent plus de risques, acquièrent plus de ressources afin d'alimenter un métabolisme nécessitant plus d'énergie et ont une probabilité de survie réduite dans des habitats avec des risques importants de prédation (Biro & Stamps 2008, Smith & Blumstein 2008, Kontiainen et al. 2009, Réale et al. 2010). Le succès reproducteur est souvent lié à la personnalité de l'individu mais il peut varier également en fonction de la combinaison des personnalités des deux parents comme chez les espèces monogames à soins biparentaux (Both et al. 2005, Schuett et al. 2010, 2011, Gabriel & Black 2012). Certaines combinaisons au sein du couple seraient plus efficaces dans leur investissement parental et auraient un meilleur succès reproducteur (Schuett et al. 2010, 2011, Burtka & Grindstaff 2015). Par exemple, au sein des couples de mésanges charbonnières ou de diamants mandarins (*Taeniopygia guttata*), les mâles et les femelles similaires dans leur comportement d'agressivité et d'exploration produisent des poussins à l'envol en meilleure condition corporelle que d'autres combinaisons de couples (Both et al. 2005, Schuett et al. 2011).

b. Dans le contexte du dérangement anthropique

Certains traits comme la témérité favoriserait la tolérance des individus au dérangement anthropique et permettrait, par exemple, la colonisation de l'habitat urbain (Evans et al. 2010, Møller 2010, Atwell et al. 2012), la persistance dans des sites touristiques (Martin & Réale 2008) ou encore la nidification à proximité d'infrastructures humaines (Carrete & Tella 2010). Chez l'accenteur mouchet (*Prunella modularis*), les individus téméraires vivent dans des sites avec une plus forte fréquentation humaine au contraire des individus timides (Holtmann et al. 2017). La distribution des individus en fonction de leur personnalité et par rapport aux

activités humaines a été principalement étudiée dans les habitats urbains (Holtmann et al. 2017) ou en comparant habitat urbain *versus* rural (Evans et al. 2010, Møller 2010, Atwell et al. 2012) mais très peu au sein même de l'environnement rural (Carrete & Tella 2010). Or, celui-ci est souvent contraint par les infrastructures humaines (axes routiers, villages, bâtiments industriels et agricoles) en plus ou moins grande densité pouvant créer un dérangement. De plus, l'impact des activités humaines pouvant être différent selon la personnalité des individus, les liens entre le phénotype des individus et leur succès reproducteur pourraient être modifiés sous l'effet d'un dérangement anthropique. Ces conséquences ont été peu examinées (voir cependant Zhao et al. 2016). Or, si le dérangement anthropique exerce des effets différents sur le succès reproducteur des individus en fonction de leur personnalité, ces effets pourraient modifier la composition phénotypique d'une population et à long terme sa viabilité face à des changements environnementaux (prédation, épidémie, disponibilité des ressources alimentaires) (Sih et al. 2012, Wolf & Weissing 2012).

4. Objectifs de la thèse et démarche générale

Mes travaux de thèse s'inscrivent dans la problématique de la compréhension des impacts du dérangement anthropique sur les réponses comportementales et physiologiques et les traits d'histoire de vie d'une espèce inféodée aux milieux agricoles, le busard cendré (*Circus pygargus*). Les enjeux de conservation de cette espèce protégée sont importants pour la viabilité des populations. En effet, le busard cendré niche au sol dans les cultures de céréales ce qui l'expose fortement aux activités agricoles (gestion mécanique des parcelles et traitements chimiques) mais aussi à d'autres activités humaines (activités récréatives comme la promenade, bruit provenant du trafic routier et des villages). Sur le site d'étude, la Zone Atelier Plaine et Val de Sèvre (ZAPVS), la population de busard cendré est suivie de manière exhaustive depuis 1995, ce qui procure une large base de données sur la répartition des nids et le comportement des individus. Notamment, la personnalité des femelles est un des paramètres mesurés depuis le début du suivi de cette population et a des conséquences sur le succès reproducteur (Arroyo et al. 2017). Enfin, ce suivi génère également un autre type de dérangement puisque le monitoring de la population implique plusieurs visites au nid et des mesures répétées sur les poussins. Ces interactions répétées avec l'Homme permettent

d'apprécier les réponses comportementales et physiologiques des poussins aux expérimentateurs. Ainsi, quatre grandes questions seront développées et constitueront les quatre chapitres de ce manuscrit :

Tout d'abord, j'ai testé l'hypothèse d'un phénomène d'habituation des poussins aux expérimentateurs à travers des visites répétées en conditions naturelles, durant une saison de reproduction. Pour cela, l'impact de manipulations successives a été évalué sur le comportement, le niveau de stress et la condition corporelle des poussins au cours des quatre visites au nid pendant l'élevage, en changeant ou non les expérimentateurs d'une visite à une autre (Chapitre I). Par la suite, j'ai testé si un patron d'appariement en fonction de la témérité des mâles et des femelles existait au sein de la population étudiée de busard cendré, ainsi que son impact sur le succès reproducteur (Chapitre II). Durant trois saisons de reproduction, la témérité des mâles et des femelles a été caractérisée à travers la distance de fuite des individus face à des approches par l'Homme (personnalité et plasticité) et le patron d'appariement des individus dans la population à travers leur témérité a été analysé. Les liens entre l'association comportementale des parents et leur succès reproducteur ont été testés. Ceci amenait naturellement à évaluer si l'association comportementale des couples dans leur témérité influençait leur investissement parental et la qualité des poussins à travers plusieurs aspects morphologiques et physiologiques (Chapitre III). Les variations inter-nids d'investissement parental des busards cendrés à travers leur approvisionnement en nourriture ont été évaluées en relation avec l'association comportementale des couples. Les effets du patron d'appariement sur la qualité des poussins ont été étudiés. Enfin, j'ai évalué si la répartition des nids de busard cendré par rapport à la proximité et la densité d'infrastructures autour des nids dépendait de la témérité des femelles, et si le succès reproducteur variait à la fois en fonction de la proximité et de la densité d'infrastructures autour des nids et de la témérité des femelles (Chapitre IV). Pour cela, je me suis appuyée sur 19 années de suivis des nids de busard cendré et une base de données géoréférencées des nids, de la personnalité des femelles et de la composition du paysage en infrastructures.

Chaque chapitre est présenté sous forme d'article dans la suite du manuscrit. La majorité de la méthodologie étant commune à l'ensemble des chapitres, une section sur les méthodes générales est présentée. Les autres méthodes spécifiques aux questions sont décrites au sein de chaque chapitre.

Méthodes générales



1. Le busard cendré (*Circus pygargus*)

Le busard cendré est un rapace diurne (Ordre des Accipitriformes) du genre *Circus* sp. qui compte, selon les phylogénies, 13 à 16 espèces à travers le monde (Simmons 2000). Ce rapace de taille moyenne (environ 43-47 cm de long et 105-120 cm d'envergure) présente un dimorphisme sexuel de coloration et de biométrie (voir la synthèse par Arroyo et al. 2004 ; Figure 3). Le mâle a un plumage gris cendré, la femelle un plumage brun, (Figure 3). Le mâle est légèrement plus petit (longueur moyenne du tarse \pm erreur standard : $59,9 \pm 1,8$ mm) et plus léger (masse : $281 \pm 28,4$ g) que la femelle (longueur moyenne du tarse : $60,8 \pm 3,3$ mm ; masse : $340 \pm 30,7$ g) (Arroyo et al. 2004 ; Figure 3). La maturité sexuelle est différente entre les sexes. Les femelles peuvent se reproduire dès l'âge d'un an lorsque les ressources alimentaires sont abondantes alors que les mâles ne se reproduisent pas avant deux ans (Salamolard et al. 2000, Arroyo et al. 2004). La longévité est similaire entre les deux sexes entre 10 et 16 ans pour le plus vieil oiseau bagué (Arroyo et al. 2004).



Figure 3 : a) Busard cendré mâle, b) femelle et c) passage de proies aérien du mâle à la femelle (© M. Passerault).

La distribution géographique du busard cendré est centrée sur la zone Paléarctique occidentale (Arroyo et al. 2004 ; Figure 4a). En France, sa répartition n'est pas homogène sur tout le pays et se concentre sur trois principaux sites de reproduction : une zone en centre-ouest (Vendée et Poitou-Charentes), une zone nord-est (Côte d'Or et Champagne-Ardenne) et une zone centre-sud (Massif central et Roussillon) (Arroyo et al. 2004 ; Figure 4b). Cette espèce de rapace a la particularité de nicher au sol dans les milieux ouverts de landes, marais ou régénérations forestières. Avec l'agriculture intensive et la réduction de ce type d'habitats

naturels, le busard cendré s'est adapté et niche maintenant principalement dans les cultures de céréales ce qui l'expose fortement aux pratiques agricoles liées à ces cultures (Salamolard et al. 2000, Arroyo et al. 2002b, Millon et al. 2002). Il n'est pas considéré comme une espèce menacée à l'échelle mondiale mais en France il est « quasi menacé » (UICN France 2016). Bien qu'il soit protégé sur tout le territoire français depuis l'arrêté ministériel du 17 avril 1981, ses populations sont en déclin, également dans plusieurs parties de l'Europe, probablement en raison des fortes pressions anthropiques de l'agriculture intensive ces dernières décennies (Butet & Leroux 2001, Comolet-Tirman et al. 2015, Le Rest et al. 2015, Santangeli et al. 2015, UICN France 2016). En absence de mesures de protection et de conservation, 60 % des poussins dans les cultures agricoles pourraient mourir lors des moissons et autres pratiques agricoles (Arroyo et al. 2002b).

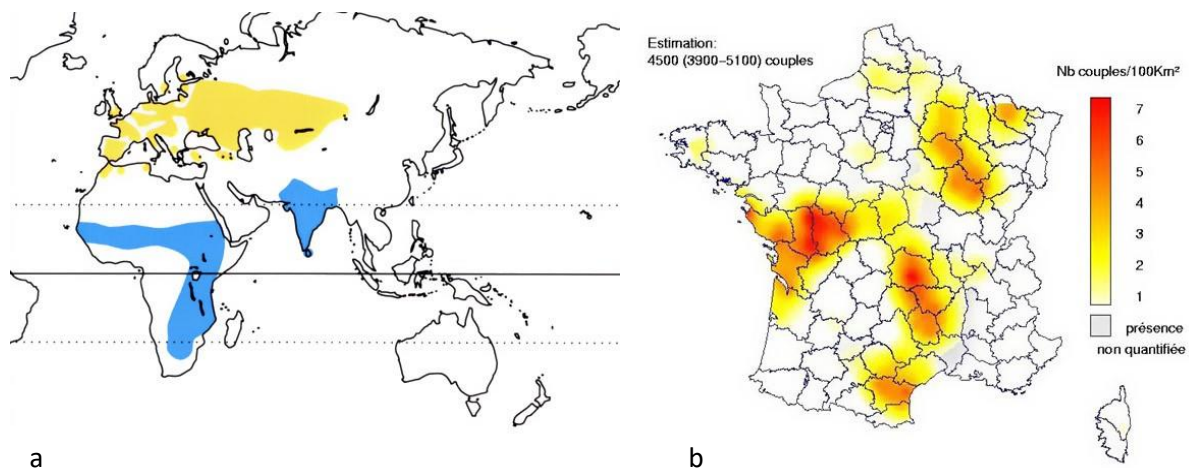


Figure 4 : a) Distribution mondiale du busard cendré avec les sites de reproduction (en jaune) localisés sur l'Europe et l'Ouest de l'Asie et les sites d'hivernage (en bleu) localisés en Afrique centrale et de l'Ouest et en Inde. B) Abondance et distribution du busard cendré en France (d'après Thiollay & Bretagnolle 2004, © Ligue pour la Protection des Oiseaux).

Cette espèce migratrice hiverne en Inde, en Afrique de l'Ouest et dans la zone sahélienne (observation de dortoirs hivernaux et reprises de bagues du Sénégal au Soudan) (Cormier & Baillon 1991, Clarke 1996, García & Arroyo 1998, Arroyo et al. 2004 ; Figure 4a). D'avril à juin, les busards cendrés reviennent de leur hivernage pour nicher en Europe et en Asie de l'Ouest dans les milieux ouverts (Arroyo et al. 2004 ; Figure 4a). Ce rapace semi-colonial niche principalement en colonie mais peut également établir son nid isolé d'autres congénères

(Arroyo et al. 2004). Le système d'appariement est principalement la monogamie malgré quelques cas rares de polyandrie notés sur notre zone d'étude (Arroyo et al. 2004). Les busards cendrés ne seraient pas fidèles à leur partenaire d'une année à l'autre (Arroyo et al. 2004) mais cette hypothèse reste à confirmer. Au moment de l'appariement, le mâle et la femelle effectuent une parade nuptiale comprenant plusieurs acrobaties aériennes avec des passages de proies du mâle à la femelle (Mougeot et al. 2006 ; Figure 3c). Les parades et accouplements peuvent se répéter pendant environ trois semaines avant la ponte (Arroyo 1999, Mougeot et al. 2006). Les busards cendrés construisent leur nid au sol de manière assez sommaire avec quelques brindilles, principalement dans les cultures de blé et d'orge sur notre zone d'étude (Arroyo et al. 2004 ; Figure 5). Les femelles commencent à pondre début mai pour les plus précoces sur notre zone d'étude, avec un intervalle de deux jours en moyenne entre deux œufs pondus et ce jusqu'à six œufs suivant l'abondance en nourriture (Arroyo et al. 2004 ; Figure 5a). L'incubation dure environ 29-30 jours et est réalisée uniquement par la femelle (García & Arroyo 2001, Arroyo et al. 2007). En effet, la particularité de cette espèce à soins biparentaux est que les rôles dans les soins à la progéniture sont différents entre les deux sexes (Arroyo et al. 2004). Pendant une grande partie de la période de reproduction, le mâle s'occupe de l'approvisionnement en proies à la fois pour la nichée et la femelle. En fin d'élevage, la femelle peut chasser tout en restant à proximité du nid et approvisionner de quelques proies la nichée mais ce taux d'approvisionnement reste inférieur à celui du mâle (García & Arroyo 2005). La défense du nid est assurée principalement par la femelle qui protège également les poussins des intempéries. Le busard cendré étant une espèce nidicole, les poussins sont entièrement dépendants des parents pour l'approvisionnement en proies et la protection (Arroyo et al. 2004 ; Figure 5). Ils prennent leur envol au bout de 30 à 35 jours d'élevage mais sont alimentés par leurs parents encore quelques jours après l'envol (Arroyo et al. 2002a ; Figure 5e).



Figure 5 : Nid de busard cendré à différents stades de développement : a) stade œuf, b) quatre à cinq jours après l'éclosion, c) trois poussins âgés entre 15 et 20 jours, d) un poussin âgé d'environ 25 jours et e) un poussin à l'envol âgé d'environ 30 jours (© C. Mangelinck, K. Monceau et J. Rabdeau).

En période de reproduction, le busard cendré se spécialise localement sur certains types de proies : les levrauts en Espagne et les micromammifères en France ou en Hollande (Arroyo 1997, Salamolard et al. 2000, Arroyo et al. 2004). En France, la principale proie est le campagnol des champs (*Microtus arvalis*) et son abondance cyclique influence directement le succès reproducteur du busard cendré (Salamolard et al. 2000, Butet & Leroux 2001, Millon et al. 2008, Millon & Bretagnolle 2008). Pendant les saisons de faible abondance en campagnols, le busard cendré peut également chasser des orthoptères et des passereaux comme l'Alouette des champs (*Alauda arvensis*) (Arroyo 1997).

2. Site d'étude : Zone Atelier Plaine et Val de Sèvre (ZAPVS)

La ZAPVS est une plaine céréalière intensive située dans le sud du département des Deux-Sèvres entre la ville de Niort à la limite nord et la forêt domaniale de Chizé au sud (46° 110 N, 0° 280 W ; Figure 6).

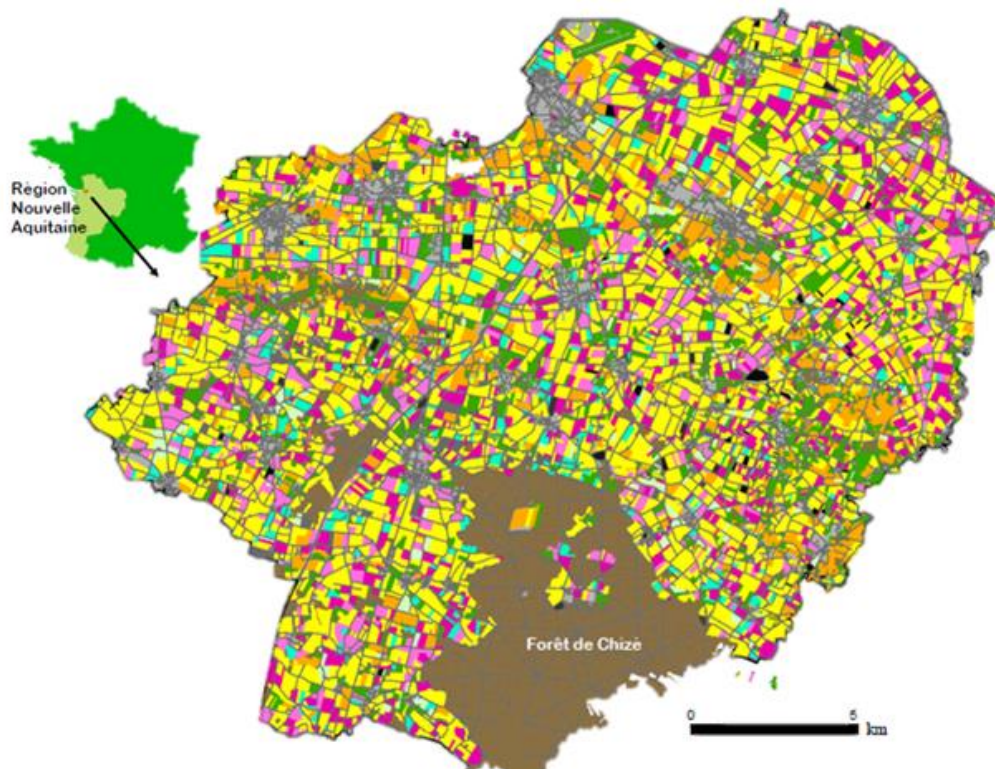


Figure 6 : Structure paysagère de la Zone Atelier Plaine et Val de Sèvre localisée dans la Région Nouvelle Aquitaine. Chaque polygone représente une parcelle agricole avec les modes d'occupation du sol symbolisés par des couleurs différentes dans la base de données géoréférencées.

Depuis la création du site d'étude en 1994 et sa désignation comme « Zone Atelier » en 2008, la ZAPVS s'est progressivement agrandie pour atteindre aujourd'hui une superficie de 435 km² (Bretagnolle et al. 2018). Depuis 2003, plus de la moitié de sa superficie bénéficie du statut de Zone de Protection Spéciale (ZPS, site NATURA 2000 désigné pour la biodiversité remarquable des espèces de la Directive Oiseaux ; Bretagnolle et al. 2018). Depuis les années 1950 et suite à l'intensification des pratiques agricoles, la topographie de cette plaine historiquement dominée par la polyculture et l'élevage, a considérablement changé (Figure 7). Ainsi, les prairies y occupaient plus de 60 % de l'espace il y a 50 ans alors qu'en 2015 elles ne représentaient plus qu'environ 10 % et cela au profit des grandes cultures céréalières notamment. D'importants remembrements ont également conduit à une réduction du linéaire de haies, du nombre de parcelles et à une augmentation de leur superficie (Figure 7).



Figure 7 : Comparaison de la structure paysagère du secteur Fors-Vaubalier sur la ZAPVS entre 1958 et 2014, à partir de photographies aériennes de l'Institut national de l'information géographique et forestière.

Depuis 1994, le parcellaire et le mode d'occupation des sols de chaque parcelle de la ZAPVS sont renseignés chaque année et géoréférencés, constituant une importante base de données spatialisées (Système d'Informations Géographiques) (Bretagnolle et al. 2018). Les modes d'occupation des sols sont notés selon plus de 30 catégories : les différentes cultures (ex : blé d'hiver, orge, tournesol, colza, maïs, luzerne), les bois, les bâtis (ex : village, bâtiment agricole et industriel), les axes routiers (dont une autoroute qui traverse la ZAPVS) et les chemins. Sur la période 2009-2016, la ZAPVS est dominée par les grandes cultures : le blé (33,8 %), le tournesol (10,4 %), le maïs (9,6 %), le colza (8,3 %) et les prairies (toutes catégories confondues : 13,5 %). Les zones en bâti y représentent 9,8 % de la surface.

Depuis 1995, la ZAPVS fait l'objet du suivi de nombreuses espèces d'oiseaux de plaine (outarde canepetière *Tetrax tetrax*, œdicnème criard *Burhinus oedicnemus*, busard Saint-Martin *Circus cyaneus*, busard des roseaux *Circus aeruginosus*) et de leurs ressources alimentaires (plantes, insectes, micromammifères).

3. Suivi de la population de busard cendré sur la ZAPVS

La population de busard cendré est suivie depuis 1995 (Bretagnolle et al 2018). Chaque année, les nids de busard cendré sont recherchés activement et exhaustivement à partir d'avril sur toute la zone d'étude. Une fois que les couples sont repérés grâce à leur comportement (passages de proie, accouplements), les nids sont localisés le plus souvent après un passage de proies (Figure 3c) lorsque la femelle retourne au nid grâce à un alignement du nid aux jumelles. Cet alignement consiste à relever des repères topographiques dans l'arrière-plan du paysage et dans l'axe nid-observateur. Les nids sont ensuite visités une à deux fois pendant l'incubation de façon à relever la taille de ponte finale (Figure 8).

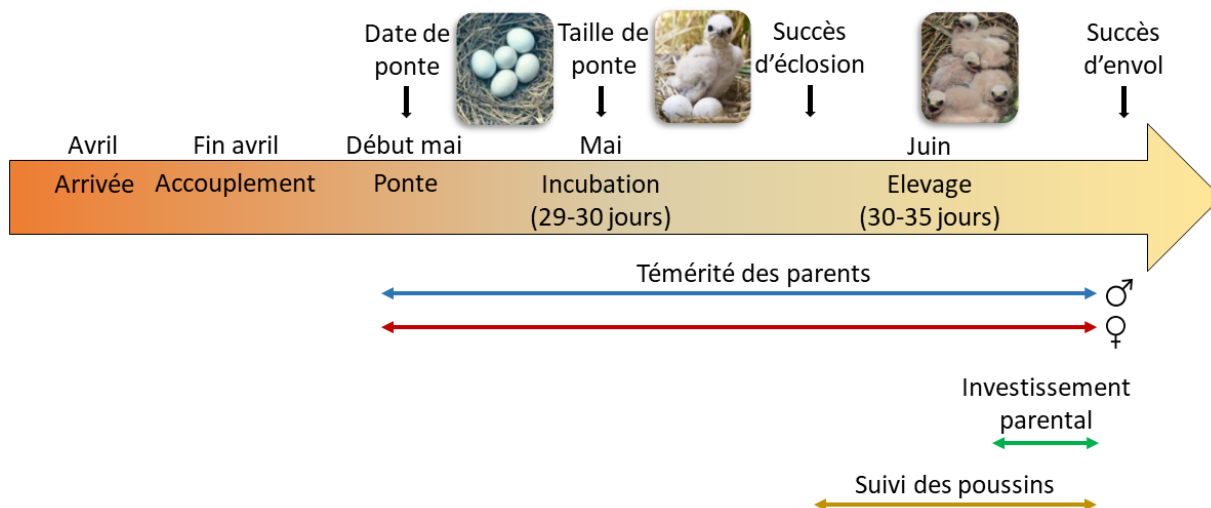


Figure 8 : Suivi de la reproduction des busards cendrés de leur arrivée sur la ZAPVS au retour de migration jusqu'à l'envol des poussins et déroulement des différents protocoles de mesures établis pour les trois saisons de reproduction (2017 à 2019).

Pendant ces visites, les œufs sont mesurés au pied à coulisse (longueur et largeur, précision : $\pm 0,1$ mm) et pesés (balance électronique modèle Pesola®, précision : $\pm 0,1$ g) ce qui permet d'estimer leur date d'éclosion (Arroyo et al. 2017). Après l'éclosion, les nids sont visités une fois par semaine pour réaliser des mesures biométriques sur les poussins jusqu'à leur envol : quatre à cinq visites suivant la taille de la nichée et l'écart d'âge entre les poussins (Figure 8). Suivant la date de moisson des céréales, le nid est délimité et/ou encerclé par du grillage lors d'une visite au nid pour éviter la dispersion et l'écrasement des poussins dans la culture au moment de la récolte. Une dernière visite est réalisée quelques jours après pour constater le succès d'envol des poussins (Figure 8).

Depuis 1995, à chaque visite de nid, plusieurs comportements des femelles sont notés comme leur distance de fuite à l'approche du nid, qui est utilisée pour caractériser leur témérité (voir section suivante ; Arroyo et al. 2017). Jusqu'à mon travail de thèse, la témérité des mâles n'avait pas été étudiée car ils sont rarement présents pendant les visites de nid. De ce fait, un protocole de mesure de distance de fuite des mâles selon d'autres modalités que pour les femelles, a été mis en place pour évaluer si cette variable permettait de caractériser leur témérité. Ces mesures comportementales pour les mâles et les femelles ont été réalisées une fois la première visite au nid effectuée et pendant tout le reste de la période de

reproduction (Figure 8). Plusieurs protocoles permettant de mesurer les réponses comportementales et physiologiques des poussins à la présence et à la manipulation par l'Homme ont également été établis. Enfin, un protocole permettant de mesurer l'investissement parental des busards cendrés dans leur approvisionnement en proies a été mis au point et réalisé en fin de période d'élevage (Figure 8). Ces différents protocoles sont présentés ci-après.

a. Distance de fuite des mâles (méthodes pour 2017, 2018, 2019)

Une fois les nids localisés, un poteau a été placé à proximité du nid en bordure de parcelle le long d'un chemin ou d'une route. La localisation exacte du poteau a été enregistrée (point GPS poteau ; Figures 9a et 10a) afin d'être utilisée ultérieurement pour estimer la distance de fuite. L'identification des mâles reposait sur des observations comportementales avec la femelle (passage de proies) et pour certains mâles, sur l'équipement de marques alaires ou de balises GPS sur le dos entre les deux ailes (Figure 10), ou encore grâce à des caractéristiques morphologiques distinguables. Lorsque l'identification du mâle n'était pas confirmée, la mesure comportementale n'était pas comptabilisée. Les mâles perchés sur leur poteau ont été approchés en se plaçant à une distance de départ d'environ 200 m en fonction de la configuration du paysage. La localisation exacte du départ de l'approche a été enregistrée (point GPS départ ; Figure 9a). L'expérimentateur marchait à vitesse constante (ca. $1,0 \text{ m}\cdot\text{s}^{-1}$) en direction du mâle sur le poteau. Lorsque celui-ci initiait un mouvement de fuite, l'expérimentateur s'arrêtait et enregistrait sa propre localisation (point GPS fuite ; Figure 9a). La distance de fuite du mâle (FID pour « flight initiation distance ») représentait alors la distance entre le point GPS du poteau et le point GPS de fuite (Figure 9a). La distance de départ était comprise entre le point GPS de départ et le point GPS du poteau (Figure 9a). Les approches de mâles ont été réalisées le matin de façon à standardiser le protocole et parce que les probabilités de trouver les mâles posés sur les poteaux étaient plus élevées le matin que le reste de la journée. Les approches ont été répétées jusqu'à quatre fois par mâle pour tester la répétabilité de la réponse comportementale, avec un intervalle de quelques jours entre chaque répétition (moyenne \pm déviation standard : $7,87 \pm 5,45$ jours). Uniquement les mâles avec au minimum deux mesures de FID ont été considérés de 2017 à 2019 (2017 : 13

mâles, 36 observations, $2,77 \pm 0,83$ mesures par individu ; 2018 : 13 mâles, 33 observations, $2,54 \pm 0,52$ mesures par individu ; 2019 : 13 mâles, 38 observations, $2,92 \pm 0,86$ mesures par individu).

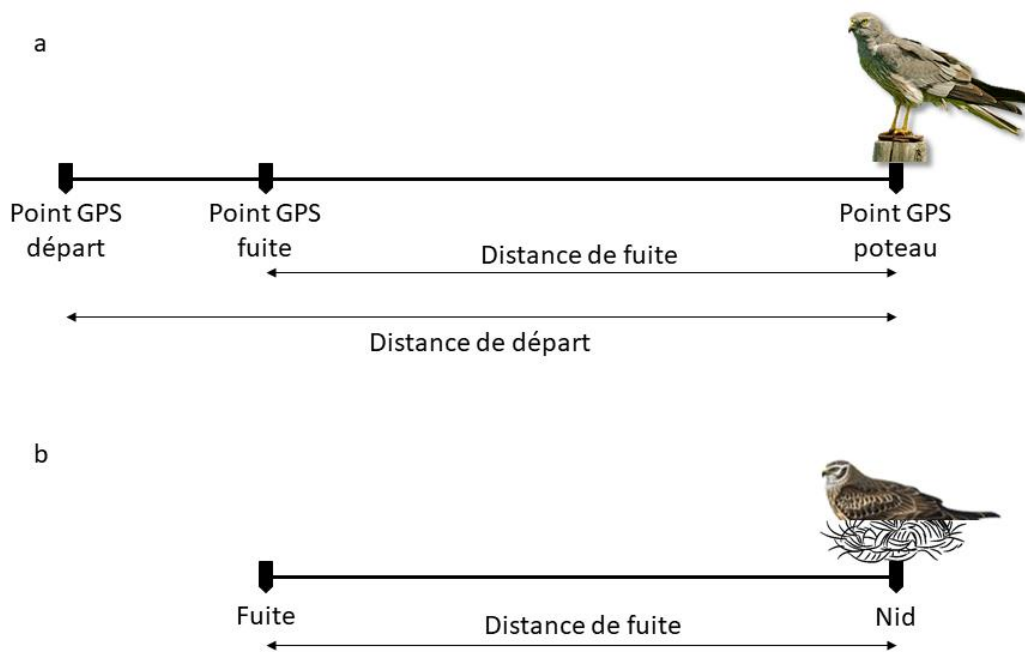


Figure 9 : Dispositif expérimental de mesures de distance de fuite chez les mâles sur un poteau (a) et les femelles présentes au nid (b). Pour les mâles, la distance de fuite était comprise entre le point GPS de fuite et le point GPS du poteau et pour la distance de départ, entre le point GPS de départ et le point GPS du poteau. Pour les femelles, la distance de fuite était comprise entre le nid où était présente la femelle et l'expérimentateur approchant au moment où la femelle s'envolait.

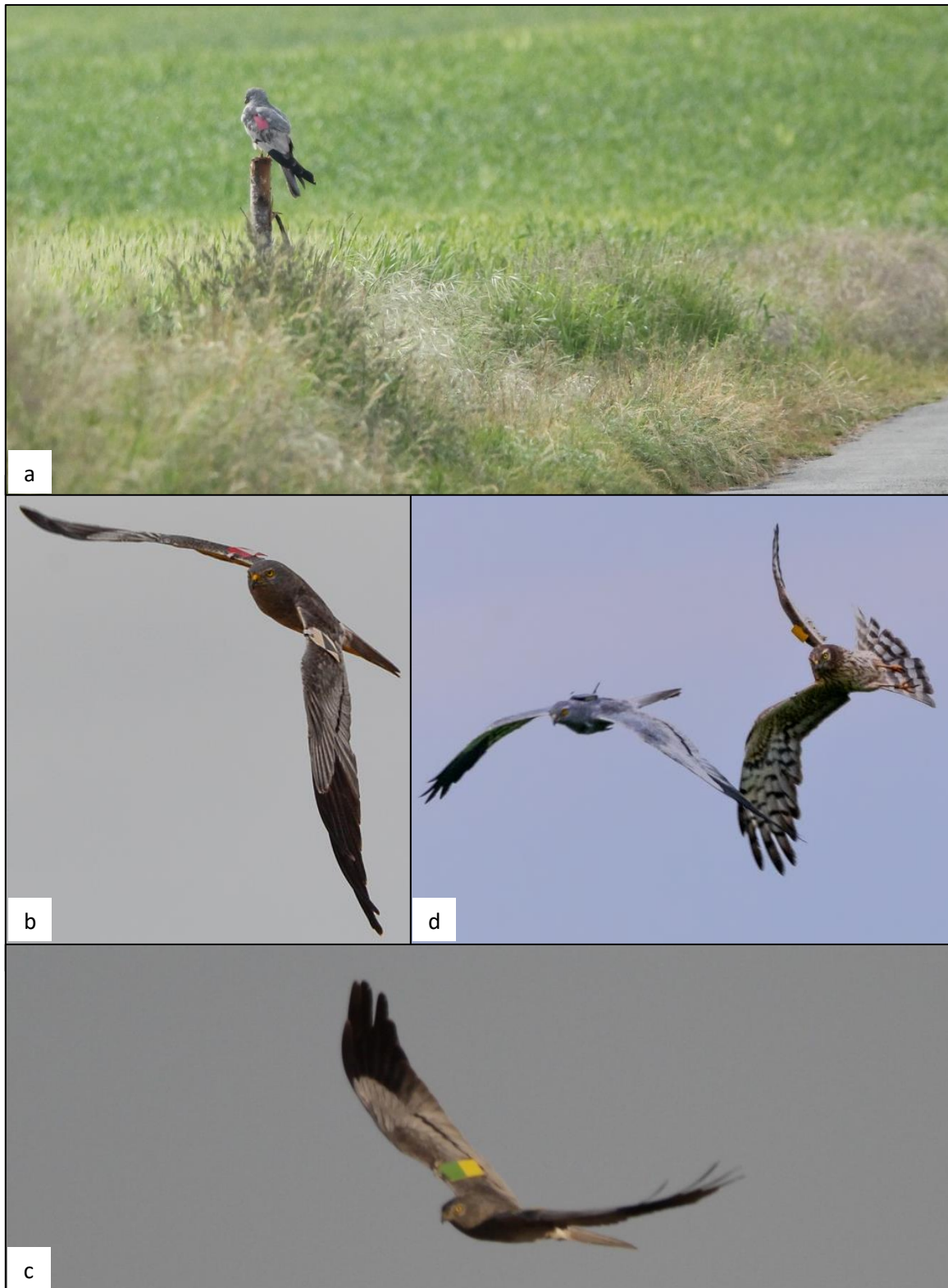


Figure 10 : a) Mâle busard cendré portant des marques alaires sur un poteau placé à proximité du nid, le long de la parcelle de culture et de la route. b et c) Mâles busards cendrés portant des marques alaires. c) Mâle équipé d'une balise GPS et femelle busard cendré portant des marques alaires (© C. Ingrand).

b. Distance de fuite des femelles (méthodes pour 2017, 2018, 2019)

Les femelles présentes au nid ont été approchées à vitesse constante (ca. $1,0 \text{ m}\cdot\text{s}^{-1}$) par les expérimentateurs (une à trois personnes en fonction des visites). La distance de fuite de la femelle représentait la distance entre le nid et les expérimentateurs au moment où la femelle s'envolait du nid (Figure 9b). Durant l'incubation, généralement un seul expérimentateur se rendait au nid ($1,12 \pm 0,40$ personnes). Durant l'élevage, en revanche, par soucis d'efficacité et de rapidité dans les mesures réalisées sur les poussins (morphométrie, comportements, prises de sang), trois personnes sauf exception visitaient les nids ($2,78 \pm 0,51$ personnes). Seules les femelles avec au minimum deux mesures de FID ont été considérées dans les analyses (2017 : 27 femelles, 79 observations, $2,93 \pm 0,78$ mesures par individu ; 2018 : 16 femelles, 42 observations, $2,63 \pm 0,80$ mesures par individu ; 2019 : 23 femelles, 60 observations, $2,63 \pm 0,80$ mesures par individu).

c. Suivi des poussins

De l'éclosion à l'envol, les nids ont été visités environ une fois par semaine (écart entre deux visites : 7 ± 2 jours) ce qui représentait quatre à cinq visites par nid pour les plus grosses nichées (cinq à six poussins) (Figures 8, 11a). A la première visite, les poussins ont été identifiés grâce à des tâches de couleur à l'arrière de leur tête à l'aide de feutres à l'eau. A l'âge de 15 jours (lors de la deuxième visite pour les poussins les plus âgés de la nichée), les poussins ont été identifiés avec des bagues en aluminium du Muséum National d'Histoire Naturelle placées au niveau d'un des tarse (Figure 11a). Au même âge, les poussins ont été également sexés en fonction de la couleur de leur iris : marron pour les femelles et gris pour les mâles (Arroyo et al. 2004).

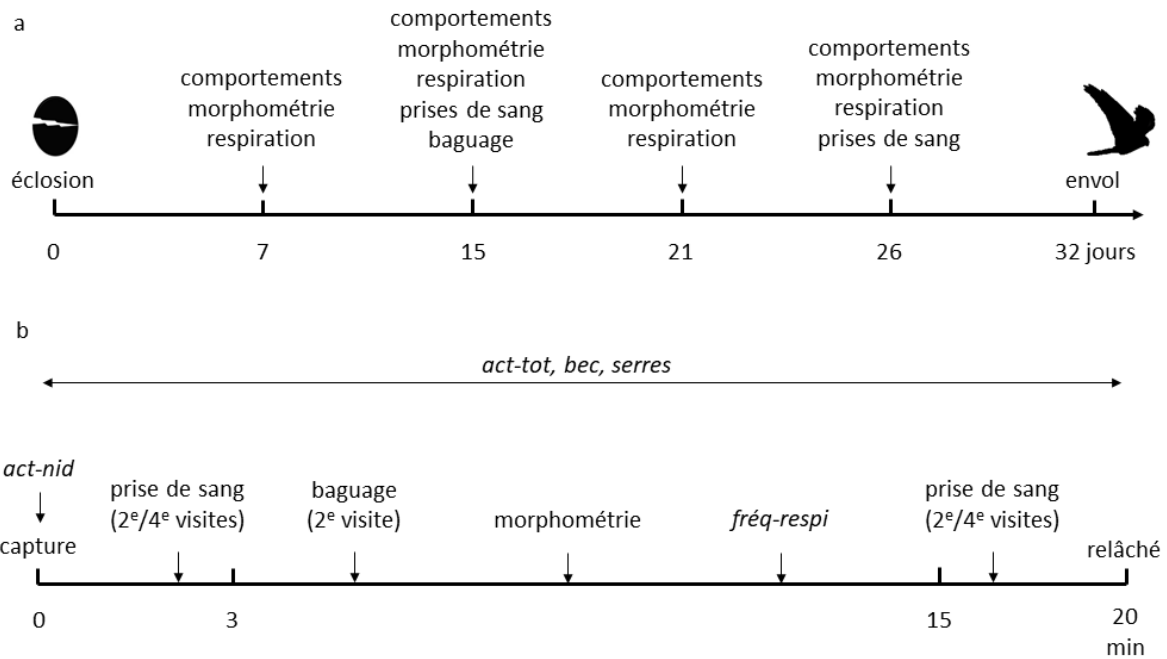


Figure 11 : a) Dispositif expérimental des quatre visites au nid pendant la période d'élevage, programmées en fonction de l'âge des poussins (± 2 jours en photos) et des différentes mesures comportementales, physiologiques et morphométriques réalisées à chaque visite (© P. Choisy, D. Authier et J. Rabdeau). b) Programmation des différentes mesures réalisées sur les poussins au sein d'une même visite de la capture au relâché des poussins au nid.

A chacune des visites, la même séquence de mesures sur les poussins a été réalisée : comportements à l'approche du nid et pendant les mesures, morphométrie et fréquence respiratoire (Figure 11). Lorsque les poussins étaient âgés de 15 jours et 26 jours (visites 2 et 4 pour les poussins les plus âgés de la nichée) deux prises de sang ont été réalisées, la première dans les trois premières minutes suivant la capture et la seconde, 15 min après la capture (Figure 11). Pendant chacune des visites, les trois expérimentateurs avaient des rôles définis :

un expérimentateur mesurait les comportements à l'approche du nid, capturait les poussins, les plaçait dans des sacs en coton et prenait des notes pendant toute la visite ; les deux autres expérimentateurs réalisaient toutes les mesures sur les poussins simultanément (un poussin par expérimentateur).

i. Comportements

A chaque visite, un score comportemental d'activité (*act-nid*) a été mesuré lorsque l'expérimentateur approchait le nid et capturait les poussins : 0 = le poussin était immobile, ou 1 = il effectuait un mouvement de recul, ou 2 = il s'enfuyait (Figure 11b). Ensuite, pendant tout le reste de la visite, trois comportements des poussins ont été mesurés : i) un score d'activité totale (*act-tot*) : 0 = le poussin restait immobile, ou 1 = présentait de rares mouvements, ou 2 = des mouvements réguliers ou 3 = des mouvements continus ; ii) un taux d'attaques avec le bec par minute (*bec*) ; iii) un taux d'attaques avec les serres par minute (*serres*) (Figure 11b).

ii. Morphométrie et fréquence respiratoire

La longueur du tarse des poussins a été mesurée avec un pied à coulisse (précision : $\pm 0,1\text{mm}$) et la longueur de l'aile pliée avec un régllet (précision : $\pm 1\text{mm}$) à chaque visite (Figure 11). Les poussins ont été pesés à chaque visite à l'aide d'une balance à ressort (Pesola® 500g, précision : $\pm 5\text{g}$; Figure 11). Lors de la dernière visite avant l'envol (26 jours), les tarses droit et gauche des poussins et leurs ailes droite et gauche ont été mesurés. La fréquence respiratoire (*freq-respi*) des poussins a été mesurée en les tenant sur le dos et en comptant le nombre de mouvements de la cage thoracique pendant 15 secondes (Figure 11b). Trois mesures successives ont été réalisées, soit sur une durée totale de 45 secondes. La fréquence respiratoire moyenne de ces trois mesures a été utilisée par la suite.

iii. Prises de sang et mesures physiologiques

Différentes mesures physiologiques ont été effectuées à partir des échantillons sanguins des poussins. Ces mesures permettaient d'estimer la qualité des poussins dans leur niveau de stress physiologique (concentration en corticostérone), leur niveau d'anémie (hématocrite) et leur qualité immunitaire (concentration en caroténoïdes et ratio hétérophile/lymphocyte).

Aux âges de 15 et 26 jours, soit pendant les visites 2 et 4 pour les poussins les plus âgés de la nichée, deux prises de sang ont été réalisées sur les poussins (environ 150 μL de sang chacune) : l'une dans les trois minutes qui suivaient la capture du poussin au nid, l'autre après 15 minutes de manipulation (Figure 11). Le premier prélèvement a été réalisé dans le but de mesurer la concentration basale en corticostérone, tandis que le second a été réalisé pour mesurer la concentration en corticostérone en réponse à la manipulation (Figure 11b). Les échantillons sanguins ont été prélevés par ponction de la veine alaire à l'aide de capillaires héparinés (Hirschmann Laborgeräte, 100 μL). Ils ont ensuite été gardés au frais (0-5°C) pendant deux à trois heures et centrifugés (10 min à 9000 tours par min, Bio Lion XC-LED12K) pour obtenir le plasma séparé des globules rouges. Les échantillons de plasma ont été stockés à -20°C jusqu'à leur analyse.

Lors de la dernière visite avant l'envol des poussins (26 jours), 10 μL supplémentaires de sang ont été prélevés avec un micro-capillaire hépariné pour mesurer l'hématocrite (voir détails plus bas). Les micro-capillaires ont été gardés au frais (0-5°C) pendant cinq heures. Au cours de cette même visite, 20 μL supplémentaires de sang ont été prélevés à l'aide de capillaires héparinés (100 μL) afin de doser les concentrations en caroténoïdes (voir détails plus bas). Les échantillons ont été gardés au frais (0-5°C) pendant deux à trois heures et centrifugés (10 min à 9000 tours par min), puis les échantillons de plasma ont été stockés à -20°C jusqu'à leur analyse. Enfin des frottis sanguins (un par poussin) ont été réalisés afin d'effectuer un comptage des différentes classes de globules blancs (voir détails plus bas).

d. Investissement parental : l'approvisionnement en proies

Les comportements d'apport de proies des parents aux poussins ont été suivis grâce à une petite caméra (MUVI™ K-series) positionnée près du nid en fin de période d'élevage et dissimulée par la végétation de la culture pour limiter une possible perturbation (Figures 8 et 12). Ces caméras ont été mises en place lors des dernières visites au nid avant l'envol des poussins dans la matinée ou en fin d'après-midi ce qui correspond aux pics d'apport de proies des busards cendrés (Clarke 1996). L'enregistrement était continu et sa durée moyenne par nid était de 2 heures et 36 minutes \pm 31 min. Au total 59 nids ont été filmés (2017 : 13 nids filmés ; 2018 : 19 nids filmés ; 2019 : 27 nids filmés mais non analysés). Au visionnage des vidéos effectué par C. Mangelinck, le nombre et la catégorie de proies (micromammifères, oiseaux et insectes) apportées par les parents ont été relevés, permettant de calculer pour chaque catégorie de proies l'approvisionnement, c'est à dire le nombre de proies délivré par heure et par poussin (Figure 12). La proportion de chaque catégorie de proies a été calculée : nombre de proies / heure / poussin divisé par nombre total de proies (toutes catégories) / heure / poussin. Une valeur de biomasse a été attribuée à chaque catégorie de proies : 50 grammes s'il s'agissait d'un oiseau, 30 g pour un micromammifère et 5 g pour un insecte (Arroyo et al. 1997). Une biomasse totale des proies délivrées par heure et par poussin a ainsi été calculée.



Figure 12 : Approvisionnement en proies par les parents busards cendrés enregistré par une caméra placée à proximité du nid. a) apport d'un micromammifère par un mâle adulte à ses poussins. b) apport d'un insecte par une femelle adulte à ses poussins (femelle avec marques alaires). c) apport d'un oiseau par une femelle adulte à ses poussins.

e. Analyses physiologiques

i. Corticostérone

Le dosage des concentrations de corticostérone (30µL de plasma) a été réalisé au laboratoire d'analyses biologiques du CEBC en suivant le protocole décrit par Lormée et al. (2003). La corticostérone a été extraite en utilisant de l'éther éthylique. Les concentrations plasmatiques de corticostérone ont été mesurées par dosage radioimmunologique en utilisant un antiserum commercial contre un conjugué de sérum d'albumine oxime bovine corticostérone-3-(O-carboxy-methyl). Deux concentrations de corticostérone (ng/µL) par poussin ont été obtenues : la concentration basale issue de l'échantillon sanguin prélevé en moins de 3 min après la capture du poussin et la concentration issue de l'échantillon sanguin prélevé 15 min après la capture. La différence entre la concentration basale et la

concentration à 15 min de corticostérone a permis d'obtenir la concentration de corticostérone en réponse à un stress aigu (Love et al. 2003, Romero & Reed 2005).

ii. Hématocrite

Les échantillons sanguins contenus dans les micro-capillaires (10 μ L) ont été centrifugés cinq heures après leur prélèvement (8 min à 9000 tours par min) afin de séparer précisément les couches de plasma et de globules rouges. Les épaisseurs des couches de globules rouges et de sang total (couches de plasma et de globules rouges) présentes dans le micro-capillaire ont été mesurées au pied à coulisse (précision : $\pm 0,1$ mm). L'hématocrite a été calculée par le ratio des épaisseurs des couches de globules rouges et de sang total (Biard et al. 2006).

iii. Caroténoïdes

Le dosage des concentrations plasmatiques en caroténoïdes a été réalisé au laboratoire d'analyses biologiques de Biogéosciences à l'Université de Bourgogne Franche-Comté en suivant le protocole décrit par Biard et al. (2010). Les échantillons de plasma (20 μ L) ont été dilués dans 180 μ L d'éthanol absolu, puis agités au vortex et centrifugés (10 min à 1500 tours par min). Les concentrations en caroténoïdes (ng/ μ L) ont été mesurées à partir d'une gamme étalon de lutéine par spectrophotométrie à 450 nm.

iv. Analyse des frottis sanguins

Les frottis sanguins ont été fixés à l'éthanol et colorés au Giesma pour la lecture au microscope (objectif à immersion grossissement x100, ZEISS®). Le nombre de leucocytes pour chaque classe (lymphocyte, hétérophile, éosinophile, monocyte, basophile) a été compté pour 8000 érythrocytes. Les parasites sanguins ont également été dénombrés. Le ratio hétérophile/lymphocyte a été calculé pour 100 leucocytes.

Chapitre 1

To change or not to change experimenters: caveats for repeated behavioural and physiological measures in Montagu's harrier

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Article

To change or not to change experimenters: caveats for repeated behavioural and physiological measures in Montagu's harrier

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Animals facing anthropogenic disturbances may exhibit different anti-predator responses depending on their perception of the risk. Experimental measures of behavioural and physiological traits may be impacted by the disturbance due to experimenter handlings and activities. In this study, we assessed the behavioural and physiological responses of Montagu's harrier chicks *Circus pygargus* from 14 nests, visited four times from hatching to fledgling by either the same (group I) or different (group NI) experimenters. Escape, aggressiveness and stress-induced corticosterone concentration increased for both groups over time but chicks from group I showed a higher increase in their behavioural and physiological responses than chicks from group NI. This increase occurred after the second visit when experimenters took the first blood sampling on nestlings. Handling by the same experimenters throughout the study did not result in habituation but in sensitization. Our results suggest that Montagu's harrier nestlings may discriminate between the people who visit their nest and respond to experimenters' identity. Therefore, we urge researchers working on behaviour and/or the physiology of stress including repeated measurements (such as animal personality studies for instance) to consider whether the experiments should or should not be conducted by the same experimenters (as usually done to minimize bias) as it can influence the outcomes of the experiments.

Keywords: aggressiveness, corticosterone, habituation, heterospecific recognition, sensitization

Introduction

Animals often consider human a potential predator (Frid and Dill 2002, Blumstein 2006, Samia et al. 2015). Human presence in animal environment and disturbances caused by anthropogenic activities may result in behavioural changes in animals such as increased escape behaviour and/or physiological stress levels through the activation of the hypothalamic–pituitary–adrenal (HPA) axis (Romero and Romero 2002, Gill 2007). To date, interactions between wild animals and humans were mostly studied in urban environments (Fernández-Juricic et al. 2001, Atwell et al. 2012, Bókony et al.



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2012, Sol et al. 2013, but see Ditmer et al. 2018) or in the context of recreational activities (Rodgers and Smith 1995, Steidl and Anthony 1996, Müllner et al. 2004, Steven et al. 2011). For example, during hunting days, little bustards *Tetrax tetrax* increase their vigilance and flight behaviours and also exhibit higher concentrations of corticosterone (stress hormone, Casas et al. 2009, Tarjuelo et al. 2015). However, if animals are repeatedly exposed to the same human-stimulus then the intensity of their behavioural/physiological response may either increase or decrease (Chace and Walsh 2006, Baudains and Lloyd 2007, Viblanc et al. 2012, Shutt et al. 2014, Lee et al. 2017).

Habituation consists in a decrease in the intensity of the behavioural and physiological response of animals repeatedly exposed to the same stimulus, presumably as they consider such stimuli harmless to them (Nisbet 2000, Cyr and Romero 2009, Blumstein 2016). Conversely, sensitization is characterized by increasing behavioural and physiological responses to a repeated stimulus (Blumstein 2016). Both processes were studied to understand the disturbance effects of tourists on wildlife in highly frequented areas. For example, Galápagos marine iguanas *Amblyrhynchus cristatus* show lower stress-induced corticosterone response in tourist areas than individuals in undisturbed areas, suggesting a habituation to human presence (Romero and Wikelski 2002). Animals repeatedly exposed to humans, may thus either habituate or sensitize to them depending on the context resulting in different behavioural and physiological responses (Rousing et al. 2005, Levey et al. 2009).

Research and conservation activities often imply repeated interactions (i.e. handling and/or exposition) of the same animal. Some effects of investigator disturbances over repeated interactions have been documented on breeding success and chick growth rate (Sandvik and Barrett 2001, Bolduc and Guillemette 2003, Blackmer et al. 2004, O'Dwyer et al. 2006, Carey 2011) and on behavioural and physiological responses (van Oers and Carere 2007, Shutt et al. 2014). In a long-term study, great tits *Parus major* that experienced more repeated handling and bleeding were more docile and easier to catch thirty days after the experiment, but had a higher breath rate than individuals that were less disturbed (van Oers and Carere 2007). However, the potential habituation/sensitization phenomena to experimenters throughout repeated measures have rarely been explored on wildlife (but see Levey et al. 2009, Marzluff et al. 2010, Lee et al. 2011, 2016, Davidson et al. 2015). In research and conservation activities, the commonest procedure is to rely on the same experimenter to measure all individuals from the study population and to take repeated measures on a same individual. This recommendation is supposed to limit potential bias of measures and handling between different experimenters. However, in this case, habituation or sensitization phenomena could occur over repeated handlings and thus impact the behavioural and physiological responses of individuals. This is especially worrying for certain kind of research topics that imply repeated measures, e.g. studies of ageing

(Love et al. 2003, McCleery et al. 2008), animal personality (which rely on several behavioural measurements on the same individuals, Carere and Maestripieri 2013 for a review) or capture–mark–recapture (Miller et al. 2005, Petit and Valiere 2006, Ryder et al. 2011).

In the present study, we investigated the impact of repeated interactions with the same versus different experimenters on the behaviour and physiology of naïve individuals in field conditions. We took advantage of our Montagu's harrier nest monitoring research program to assess the effect of repeated visits and handling on the behaviour, stress of the chicks during the nestling phase and body condition at fledgling which is an important parameter that determines the post-fledgling survival in birds (Wiens et al. 2006, Schwagmeyer and Mock 2008, Naef-Daenzer and Gruebler 2016). Two different modalities of nest visits were done: nests were visited by either the very same experimenters during the whole survey or by different experimenters from one visit to the next. We hypothesized that although the physiological and behavioural responses of the nestlings may globally increase during the monitoring due to ontogenetic changes, these responses should increase less for those repeatedly manipulated by the same experimenters than for those visited by different experimenters under a habituation process. We expected that chicks visited by different experimenters throughout the study should have higher behavioural and physiological stress responses than those visited by same experimenters. As corticosterone secretion has an impact on fledgling condition (Spencer and Verhulst 2007, Tilgar et al. 2017), chicks visited by different experimenters should exhibit a lower body condition.

Material and methods

Study site and model species

This study took place within the LTSER Zone Atelier Plaine & Val de Sèvre (western France, 46°110N, 0°280W), covering ca 435 km² of farmland where the population of Montagu's harrier *Circus pygargus* has been monitored since 1994 (Bretagnolle et al. 2018). The Montagu's harrier is a farmland flagship and protected species that nests on the ground mainly in cereal crops (Arroyo et al. 2002), making chicks and adults vulnerable to agricultural work (harvests) but isolated from human presence (Arroyo et al. 2017). The Montagu's harrier population is decreasing in France probably due to the intensification of agricultural practices in the last few decades, thus making them of high conservational priority but also limiting our sample size (Butet and Leroux 2001, Comolet-Tirman et al. 2015, Le Rest et al. 2015). Adult females lay up to six eggs according to the year and available resources which consist mainly of common voles *Microtus arvalis* (Millon et al. 2008). The most common pattern in this species is an average of two-days interval between laying consecutive eggs and one-day interval on average at

hatching (Arroyo et al. 2004). The incubation period lasts 29 d and 30–35 d for the rearing period (García and Arroyo 2001, Arroyo et al. 2007).

Experimental design

Overall design

Montagu's harrier nests found in 2017 were visited twice during incubation and four times during the chick rearing period (from hatching to fledgling) every week (lag between visits: 7 ± 2 d, $n=96$ visits) (Fig. 1a). To plan nest visits during the rearing period, the hatching date was estimated based on egg biometrics taken during the incubation period visits following Arroyo et al. (2017). Before the first nest visit, nestlings had never been manipulated by humans. As nests were discovered progressively during the breeding season, they were alternately assigned to each experimental group (or randomly assigned to each group if discovered on the same day), so that time of breeding did not differ between the two experimental groups. In the first group called thereafter group I (for identical), the experimenters were always the same three people with the very same role at each visit and for each nest (Supplementary material Appendix 1 Table A1): experimenter A captured the chicks at the nest and took notes during the chick handling by experimenters B and C who performed the measures simultaneously on two chicks from the same nest (i.e. one chick per experimenter). Chicks were randomly handled (i.e. manipulated and measured) by the experimenter B or C from one visit to the next to avoid handling and measurement bias. In the second experimental group, called thereafter group NI (for non-identical), a combination of three experimenters was selected for each visit among nine qualified manipulators including the three of the experimental group I (A, B and C) to prevent potential measurement bias for behavioural scores (Supplementary material Appendix 1 Table A1). Each experimenter combination

for the group NI depended on people availability and was changed from a visit to the next. The roles of experimenters in the group NI were randomly assigned at each visit (i.e. one experimenter captured the chicks at the nest and took notes and two experimenters performed the measures simultaneously on two chicks, Supplementary material Appendix 1 Table A1). In both experimental groups, the experimenters were qualified and trained to manipulate this species and to take blood sampling. They were all wearing grey fishing pocket vests over sober-colours t-shirt thus making the visible part of their body to chicks homogeneous. Thus, the identity effect that we tested relies on visual/olfactory/auditory cues due to the people doing the experiments as well as the way they manipulated the birds.

During the monitoring program, all nests found in the study area were monitored, which represents 19 Montagu's harrier nests. We excluded five of them which failed to breed due to chick predation before fledging. In the 14 remaining nests, twelve chicks were excluded: seven chicks died before fledging (because of starvation and/or predation, group I=5 dead chicks and group NI=2 dead chicks; Fisher test: $p=0.40$); five chicks (group I=2 chicks and group NI=3 chicks) hatched with a delay of at least four days thus getting one more exposure to human (they were too young at the nest visit 2 for blood sampling, thus the experimental design of visit was delayed for them). Consequently, in nests with more than two chicks, only the two first chicks were considered in this study. Group I thus consisted of seven nests and 11 chicks (mean \pm standard deviation, SD: 1.57 ± 0.53 chicks per nest) and group NI consisted of seven nests and 13 chicks (1.86 ± 0.38 chicks per nest). Nestlings were individually identified at the first visit with water paint colours on the back of their head. They were banded at 15 d-old during visit 2 with a numbered aluminium ring from the Muséum National d'Histoire Naturelle (Fig. 1a). Chicks were sexed by iris

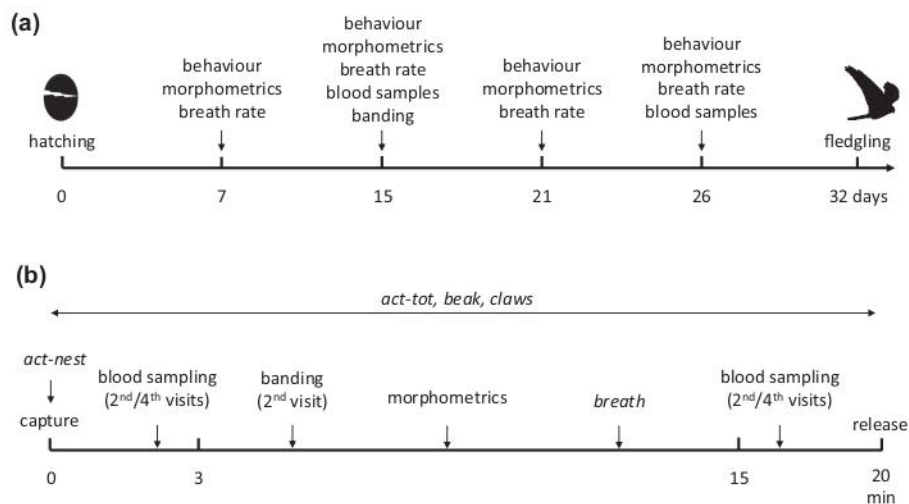


Figure 1. Summary of experimental design with: (a) the schedule of the four nest visits during the rearing period depending on the estimation of chick age (± 2 d); (b) the schedule of behavioural, physiological and morphometric measures on chicks at each nest visit.

colour at 15 d-old (Arroyo 2002). The sex ratio within each experimental group was balanced (group I: seven females and four males, binomial test: $p=0.55$ and group NI: four females and nine males, $p=0.27$) with no difference between groups (Pearson's Chi-squared test: $\chi^2=1.44$, 1 df, $p=0.23$). During the rearing period, four nest visits of ca 20 min (mean \pm SD, group I: 20.0 ± 10.56 min; group NI: 20.53 ± 11.13 min) were conducted with the same sequence of measures on chicks at each visit: behavioural scores, morphological measurements (wing, tarsus, tail length and body mass) that we used to characterize chick body condition, breath rate and blood sampling for corticosterone titration (Fig. 1b). Visits with blood sampling were longer (visits 2 and 4: 27.27 ± 9.84 min) than visits without blood sampling (visits 1 and 3: 13.31 ± 6.34 min).

All nest visits were made during sunny days (no rain) mainly either early in the morning (before 11:00 am) or after 04:00 pm to avoid thermal stress for the chicks, which could impair their development and their corticosterone concentrations (Lobato et al. 2008, Pérez et al. 2008, Rodríguez and Barba 2016).

Behavioural measures

At each visit, one behaviour was measured when the experimenter approached to the nest, captured the chicks and placed them in cotton bags (Fig. 1b). 1) Their activity at the nest approach (thereafter 'act-nest') was measured: chicks stayed immobile=0, or displayed a retreat movement=1, or ran away=2. Three behaviours were also scored during handling (Fig. 1b): 1) total activity score (thereafter 'act-tot'): chicks either stayed immobile=0, or displayed rare movements=1, or regular movements=2, or continuous movements=3; 2) rate of beak attacks per minute (thereafter 'beak'); 3) rate of attacks with claws per minute (thereafter 'claws'). To summarize, each nestling was scored for four behavioural traits: act-nest, act-tot, beak and claws.

Morphometrics

At each visit, nestlings were measured twice for right tarsus length with a digital calliper (accuracy: ± 0.1 mm), right wing length (maximum chord) and tail length using a ruler (accuracy: ± 1 mm). At visit 4, left tarsus length was also measured. Before their release at the nest, chicks were weighed with spring scale (Pesola 500 g, accuracy: ± 5 g). Body condition was estimated by the scale mass index developed by Peig and Green (2009) as follows:

$$SMI_i = Mass_i \times \left(\frac{\text{Mean tarsus length of the group}}{\text{Tarsus length}_i} \right)^{b_{sma}}$$

where b_{sma} is the slope of the major axis regression of log (body mass) on log (mean of the two measures of the left and right tarsus length) following the standard major axis method for each chick i (using 'smatr' package, Lenth 2016).

Physiological measures

At each nest visit, breath rate of chicks (thereafter 'breath') was assessed after morphological measurements as an indicator of a physiological stress response (Carere and van Oers 2004, van Oers and Carere 2007, Torné-Noguera et al. 2014; Fig. 1). Chicks were handled in back position and the number of thoracic movements were counted during 15 s. Three consecutive measures were made in one continuous observation for a total duration of 45 s and averaged to give the breath score (Fig. 1b). At the second visit (15 ± 2 d old chicks) and at the fourth visit (26 ± 2 d old chicks), two blood samples (150 μ l each) were taken from the puncture of the brachial vein using heparinized capillaries at the beginning and the end of handling (Fig. 1). The first blood sample was taken within the first three minutes of the visit to measure the baseline corticosterone concentration and the second one was performed after 15 min (mean \pm SD, group I: 16.79 ± 2.29 min; group NI: 22.28 ± 6.61 min) of handling (Fig. 1b). We calculated for each chick the stress-induced corticosterone concentration as the difference between corticosterone concentration at 15 min and baseline corticosterone concentration (Love et al. 2003, Romero and Reed 2005). Blood samples were kept refrigerated (0–5°C) for two to three hours and then centrifuged (10 min at 9000 rpm, Bio Lion XC-LED12K) to obtain the plasma, and then were stored at -20°C until titration by radioimmunoassay following Lormée et al. (2003). Among the 24 chicks, some individuals were not included in the analyses as we failed to collect enough blood for titration. Thus, two chicks (one from each group) were excluded for baseline corticosterone analysis and eight individuals (two from group I and six from group NI) for stress-induced corticosterone analysis.

Statistical analyses

Chick behavioural and physiological responses were analysed according to the group of experimenters and across the four nest visits.

First, two principal component analyses (PCA) were carried out to get two synthetic variables reflecting chick global activity and chick aggressiveness toward experimenters respectively. The PCAs were performed using a singular value decomposition of the centred and scaled (standardized) data matrix (Crawley 2012). The first PCA included act-nest and act-tot to obtain a synthetic score of global activity. The first axis of the PCA, PC1-activity was positively correlated with act-nest ($r=0.76$) and act-tot ($r=0.76$). We retained it as its eigenvalue was above one and accounted for 57.4% of the overall variance (Supplementary material Appendix 1 Fig. A1). The second PCA included beak and claws to obtain a synthetic score of aggressiveness toward experimenters. The first axis of this PCA, PC1-attacks, was positively correlated with beak ($r=0.83$) and claws ($r=0.83$), and we retained it as its eigenvalue was above one and accounted for 68.4% of the overall variance (Supplementary material Appendix 1 Fig. A1).

We used PC1-activity, PC1-attacks, baseline corticosterone, stress-induced corticosterone and breath as response variables. The normality and the homoscedasticity were checked with Shapiro–Wilk and Levene tests respectively. Then, the differences between groups (I versus NI) were analysed on PC1-activity, PC1-attacks, breath, basal and stress-induced corticosterone concentrations using linear mixed-effects models (LMMs, i.e. one per response variable, using ‘lme4’ package, Hothorn et al. 2008). These LMMs included as fixed effects the experimental group, the rank of the nest visit and its interaction with the group, and the sex of chicks to account for potential effect of sexual dimorphism (Weimerskirch et al. 2002, Lormée et al. 2003, Ellenberg et al. 2009). Pre-fledging body condition was compared between groups using a LMM including sex to control for sexual dimorphism. Since chicks from the same nests were not independent, chick identity nested within nest identity were set as random effects in all models except for body condition analysis. For the body condition model, only nest identity was included as random effect (one measure per chick). For each model, the statistical significance of each parameter was assessed through model comparison with likelihood ratio-based χ^2 -statistics (Fox and Weisberg 2011). Significant effects of the rank of nest visits in interaction with the group or in single effect were then tested, using post hoc tests based on least-squares means associated with Benjamini–Hochberg’s correction for multiple comparisons, in order to control for false discovery rate (with ‘lsmeans’ and ‘multcomp’ packages, Warton et al. 2012, Bates et al. 2015 respectively, Benjamini and Hochberg 1995).

Statistics were performed with R software (ver. 3.5.1, R Development Core Team).

Ethics statement

We released all birds involved in the present study at their site of capture (i.e. their nest) after each handling. Bird manipulation was allowed by a permit of the CRBPO (Centre de Recherches sur la Biologie des Populations d’Oiseaux – Museum National d’Histoire Naturelle, licence #1308). The methods used for the capture, handling, banding and blood sampling comply with French guidelines for ethical use of animals in research.

Data deposition

Data available from the Dryad Digital Repository: <<https://doi.org/10.5061/dryad.b8r04br>> (Rabdeau et al. 2019).

Results

Behaviour of the chicks

During the course of the monitoring, the overall activity (PC1-activity) of the chicks increased for both sexes similarly (Table 1, Supplementary material Appendix 1 Table A3,

Table 1. Summary of LMMs for testing the effects of the experimental group (I versus NI), the rank of the nest visits and the sex of the chicks on behavioural and physiological responses of Montagu’s harrier chicks using the model comparison (including statistic values and degrees of freedom). Significant effects are in bold. If the interaction group \times visit rank was significant, simple effects (group and visit rank) cannot be tested. The estimates of each effects included in each model are presented in Supplementary material Appendix 1 Table A3.

Explanatory variables	χ^2	df	p
PC1-activity			
Group	0.07	1	0.79
Visit rank	58.09	3	< 0.0001
Sex	0.03	1	0.87
Group \times Visit rank	28.56	3	< 0.0001
PC1-attacks			
Group	2.28	1	0.86
Visit rank	38.68	3	< 0.0001
Sex	0.84	1	0.36
Group \times Visit rank	19.93	3	0.0002
Baseline corticosterone			
Group	0.26	1	0.61
Visit rank	3.54	1	0.06
Sex	0.0007	1	0.98
Group \times Visit rank	1.51	1	0.22
Stress-induced corticosterone			
Group	3.57	1	0.06
Visit rank	26.63	1	< 0.0001
Sex	0.51	1	0.47
Group \times Visit rank	5.25	1	0.02
Respiratory frequency – breath			
Group	1.0	1	0.32
Visit rank	0.34	3	0.95
Sex	0.007	1	0.94
Group \times Visit rank	5.48	3	0.14
Body condition			
Group	1.74	1	0.19
Sex	1.02	1	0.31

Fig. 2a). However, this trend differed between the two groups of chicks (Table 1, Supplementary material Appendix 1 Table A3). Initially, at visit 1, chicks behaved similarly in the two groups (post hoc test: $p=0.82$); whereas at visit 2 chicks from group NI were more active than chicks from group I ($p=0.02$; Fig. 2a). At visits 3 and 4, the activity of chicks from group I increased (visit 2 versus visit 3: $p=0.02$ and visit 3 versus visit 4: $p<0.001$), but no difference was observed for chicks from group NI (visit 2 versus visit 3: $p=0.46$ and visit 3 versus visit 4: $p=0.48$). No difference in activity was detected between groups at visit 3 ($p=0.34$), but at visit 4 chicks from group I were more active than chicks from group NI ($p=0.02$; Fig. 2a).

The aggressiveness of the chicks toward experimenters (PC1-attacks) increased during the monitoring with no difference between sexes (Table 1, Supplementary material Appendix 1 Table A3, Fig. 2b). Similarly to chick activity, this increase differed between the two groups (Table 1, Supplementary material Appendix 1 Table A3). At visits 1 and 2, chicks behaved similarly in the two groups (post hoc test, $p=0.89$ and $p=0.33$ respectively; Fig. 2b). Then,

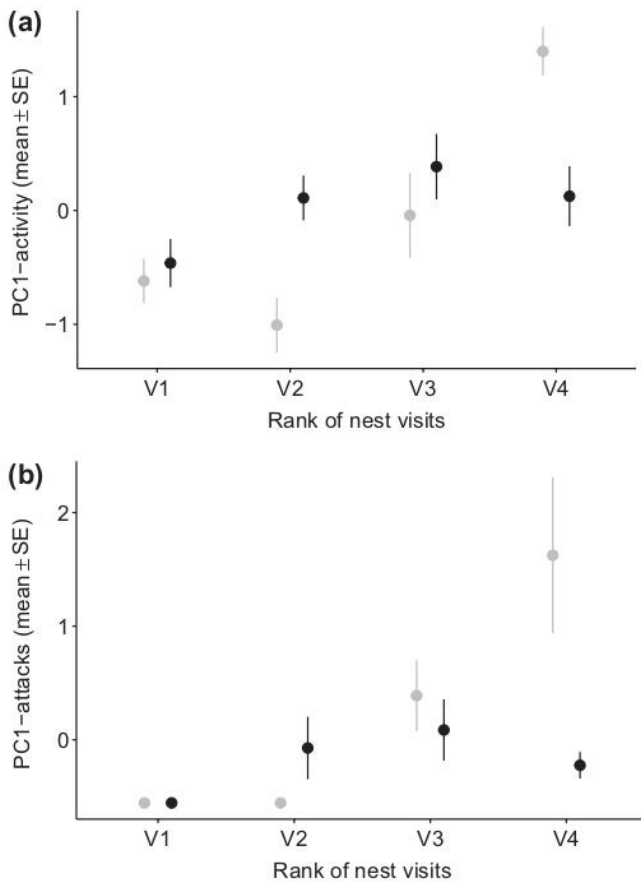


Figure 2. Effects of the group (grey=group I, seven nests and 11 chicks; black=group NI, seven nests and 13 chicks) on (a) the overall activity (PC1-activity mean \pm SE) and (b) the aggressiveness (PC1-attacks mean \pm SE) depending on the rank of nest visits.

aggressiveness of chicks from group NI did not change from visits 2 to 3 and between visits 3 and 4 (all $p > 0.52$); but the number of attacks of chicks from group I tended to increase from visit 2 to 3 ($p = 0.07$) and significantly increased from visit 3 to 4 ($p = 0.01$; Fig. 2b). No difference in aggressiveness was detected between groups at visit 3 ($p = 0.71$), but at visit 4, chicks from group I were more aggressive than chicks from group NI ($p < 0.001$; Fig. 2b).

Chick physiological stress and body condition before fledging

The respiratory frequency did not vary significantly during the monitoring neither with sex nor with experimental group (Table 1, Supplementary material Appendix 1 Table A3). The baseline corticosterone concentration tended to be higher at visit 4 than at visit 2 and was not affected by the experimental group nor the sex of chicks (Table 1, Supplementary material Appendix 1 Table A3, Fig. 3a). The stress-induced corticosterone concentration was not significantly different between the two groups at visit 2 (post hoc test, $p = 0.38$) and at visit 4

($p = 0.38$) (Table 1, Supplementary material Appendix 1 Table A3, Fig. 3b). However, the interaction between visit and group significantly affected the stress-induced corticosterone concentration (Table 1, Supplementary material Appendix 1 Table A3, Fig. 3b). While stress-induced concentration did not differ between the two visits for chicks in group NI (visit 2 versus visit 4: $p = 0.23$), it was significantly higher at visit 4 than at visit 2 for individuals in group I (visit 2 versus visit 4: $p < 0.001$). At visit 4, chicks from the two groups did not differ significantly in body condition (mean SMI \pm SD, group I: 305.8 ± 26.0 g and group NI: 300.4 ± 22.8 g) nor with sex (Table 1, Supplementary material Appendix 1 Table A3).

Discussion

In this study, Montagu's harrier nests were regularly visited either by the same experimenters (group I) or by different experimenters (group NI), the only parameter that differed in our experimental design. The behaviour of the chicks was scored, and their physiological stress was estimated thus allowing to assess the difference in behavioural and physiological responses of naïve individuals between the two experimental groups. Chicks from the two experimental groups showed an increase in different behavioural and physiological responses but their dynamics were different between the two groups over repeated visits. Although the respiratory frequency is often used to measure the stress (Carere and van Oers 2004, van Oers and Carere 2007, Torné-Noguera et al. 2014), no effect was observed in the present study. Finally, the body condition of the chicks before fledging did not differ between the two groups.

Ontogenetic changes in behaviour and physiology

Our results showed a global increase with the rank of visit in the activity and attack behaviours and stress-induced corticosterone concentrations of chicks for the two experimental groups. During the course of the monitoring, Montagu's harrier chicks, as an altricial species, developed their physical and cognitive abilities during their ontogeny (Arendt 1997, Arroyo 2002). Chicks became more active, aggressive and defensive toward the experimenters (Thomas 1977). Similarly, the development of the HPA axis led to an increase in the basal and stress-induced concentrations of corticosterone (review by Wada 2008), which is quite clear in the present case.

Different dynamics in chick behaviour and physiology between the two groups

Chicks from the two experimental groups showed different dynamics in their behavioural responses. In the first part of the monitoring (visit 1–2), chicks manipulated by the same experimenters exhibited lower reactivity than those

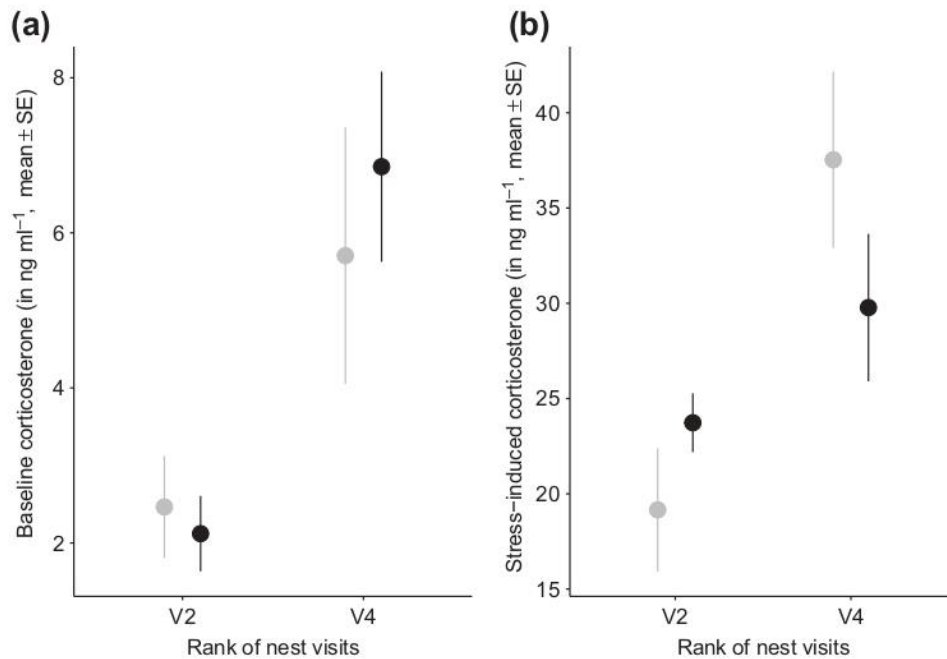


Figure 3. Effects of the group (grey = group I; black = group NI) and the rank of nest visits on (a) the baseline concentration of corticosterone (group I: seven nests and 10 chicks; group NI: seven nests and 12 chicks) and (b) the stress-induced concentration of corticosterone (group I: six nests and nine chicks; group NI: four nests and seven chicks) (mean \pm SE) in Montagu's harrier chicks.

manipulated by different people indicating lower stress. Once the second visit was done, however, the behavioural and physiological changes were more pronounced for the chicks manipulated by the same experimenters. At the visit 4, chicks from the group with the same experimenters were more active and aggressive, i.e. the reversed situation compared to second visit. This pattern is the opposite of the prediction linked with habituation. Indeed, chicks visited by identical experimenters were more stressed and aggressive at the end of the experiment suggesting a sensitization phenomenon (Blumstein 2016). Behavioural and physiological responses of chicks could be influenced by the behavioural types of adults and their calls during nest visits or environmental conditions such as anthropic disturbances (Platzen and Magrath 2004, Almasi et al. 2015, Arroyo et al. 2017). However, nests were randomly assigned to the two groups, thus these factors could not explain the differences we observed. The differences in chick behavioural and physiological responses between the two groups could result from differences in the way the experimenters handled chicks between the two groups. However, in the group with the same experimenters, chicks were randomly handled by two experimenters who were also included in the other experimental group. Although this explanation is less conceivable, this hypothesis should be properly tested to exclude such an effect. Finally, another explanation implies two major associated facts: 1) Montagu's harrier chicks identified an event during visit 2 that can be qualified as harmful for them and 2) chick could be sensitized after this visit 2.

Blood sampling as a negative event associated with the experimenters

The differences in the behaviour and the physiology of the chicks between the two experimental groups were observed after the second visit, which differed from the first one by the realization of two blood sampling events. Blood sampling seems to be aversive for chicks (Domjan 2005), although great care was taken to minimize stress and pain during handling and sampling (only done by trained experimenters, habituated to manipulate and bleed wild birds). This phenomenon has also been evidenced in yellow-eyed penguins *Megadyptes antipodes*: individuals previously blood sampled are less likely to habituate at next interaction (Ellenberg et al. 2009). The total volume of blood collected for the first and second samples was minimized and made profitable for other further analyses (immunology, genetics, pesticide titration). Based on French guidelines, the blood samples collected at visit 2 and 4 were far below the maximal volume that is possible to collect without an impairment of the chick development (10% of the total volume of blood, which represents 6% of the body mass of nestlings of more than 150 g, i.e. 900 μ l). Moreover, recent experiments and review suggest that blood sampling (from the brachial vein like in the present case) does not alter the development of nestlings (Sheldon et al. 2008).

According to our result, blood sampling by itself is not the problem but the association with traits of the experimenters. Indeed, blood sampling alone cannot explain the pattern we observed, otherwise the large increase of the behavioural and physiological responses should be the same

in both experimental groups. Instead, nestlings would associate the blood sampling event to traits of the three experimenters through a fear conditioning mechanism and a sensitization process would occur across nest visits (see Davis 2002, Domjan 2005, Anderson et al. 2010, Marzluff et al. 2010, Blumstein 2016 for a review). This result suggests that Montagu's harrier nestlings could recognize and discriminate humans over nest visits; or at least were able to make an association between traits of our experimental group I with the manipulation that did not occur for group NI. In other bird species, individuals showed more defensive behaviours toward the humans who visited their nest or captured them, than toward neutral humans who had no previous interaction with them (Levey et al. 2009, Marzluff et al. 2010, Lee et al. 2011, 2016, Davidson et al. 2015). For example, in the American crow *Corvus brachyrhynchos*, individuals trapped by an experimenter wearing the 'dangerous mask', scold all people wearing this mask after trapping (Marzluff et al. 2010). Several species of invertebrates and vertebrates are able to discriminate among humans (Boysen 1994, Kendrick et al. 2001, Davis 2002, Davis and Heslop 2004, Adachi et al. 2007, Dittrich et al. 2010). Other studies highlighted that Montagu's harrier is able to discriminate among different species (Arroyo et al. 2001, García 2003). Heterospecific discrimination would allow adjusting the defence investment and its costs for individuals (Arroyo et al. 2001, Marzluff et al. 2010). Our results suggest that this heterospecific discrimination toward humans could be more precise; they may recognize human individual, but the exact nature of the cues they used remain unknown in the present case. Moreover, the present study was conducted on chicks, i.e. naïve individuals not exposed to humans before our nest visits compared to other field studies on adult individuals and pre-exposed to humans (Slobodchikoff et al. 1991, Levey et al. 2009, Marzluff et al. 2010, Lee et al. 2011, 2016, Davidson et al. 2015). Montagu's harriers could have cognitive and perceptual abilities to discriminate human individuals, similarly to other species known for their high cognitive ability such as pigeons, parrots and corvids. This hypothesis should, however, be properly tested and the cues (visual/olfactory/auditory/the way experimenters handled birds) used to discriminate humans should be investigated with different masks to test the use of visage cues (Lefebvre et al. 2004, Emery 2006, Iwaniuk et al. 2009, Levey et al. 2009, Marzluff et al. 2010, Belguermi et al. 2011, Lee et al. 2011, 2016, Stephan et al. 2012).

Methodological caveats

One important outcome of the present study is to show the differential effect of the experimenters' identity. Indeed, most of the time, the impact of investigators is seen from the disturbance effect point of view either on breeding success (Bolduc and Guillemette 2003, Blackmer et al. 2004, Ibáñez-Álamo et al. 2012), growth of chicks (Sandvik and Barrett 2001, O'Dwyer et al. 2006, Carey 2009, 2011), or on behaviour and physiology (van Oers and Carere 2007)

but rarely, to our knowledge, considering the identity of the people in charge of the experiment on wildlife fauna (see, however, Slobodchikoff et al. 1991, Levey et al. 2009, Marzluff et al. 2010, Lee et al. 2011, 2016, Davidson et al. 2015). Basically, to limit bias due to experimenter effect, measures are done by the same people. Our study shows that depending on whether the model species is able to habituate or sensitize to humans, the outcomes of the behavioural and physiological measures might be either exacerbated or limited (Cibulski et al. 2014). In our study, chicks visited and handled by the same three experimenters were more aggressive and stressed than those manipulated by different people. Thankfully, the overall pattern of behavioural and physiological responses is similar in both groups suggesting that the global methodology is robust despite the limitation of the sample size. The three experimenters in the group I operated also in the group NI with six other experimenters in different combinations at each visit, which allow avoiding potential bias for behavioural scoring.

Regarding the assessment of the behavioural response to human-mediated disturbance, keeping or changing experimenters have contrasted advantages. Mostly, conserving the same experimenters limits the bias from different manipulators, but does not reflect a true exposition to humans in real life, i.e. during their life wild animals are expected to be confronted to many different humans. Thus, depending on the question (research or conservation), taking habituation/sensitization processes into account when repeated measurements are required, is unavoidable. It is particularly important to consider the sensitization process during research and conservation activities since it is linked to an increase in corticosterone due to stress during post-natal phase. Although the mechanisms are complex, an increased corticosterone secretion during nestling phase may compromise chick developmental plasticity in stopping (Spencer and Verhulst 2007) or enhancing growth rate (Tilgar et al. 2017), may modulate their future behaviour (Müller et al. 2009, Boogert et al. 2014), may promote a higher susceptibility to oxidative stress (Noguera et al. 2017) and/or may impact immunity (Chin et al. 2013, Schmidt et al. 2015, Virgin and Rosvall 2018). These consequences may alter chick survival, being critical for the conservation of endangered species. In the present case, no effect was detected on body condition before fledgling, although a link between corticosterone and body condition was highlighted in other studies (Spencer and Verhulst 2007, Tilgar et al. 2017). This is especially important in migratory species like the Montagu's harrier. However, further investigations should be undertaken since long-term effects on life history traits cannot be excluded.

Concluding remarks

Although our sample size was limited due to the conservational status of the Montagu's harrier, our results show important differences in the response to variation in the identity of observers that are rarely considered and may bias the interpretation of the observed patterns. We

therefore urge behavioural ecologists and conservationists to 1) take into account whether changing or not experimenters in cases involving repeated interactions with the organisms in regard to the probability of habituation or sensitization, and/or to 2) consider this methodological issue when planning projects and interpreting their results. We also highlight that habituation/sensitization might also be problematic for meta-analyses and comparisons between studies.

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Supplementary material (available online as Appendix jav-02160 at <www.avianbiology.org/appendix/jav-02160>). Appendix 1.

Chapitre 2

Assortative pairing for boldness and consequences on reproductive success in Montagu's harrier

En préparation pour Animal Behaviour



**Assortative pairing for boldness and consequences
on reproductive success in Montagu's harrier**

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In preparation for Animal Behaviour

Abstract

Personality and plasticity of partners may influence the reproductive success depending on the behavioural association within pairs. However, studies testing this hypothesis are rarely made and this is even more scarce in the case of monogamous species with bi-parental care and different roles between the sexes. The present study assessed whether the pairing pattern based on individual boldness and boldness plasticity may influence the reproductive success in the Montagu's harrier (*Circus pygargus*), a species with sex-specific care. Males and females were paired by a positive assortative pattern for both boldness and plasticity. Four indices were extracted from the pairing pattern to characterize the behavioural association within pairs: pair boldness, boldness similarity within pairs, pair plasticity, plasticity similarity within pairs. The effects of the parents' behaviour on the reproductive success were then tested through these four indices. A significant effect of plasticity similarity within pairs on the reproductive success was established, less similar pairs in plasticity having a higher reproductive success than more similar pairs. These results allow for discussion on the origin of the pairing pattern. Sexual selection could not be the major driver of pairing pattern and alternative ecological processes leading to assortative pairing are discussed.

Keywords: behavioural reaction norms, behavioural similarity, flight initiation distance, personality, plasticity, sexual selection.

Introduction

Random mating rarely occurs through the Animal kingdom and assortative pairing for genetic or phenotypic traits is prevalent across many species (review in Jiang et al. 2013). Assortative pairing may be either positive if males and females mate according to similar trait values or negative if they are paired for opposite values of the trait (also called disassortative pairing). Many studies have investigated pairing patterns in regard to genetic traits (major histocompatibility complex, Bonneaud et al 2006, Ortego et al. 2009), age (Black and Owen 1995, Ludwig and Becker 2008), body size (da Silva Castiglioni and Bond-Buckup 2008, Pack et al. 2012), coloration (MacDougall and Montgomerie 2003, Pérez i de Lanuza et al. 2013, Fargevieille et al. 2017) and behavioural traits (Gabriel and Black 2012). Among the latter, personality traits (i.e. constant behavioural differences among individuals, Réale et al. 2007, Dingemanse et al. 2010) such as exploration (Both et al. 2005), aggressiveness (Harris and Siefferman 2014), activity (Montiglio et al. 2016) or boldness (Traisnel and Pichegru 2017) were particularly investigated (review in Schuett et al. 2010). Assortative mating may result either from sexual selection (i.e., mate choice, Schuett et al. 2010), or from non-random spatial or temporal distribution of phenotypes/genotypes within the environment (Ferrer and Penteriani 2003, Flockhart and Wiebe 2007, Servedio and Boughman 2017). Whatever its origin, assortative pairing may provide fitness benefits to both members of the pair (Masumoto 1999, Daunt et al. 2003, Ariyomo and Watt 2012) through an increase in reproductive success (Faivre et al. 2001, Masello and Quillfeldt 2003, Bitton et al. 2008). However, if the pattern does not result from sexual selection, fitness benefits are not necessarily expected (Ferrer and Penteriani 2003, Flockhart and Wiebe 2007).

Personality traits within pairs may determine the reproductive success through the link with parental investment, especially in species with bi-parental care (Kontiainen et al. 2009, Schuett et al. 2010, 2011a). Pairs with similar aggressiveness in zebra finches (*Taeniopygia guttata*) have chicks in better body condition at fledging, perhaps because of reduced sexual conflicts between the parents over food provisioning (Schuett et al. 2011a). Behavioural similarity between partners may thus provide greater behavioural compatibility, higher parental investment and consequently, fitness benefits through higher reproductive success

(Spoon et al. 2006, Royle et al. 2010, Schuett et al. 2010, Gabriel and Black 2012, Ihle et al. 2015). Similar pair members would be more effective in cooperating and coordinating their activities, for instance in food provisioning and/or nest defence (Spoon et al. 2006, Schuett et al. 2010, 2011a, Gabriel and Black 2012, Harris and Siefferman 2014, Burtka and Grindstaff 2015). In addition, pairing pattern may also occur for behavioural plasticity (Royle et al. 2010, Schuett et al. 2011a). Behavioural plasticity represents the ability of individuals to be flexible in their behaviour depending on environmental conditions and is often linked to personality (Nussey et al. 2007, Dingemanse et al. 2010, Betini and Norris 2012). For example, shy and less aggressive individuals show a greater behavioural plasticity than bold and aggressive individuals, because they are more sensitive to changing environmental conditions (Koolhaas et al. 1999, 2010, Kareklas et al. 2016, Zidar et al. 2017). However, investigating fitness consequences of pairing pattern while simultaneously accounting for personality and behavioural plasticity remains scarce (Royle et al. 2010, Schuett et al. 2010, 2011a). A single study examined the effects of personality and behavioural plasticity within pairs on reproductive success (Schuett et al. 2011a). In this study, zebra finch pairs with similar exploration plasticity (both partners with either low plasticity or high plasticity) have nestlings in better body condition than dissimilar pairs possibly due to higher coordination in parental care.

To date, most studies were conducted on species with bi-parental care and similar roles for males and females, such as great tits (*Parus major*), zebra finches or African penguins (*Spheniscus demersus*) (Dingemanse et al. 2004, Both et al. 2005, Schuett et al. 2011a, b, Traisnel and Pichegru 2017). Studies on species with sex-specific roles are rare. For these species, the behavioural similarity within the pair could be of interest because sexual conflicts are more likely to occur in these species (see the review by Royle et al. 2010). Indeed, if one partner reduced its parental investment, the other one could not compensate (Royle et al. 2010). Thus, the similarity in personality and plasticity between partners may reduce sexual conflicts and consequently may improve their reproductive success. In the present study, these hypotheses were tested in Montagu's harrier (*Circus pygargus*), a raptor species with differences in parental care between sexes. Basically, the female is responsible for incubation and care at nest while the male hunts and brings the prey to feed his female and chicks (see the review by Arroyo et al. 2004). Nest defence is performed by both sexes but occurs at

different distances from the nest, the female staying nearby most of the time while the male may be away for long periods during hunting session (Mougeot et al. 2001, 2006). A long-term study previously showed the link between female boldness and their reproductive success; shy females having more nest failures and lower productivity than bold females (Arroyo et al. 2017). However, male boldness has never been investigated to date and its effect on reproductive success has not been tested either alone or associated to female behaviour. Our present study explored pairing pattern and the influence of boldness trait (boldness thereafter) and boldness plasticity (plasticity thereafter) within Montagu's harrier pairs on their reproductive success. First, boldness and plasticity were assessed for both sexes during three consecutive breeding seasons. Second, the pairing pattern between males and females according to their boldness and plasticity was investigated. Finally, the link of the behavioural association within pairs on the reproductive success was assessed depending on four indices in boldness and plasticity on the level of the pair and on the similarity of the partners within pairs: pair boldness, boldness similarity within pairs, pair plasticity, plasticity similarity within pairs. We predicted that mates should be paired by a positive assortment for boldness and plasticity because behavioural similarity within pairs should decrease sexual conflicts. The reproductive success from pairs should be associated to the similarity in boldness and plasticity between partners and more similar pairs should have a higher reproductive success.

Material & Methods

Study site and model species

The present study was done on a population of Montagu's harriers extensively monitored since 1995 within the LTSER (Long-Term Socio-Ecological Research) Zone Atelier Plaine & Val de Sèvre (ZAPVS thereafter, western France, 46° 110 N, 0° 280 W) covering ca. 435 km² of intensive farmland habitat (Bretagnolle et al. 2018). The Montagu's harrier is a flagship and protected species that nests on the ground mainly in cereal crops, making chicks and adults vulnerable to agricultural work, especially at harvesting period of cereals (Arroyo et al. 2002, Millon et al. 2002). The number of Montagu's harrier pairs nesting on the ZAPVS varies according to abundance of small mammals and especially its main prey, the common vole

(*Microtus arvalis*) (Millon et al. 2008, Millon and Bretagnolle 2008). In years of high abundance in micromammals, up to 100 pairs of Montagu's harriers may nest in ZAPVS (Arroyo et al. 2003), but over the last three years (2017 to 2019), up to ca. 40 pairs did so. In France, Montagu's harrier population is decreasing probably due to the intensification of agricultural practices in the last decades, thus making them of high conservational priority (Butet and Leroux 2001, Santangeli et al. 2015). Adult females lay up to six eggs (Millon et al. 2008). The incubation period usually begins in early May for the earliest pairs, lasts 29 days on average, while rearing period lasts for 30-35 days (García and Arroyo 2001, Arroyo et al. 2004).

Nest monitoring and reproductive success

In 2017, 2018 and 2019, Montagu's harrier nests were localized in the ZAPVS (43, 40 and 40 nests respectively) and visited twice during incubation and five times during chick rearing period (from hatching to fledgling; lag time between visits: 7 ± 2 days), depending on brood size and differences in hatching dates. During incubation, egg length and width were taken using a calliper (accuracy: ± 0.1 mm) and mass was measured using Pesola® electronic scale (accuracy: ± 0.1 g) in order to estimate the hatching date, following Arroyo et al. (2017). The final clutch size was also noted. After hatching, the first visit occurred when first nestlings were 7 ± 2 days old; the two first visits allowed counting the number of hatched eggs to estimate hatching failure. At the end of the breeding period, a last visit was conducted to check fledgling success. Behavioural measures on female and male adults were performed from nest discovering and up to chick fledging (i.e., from the end of April to July).

Adult behavioural measures

The flight initiation distance (FID), corresponding to the distance at which the individual begins to flight when a predator - including human - approaches, is considered an anti-predator behaviour and it is often used to estimate the individual boldness (Frid and Dill 2002, Blumstein 2006, Carrete and Tella 2010, Selmann et al. 2012, Møller et al. 2013). Therefore, we measured FID for Montagu's harrier males and females in order to estimate their boldness.

Male boldness measure

Once a nest was located in a field crop, a pole was positioned close to the nest in the border of the field along a path or a road, allowing the male to perch and its GPS location was noted (Fig. 1). Male individual identification was systematically checked with behavioural observations between male and female (e.g., food provisioning) and in addition, wing tags, the presence of GPS device (see Schlaich et al. 2016) or morphological features allowed to identify males. Every morning between 23th of May and 16th of July, between 6am and 12pm, with favourable weather conditions (no rain and wind below 30 km.h⁻¹), the observer looked for males standing on their pole. When one was found, it was approached by the experimenter from a starting distance of ca. 200 m depending on landscape configuration (“starting GPS position”, Fig. 1). Then, the experimenter began walking straight towards the focal individual at a constant speed (ca. 1.0 m.s⁻¹). When the focal individual initiated a flight, the experimenter stopped and recorded his GPS coordinates (“flying GPS position”) in order to calculate the FID of the individual (Fig. 1). At least two measures per individual were performed over the breeding season with an interval of some days between repeats (mean ± standard deviation: 7.87 ± 5.45 days, N = 107 observations). FID was measured for 13 males in 2017 (36 observations; 2.77 ± 0.83 measures per individual), 13 males in 2018 (33 observations; 2.54 ± 0.52 measures per individual) and 13 males in 2019 (38 observations; 2.92 ± 0.86 measures per individual) with no differences among males in number of repeated measures (Chi-squared test: $\chi^2 = 9.13$, df = 38, P = 1.0). Different factors that can influence FID were recorded at each approach: the exact starting distance, the rank of approaches for a given individual and the brood stage.

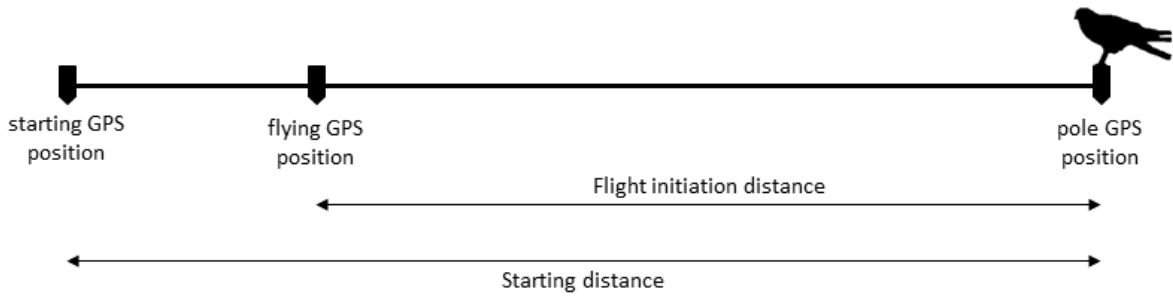


Figure 1. Experimental design to measure male FID (flight initiation distance). During each approach, the distance between the starting GPS position and the pole GPS position (i.e. position of the focal male) allowed calculating the starting distance. The flight initiation distance represented the distance between the flying GPS position and the pole GPS position.

Female boldness measure

As females rarely use pole during incubation, FID measures were done during nest approach. Female FID was measured as the distance at which female on the nest initiated a flight when experimenter approached during nest visits (see details in Arroyo et al. 2017). The experimenters (from 1 to 3 people depending on the visit, see below) walked to the nest at a constant speed (ca. $1.0 \text{ m}\cdot\text{s}^{-1}$) across the field crop. When the female flew out the nest, the experimenter stopped and counted his steps to the nest in order to measure the FID of the female. The number of experimenters was mainly one person during incubation visits (1.12 ± 0.40 , $N = 113$ visits) and mainly three people for visits during the rearing period (2.78 ± 0.51 , $N = 290$ visits). Female FID was measured at least twice for 27 females in 2017 (79 observations, 2.93 ± 0.78 measures per individual), 16 in 2018 (42 observations, 2.63 ± 0.80 measures per individual) and 23 in 2019 (60 observations, 2.63 ± 0.80 measures per individual). At each nest visit, different factors that can influence FID were noted: the rank of nest visit and the brood stage. Additionally, as nests are hidden in crop vegetation, perturbation detection by the females mainly relies on noise cues so the number of experimenters were also noted (i.e. the more experimenters they are, the more noise there is).

Statistical analyses

FID repeatability

Montagu's harrier FID repeatability over time was analysed for sexes and years separately. The repeatability is a condition to meet to consider a behavioural trait as a personality trait. The normality and the homoscedasticity of FID were checked with Shapiro-Wilk and Levene tests respectively. The FID repeatability coefficients (R thereafter, ranging from 0: non-consistent to 1: highly consistent) were estimated using linear mixed-effects models (LMMs) using 'rptR' (Nakagawa and Schielzeth 2010) and 'lmerTest' packages (Kuznetsova et al. 2014) including individual's identity as random effect.

Factors influencing FID

LMMs were also used to explore the effects of various factors including for males: the starting distance, the rank of approaches and the brood stage, and for females: the number of experimenters, the rank of nest visits and the brood stage on FID. This was achieved following the *Within-subject centering* (WSC) method proposed by van de Pol and Wright (2009). This method is advocated for the analysis of behavioural reaction norms and for *in natura* studies, when individuals cannot be measured in the same conditions (see Dingemanse et al. 2010). It enables discriminating between the behavioural variability linked to within-individual variation (i.e., behavioural plasticity across contexts) and the behavioural variability linked to between-individual variation (i.e., some behaviours occurred more often in specific environmental conditions). In the present study, males could not be measured at the same starting distance due to differences in landscape configuration around each pole. Consequently, the WSC method allows identifying whether the behavioural response is explained by a between-individual or by a within-individual effect from the explanatory variable (van de Pol and Wright 2009). All factors were first centred and scaled. Then, for each individual, the mean value of each factor (for males: starting distance and brood stage; for females: the number of experimenters and brood stage) was calculated, as representing between-individual variability (*between* thereafter; van de Pol and Wright 2009, Dingemanse

et al. 2010). Then, for each observation of an individual, the deviation from this mean value was calculated, and represented the within-individual variability (*within* thereafter; van de Pol and Wright 2009, Dingemanse et al. 2010). *Between* and *within* effects for each factor were included in LMMs, with individual's identity included as random effect. The random intercept allowed estimating the personality of each individual (see below). The rank of approaches was included as fixed effect in order to examine potential effects of habituation/sensitization across repeated approaches. For each LMM (one per sex), the normality of residuals was tested and compliant. For both sexes, the brood stage was correlated to the rank of approaches (Spearman's rank correlation test, males: $\rho = 0.27$; 95% CI = [0.09; 0.44] and females: $\rho = 0.70$; 95% CI = [0.60; 0.77]). In females, the number of experimenters was also highly correlated to the rank of approaches ($\rho = 0.62$; 95% CI = [0.52; 0.72]). Consequently, for males, only the rank of approaches as fixed effect and the starting distance in *between* and *within* effects were conserved in the LMM. For females, only the rank of approaches was conserved as fixed effect in the LMM.

Individual boldness and boldness plasticity based on FID

From LMMs described above, differences among individuals in their plasticity, i.e., the relationship between FID and the starting distance for males or the rank of approaches for females were assessed (van de Pol and Wright 2009, Dingemanse et al. 2010, 2012, Carter et al. 2012). For each sex, LMM with both random intercept on the individual's identity and random slope on the considered factor was compared to the same LMM without random slope, using a likelihood-ratio Chi-squared test in order to assess whether individuals differed from each other in their plasticity. Results from LMMs with random slope and random intercept were presented in this study. Finally, intercept and slope from each individual were extracted from LMM with random slope and random intercept for each sex. Consequently, for each individual, the intercept represented the boldness personality (called "boldness" thereafter, high values represented shy individuals; low values represented bold individuals) and the slope represented the plasticity for boldness (called "plasticity" thereafter, high values represented plastic individuals; low values represented not-plastic individuals). Thereafter, the absolute value of the slope (i.e., whether the individuals are plastic or not) for each

individual were used. For each sex, the link between boldness and plasticity was tested using Spearman's rank correlation test because variable did not meet the assumption of normality.

Pairing patterns

Boldness and plasticity were used separately in Spearman's rank correlation tests to test how males and females were paired (n = 30 pairs; 2017: 12 pairs; 2018: 6 pairs; 2019: 12 pairs). Following Cohen et al. (1988), a correlation coefficient (ρ) of 0.1, 0.3 or 0.5 were considered as "small", "medium" or "large" effect respectively. In addition, four indices for each pair were calculated in order to characterize their behavioural association: pair boldness, boldness similarity within pairs, pair plasticity, plasticity similarity within pairs. To do so, two principal component analyses (PCA) were performed (one for boldness and one for plasticity) using a singular value decomposition of the centred and scaled data matrix (Crawley 2012). The first PCA included female and male boldness. The first axis of the PCA was correlated with female boldness ($r = 0.83$) and male boldness ($r = 0.83$) and accounted for 68.9% of the overall variance. The second axis of the PCA was correlated with female boldness ($r = -0.56$) and male boldness ($r = 0.56$) and accounted for 31.1% of the overall variance. Both axes were retained, the first axis represented the pair boldness (gradient from bold pairs with low values to shy pairs with high values) and the absolute values of the second axis represented the boldness similarity within pairs (gradient from more similar pairs with low values to less similar pairs with high values). The second PCA included female and male plasticity. The first axis of the PCA was correlated with female plasticity ($r = 0.75$) and male plasticity ($r = 0.75$) and accounted for 55.8% of the overall variance. The second axis of the PCA was correlated with female plasticity ($r = -0.67$) and male plasticity ($r = 0.67$) and accounted for 44.2% of the overall variance. Both axes were retained, the first axis represented the pair plasticity (gradient from not-plastic pairs with low values to plastic pairs with high values) and the absolute values of the second axis represented the plasticity similarity within pairs (gradient from more similar pairs with low values to less similar pairs with high values).

Effects of pairing patterns on pair reproductive success

To assess the link between the behavioural association within pairs and the reproductive success, different reproductive parameters were used: i/ the final clutch size, ii/ the number of hatched eggs, iii/ the number of fledglings, iv/ the hatching success (hatched eggs / clutch size), v/ the fledging success (fledglings / hatched eggs) and vi/ the overall success (fledglings / clutch size). Then, the effects of pair boldness, boldness similarity, pair plasticity and plasticity similarity on each of these six parameters were tested with either generalized linear mixed-effects models with a negative binomial-error (NBGLMM) using “quasi-Poisson” parameterization (i.e., variance strictly proportional to the mean, from ‘glmmADMB’ package, Bolker et al. 2012) for parameters i-iii and generalized linear mixed-effects models with a binomial-error distribution (BGLMM) for parameters iv-vi. For each of the six reproductive parameters, models were performed once for pair boldness and boldness similarity at the same time and once for pair plasticity and plasticity similarity at the same time (12 models). For each model, the year was included as random effect because the same female and male could nest through different years.

For all models (LMMs, NBGLMM, BGLMM), model comparisons with likelihood ratio-based χ^2 -statistics were used to estimate statistical significance of the explicative variables (using ‘car’ package, Fox and Weisberg 2011, Fox et al. 2012). All statistics were performed with R software (v. 3.5.1, R Development Core Team 2018).

Ethics statement

Nest visits were allowed by a permit of the CRBPO (Centre de Recherches sur la Biologie des Populations d’Oiseaux - Museum National d’Histoire Naturelle, licence #1308).

Results

Flight initiation distance (FID) and its repeatability

A summary of the FID distances for males and females as well as their repeatability are given in Table 1. Within males, the FID increased with longer starting distances (i.e., the *within* effect; Table 2; Fig. 2a). The *between* effect suggested significant variations among males in their FID and also indicated that males were measured in different landscapes (and thus starting distances, Table 2). However, the rank of approaches did not have any effect on male FID (Table 2). Conversely, in females, FID increased with rank of approaches (Table 2; Fig. 2b).

Table 1. Mean and 95% confidence interval of FID (meters) and FID repeatability (R) of individuals for both sexes in Montagu's harrier adults. Values of R significantly > 0 are in bold.

Years	Mean [95%CI]	R [95%CI]
<i>Males</i>		
2017	169.39 [154.26; 184.52]	0.41 [0.01; 0.70]
2018	170.33 [157.52; 183.14]	0.65 [0.26; 0.85]
2019	176.71 [166.80; 186.63]	0.50 [0.09; 0.74]
<i>Females</i>		
2017	5.84 [4.80; 6.89]	0.37 [0.08; 0.60]
2018	6.36 [4.52; 8.20]	0.61 [0.25; 0.81]
2019	6.98 [4.86; 9.11]	0.34 [0.02; 0.60]

Table 2. Effects of factors on FID of individuals for both sexes analysed by LMMs with random intercept on individuals and random slopes on factor (for males: starting distance in *within* effect; for females:

rank of approaches). Significance of each explanatory variable is tested using model comparison with likelihood ratio-based χ^2 -statistics (including statistic values, degrees of freedom and p values). Significant effects are in bold.

Explanatory variables	χ^2	<i>P</i>
<i>Males</i>		
Rank of approaches	0.61	0.43
Starting distance <small>between</small>	39.22	<0.0001
Starting distance <small>within</small>	14.07	<0.001
<i>Females</i>		
Rank of approaches	9.26	0.002

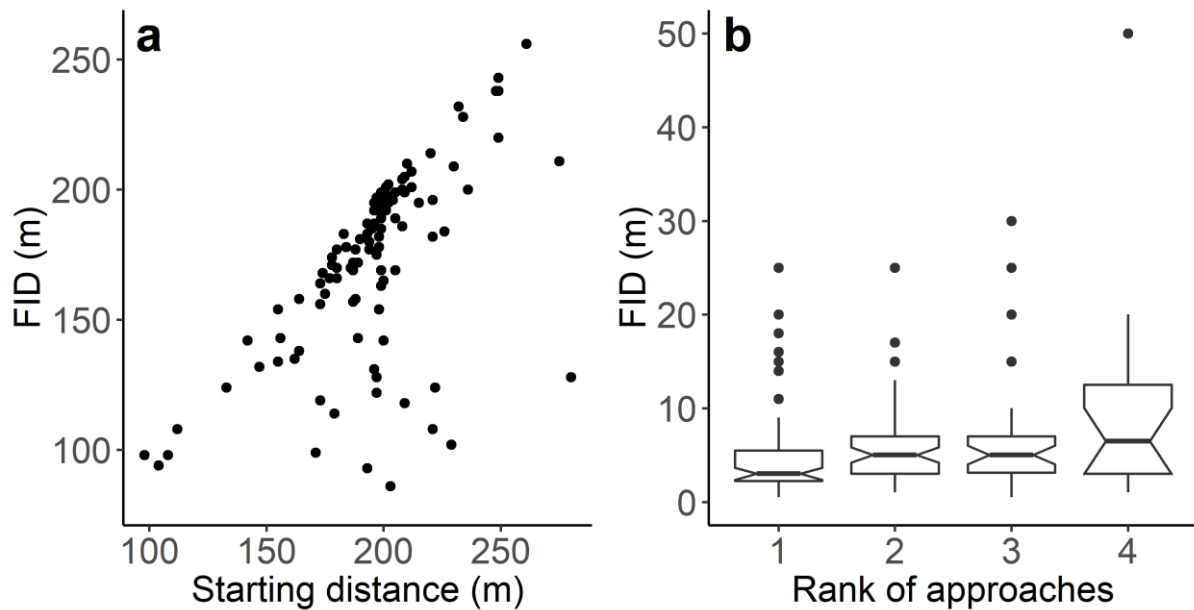


Figure 2. Effects of a) the starting distance (meters) for males and b) the rank of approaches for females on FID (meters). a) Points represented each approach measuring FID depending on the starting distance for all males. b) Boxplot represented the median (heavy lines within boxes), 25% and 75% quartiles (boxes), 1.5 times the interquartile range (whiskers) and data points outside the whiskers, for the FID of females depending on the rank of approaches.

Individual boldness and plasticity, and pairing patterns

Males did not differ from each other in their plasticity (i.e., slope, likelihood ratio test: $\chi^2 = 2.28$; $df = 1$; $P = 0.13$). Male boldness was positively correlated with their plasticity (Spearman's rank correlation test: $\rho = 0.55$; 95% CI = [0.25; 0.76]; Fig. 3a). Conversely, females differed from each other in their plasticity (likelihood ratio test: $\chi^2 = 14.75$; $df = 1$; $P < 0.001$), but as in males their boldness was positively correlated with their plasticity ($\rho = 0.65$; 95% CI = [0.47; 0.79]; Fig. 3b).

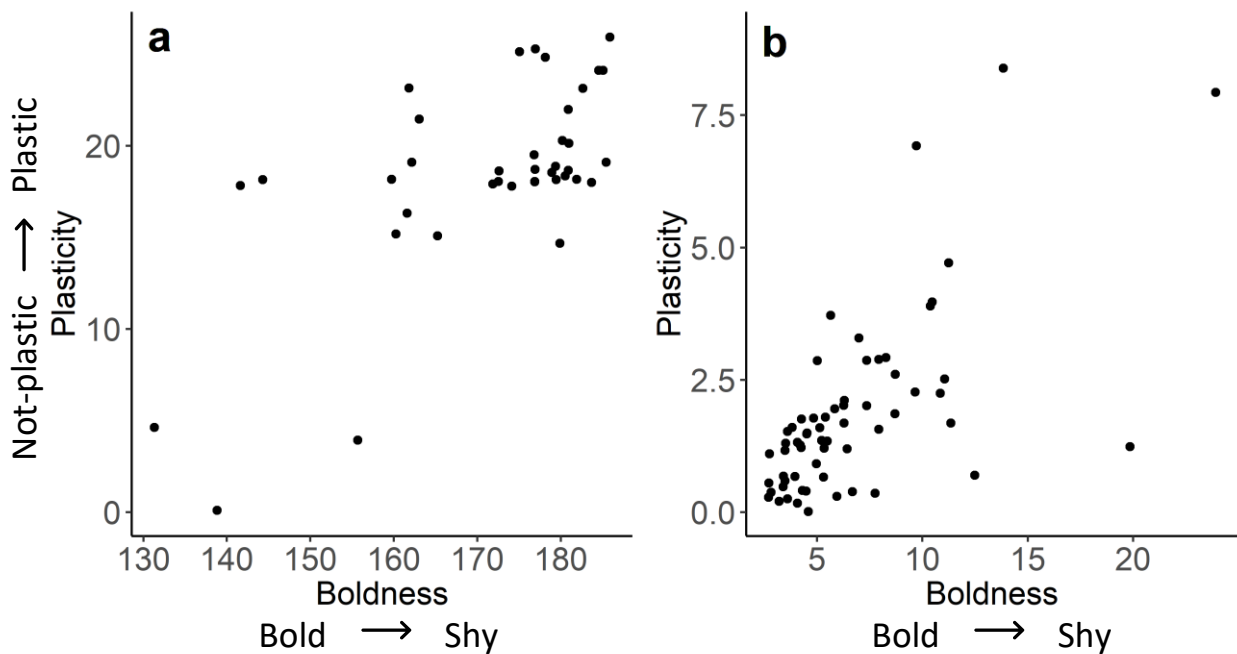


Figure 3. Observed values of boldness and plasticity from each individual for a) males and b) females. For each individual, boldness and plasticity were estimated by intercept and slope from LMM for each sex separately.

Male and female boldness were positively correlated when pooling years ($\rho = 0.53$; 95% CI = [0.17; 0.77]; Fig. 4a). When considering each year separately, the magnitude of the correlations was medium (2019: $\rho = 0.36$; 95% CI = [-0.39; 0.86]) to large (2017: $\rho = 0.72$; 95% CI = [0.10; 0.96]; 2018: $\rho = 0.54$; 95% CI = [-1.0; 0.80]). The confidence intervals for each year overlapped among them, thus the correlations are considered similar. The sample size (2017: 12 pairs, 2018: six pairs, 2019: 12 pairs) could explain a lack of statistical power. Power

analyses showed that the correlation for 2018 and 2019 would be significant with sample sizes around five times larger than they were. Male and female plasticity showed a medium correlation when considering all years pooled ($\rho = 0.29$; 95% CI = [-0.08; 0.62], Fig. 4b). A lack of statistical power could also explain that the confidence interval slightly overlapped 0 ($n = 30$ pairs). Power analyses showed that with a sample size three times larger, the correlation would be significant. When considering each season separately, correlations were medium (2018: $\rho = 0.26$; 95% CI = [-0.94; 1.0]; 2019: $\rho = 0.29$; 95% CI = [-0.34; 0.72]) to large (2017: $\rho = 0.45$; 95% CI = [-0.35; 0.85]). The confidence intervals from each year overlapped among them, thus the correlations are considered similar.

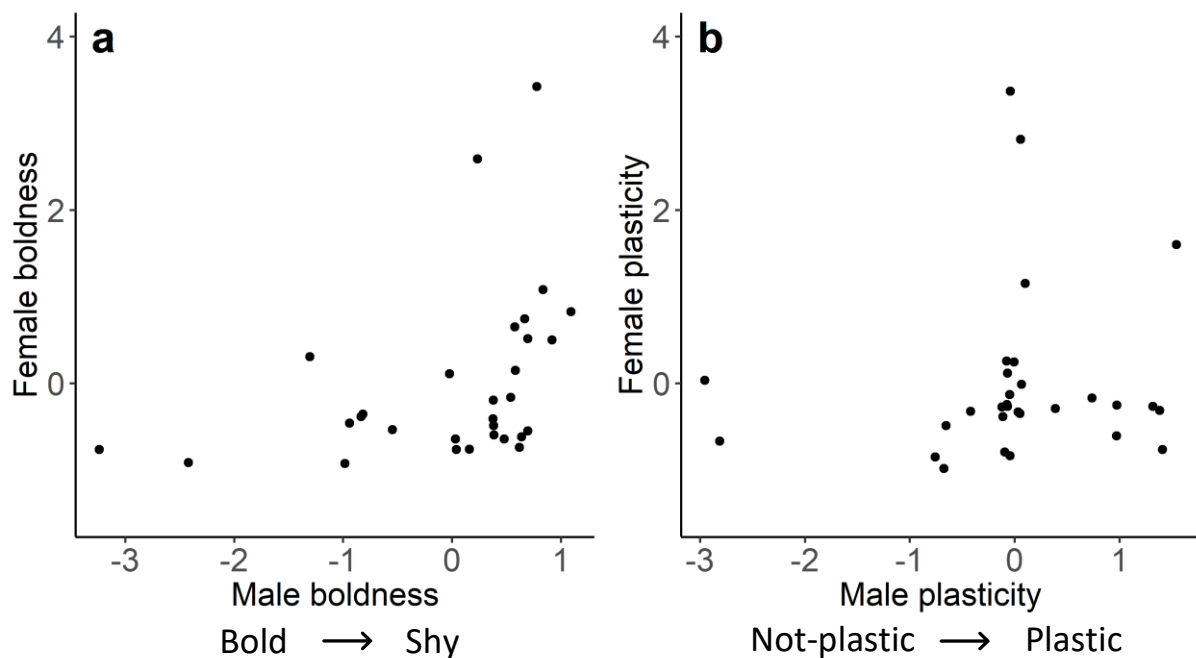


Figure 4. Observed pairing values of a) boldness within pairs and b) plasticity within pairs. Positive assortative pairing patterns in a) boldness and b) plasticity. Each point represented the behavioural combination of each pair from a) boldness of each partner and b) boldness plasticity of each partner.

Effects of boldness and plasticity pairing patterns on reproductive success

A summary of the reproductive success parameters depending on years are given in Table 3. Pair boldness and boldness similarity within pairs did not have any effect on any reproductive parameters (Table 4).

Table 3. Mean \pm standard deviation (range) of reproductive success parameters for Montagu's harrier pairs for each year.

Reproductive success parameters	2017 (N = 12)	2018 (N = 6)	2019 (N = 12)
Clutch size	3.42 \pm 0.79 (2-4)	4.17 \pm 0.75 (3-5)	4.33 \pm 0.65 (3-5)
Hatched eggs	2.08 \pm 1.24 (0-4)	3.83 \pm 0.75 (3-5)	3.33 \pm 1.16 (1-5)
Fledglings	1.25 \pm 1.22 (0-3)	3.17 \pm 0.75 (2-4)	2.92 \pm 1.44 (0-5)
Hatching success	0.60 \pm 0.33 (0-1)	0.93 \pm 0.12 (0.75-1)	0.77 \pm 0.23 (0.25-1)
Fledging success	0.47 \pm 0.44 (0-1)	0.84 \pm 0.19 (0.6-1)	0.87 \pm 0.30 (0-1)
Overall success	0.38 \pm 0.37 (0-1)	0.77 \pm 0.19 (0.6-1)	0.67 \pm 0.29 (0-1)

Table 4. Effects of the boldness similarity and pair boldness on reproductive success. Significance of each explanatory variable was tested using model comparison with likelihood ratio-based χ^2 -statistics (including statistic values, degrees of freedom and p values).

Explanatory variables	χ^2	P
<i>Clutch size</i> ¹		
Boldness similarity	0.01	0.91
Pair boldness	0.07	0.80
<i>Hatched eggs</i> ¹		
Boldness similarity	0.33	0.57
Pair boldness	0.002	0.97
<i>Fledglings</i> ¹		
Boldness similarity	0.65	0.42
Pair boldness	0.06	0.81
<i>Hatching success</i> ²		
Boldness similarity	1.35	0.25
Pair boldness	0.38	0.54
<i>Fledging success</i> ²		
Boldness similarity	0.31	0.58
Pair boldness	0.04	0.84

<i>Overall success</i> ²		
Boldness similarity	1.53	0.22
Pair boldness	0.06	0.81

¹ NBGLMM; ² BGLMM

Pair plasticity had no effect on any reproductive parameters (Table 5). However, the proportion of hatched eggs which succeeded to become fledglings significantly varied depending on plasticity similarity within pairs: the fledging success was greater for less similar pairs (Table 5; Fig. 5a). Likewise, less similar pairs in plasticity had a greater overall success with a higher proportion of laid eggs which succeeded to become fledglings (Table 5; Fig. 5b). Other reproductive parameters were not impacted by plasticity similarity within pairs (Table 5).

Table 5. Effects of the plasticity similarity and pair plasticity on reproductive success. Significance of each explanatory variable was tested using model comparison with likelihood ratio-based χ^2 -statistics (including statistic values, degrees of freedom and p values). Significant effects are in bold.

Explanatory variables	χ^2	<i>P</i>
<i>Clutch size</i> ¹		
Plasticity similarity	0.12	0.73
Pair plasticity	0.21	0.65
<i>Hatched eggs</i> ¹		
Plasticity similarity	0.25	0.62
Pair plasticity	0.12	0.73
<i>Fledglings</i> ¹		
Plasticity similarity	1.73	0.19
Pair plasticity	0.57	0.45
<i>Hatching success</i> ²		
Plasticity similarity	2.78	0.10
Pair plasticity	0.003	0.96

<i>Fledging success</i> ²		
Plasticity similarity	4.66	0.03
Pair plasticity	2.88	0.09
<i>Overall success</i> ²		
Plasticity similarity	6.57	0.01
Pair plasticity	0.69	0.41

¹ NBGLMM; ² BGLMM

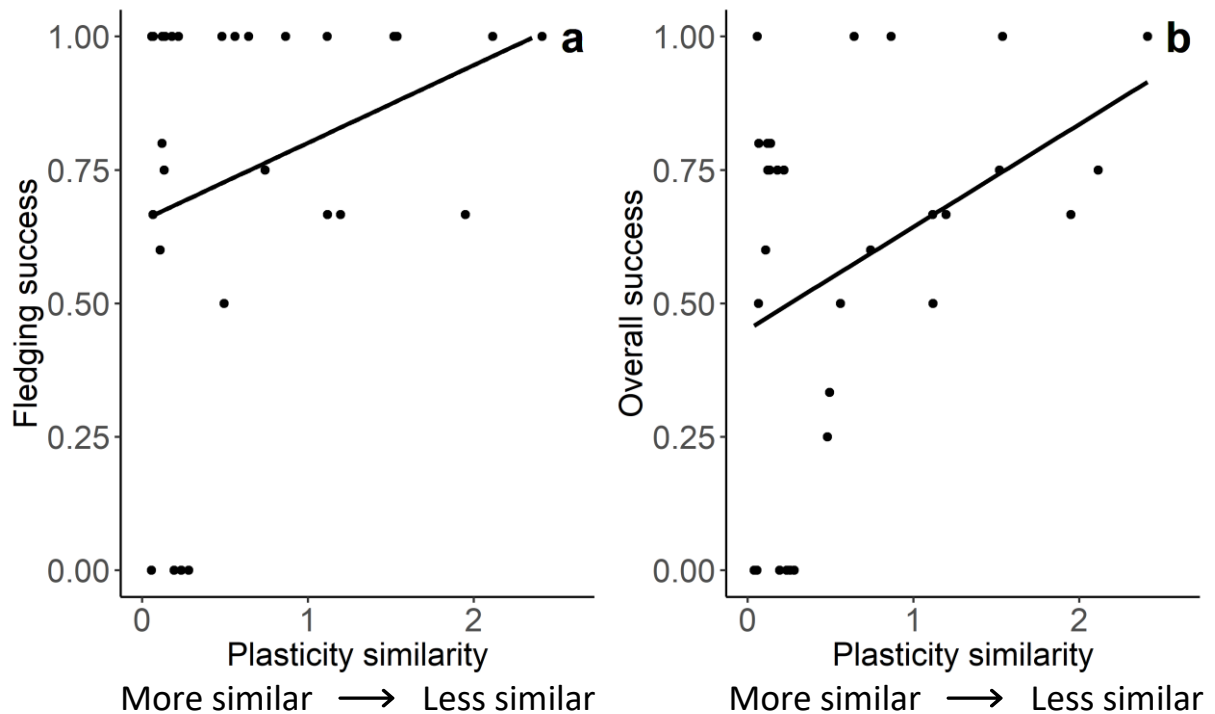


Figure 5. Effect of the plasticity similarity within pairs on a) the fledging success (fledglings / hatched eggs proportion) and on b) the overall success (fledglings / clutch size proportion). Linear lines represented predicted a) fledging success and b) overall success depending on the plasticity similarity.

Discussion

This study investigated simultaneously the pairing pattern of Montagu's harriers for both boldness and plasticity and the consequences on their reproductive success. FID as a boldness proxy was repeatable in both sexes and varied with the experimenter starting distance for males and the rank of approaches for females. For both sexes, individual boldness was positively correlated with their plasticity and males and females were positively associated for both boldness and plasticity. Only the plasticity similarity within pairs was linked to the fledging success and the overall success; less similar pairs in plasticity having higher proportions of laid eggs and hatched eggs that succeeded to become fledglings.

From FID to boldness

In Montagu's harrier, FID was repeatable across time in both sexes thus reflecting consistent differences among individuals in their risk-taking behaviour, similarly to other species (Réale et al. 2007, Bell et al. 2009, Carrete and Tella 2010, Carter et al. 2012, Seltmann et al. 2012, Carere and Maestriperi 2013, Møller 2014). However, our result for females was different from the study by Arroyo et al. (2017) performed on previous years but on the same population, which failed to evidence any repeatability for this behaviour. Further investigations are thus necessary to understand this discrepancy.

Although the FID was repeatable in both sexes, variations in experimental factors influenced FID of Montagu's harriers. For males, the FID increased with increasing experimenter starting distance. This result was in line with other studies in birds and mammals (Blumstein 2003, 2006). The animal would flee early in order to reduce the costs associated to an escape when it detects a predator (Blumstein 2006). Montagu's harrier females increased their FID throughout repeated approaches. This may be explained by the link between the rank of the approaches and the number of experimenters who visited the nest (see Material & methods). Females increased their FID over time because the number of experimenters was higher in the last approaches. The noise caused by approaches would be higher with increasing number of experimenters, as well as the predation risk perception

resulting in longer FID as also shown in Crimson Rosellas (*Platycecus elegans*) (Geist et al. 2005). Another explanation would be a sensitization process as the intensity of behavioural response, FID, increased over repeated nest visits (sensitization process described by Blumstein 2016, Rabdeau et al. 2019).

Male and female boldness and plasticity

Our Montagu's harrier study population showed differences in boldness among individuals and some degree of plasticity. It is in line with the concept of behavioural reaction norms suggesting that individuals from a same population may vary in both their average behaviour (personality) and their behavioural response over changing environmental conditions (plasticity) (Dingemanse et al. 2010). Males did not differ from each other in their plasticity while females did. This could be explained by different selection pressures (Dingemanse et al. 2010). For both sexes, individual boldness and plasticity were positively correlated, shy individuals had a greater plasticity than bold individuals. For instance, in three-spined sticklebacks (*Gasterosteus aculeatus*), shy individuals increased the amount of time spend out of cover over time while bold individuals differ little (Jolles et al. 2019). Shy individuals would be more sensitive to environmental stimuli and could thus adjust more their behaviour according to changing conditions while bold individuals would be less sensitive to changes and develop routines (Koolhaas et al. 1999, 2010, Kareklas et al. 2016, Zidar et al. 2017). These differences in behavioural plasticity is related to the adaptive character of boldness with bold individuals that better success in consistent environmental conditions and shy individuals that better success in changing environmental conditions (Koolhaas et al. 2010).

Pairing patterns for boldness and plasticity and reproductive consequences

Montagu's harriers showed a positive assortative pairing for both boldness and plasticity. Positive assortative pairing pattern has been shown for personality traits, but not for their plasticity, in different species in which partners have similar roles in parental care such as the eastern bluebird (*Sialia sialis*), Steller's jay (*Cyanocitta stelleri*) and African penguins (*Spheniscus demersus*) (Gabriel and Black 2012, Harris and Siefferman 2014, Burtka and

Grindstaff 2015, Traisnel and Pichegru 2017). To our knowledges, our study is the first to assess the pairing pattern depending on both personality and plasticity traits in a species with sex-specific care. Our study showed that even with different sex roles, males and females can also be positively associated for boldness and plasticity. Such a pattern may increase the fitness of the individuals by reducing conflicts within pairs and increasing the behavioural coordination (Royle et al. 2002, Both et al. 2005, Royle et al. 2010, Schuett et al. 2010, 2011a, Gabriel and Black 2012). For example, in Steller's jay, more similar pairs in exploration traits have a higher fledging success than dissimilar pairs (Gabriel and Black 2012). In zebra finch, pairs with similar behavioural plasticity in exploration raised chicks in better body condition than dissimilar pairs (Schuett et al. 2011a). In our study, only plasticity similarity within pairs influenced the fledging success and the overall success. Less similar pairs had greater proportions of laid eggs and hatched eggs which succeeded to become fledglings. This result contrasts with those shown in zebra finch (Schuett et al. 2011a). In our study, a consistent parent with a certain predictability paired with a plastic parent that adjusts his behavioural response to variations of environmental conditions could provide a better parental investment and would explain this result. However, further investigations are needed in parental investment for example, because benefits from less similar pairs in plasticity were not found on other reproductive parameters. The similarity within pairs in boldness did not influence any reproductive parameters. There would be not benefits from positive pairing patterns for boldness and plasticity. Moreover, no reproductive parameters varied depending on pair boldness and plasticity. Thus, the two positive assortative pairing patterns did not seem to be linked to evolutive strategies providing fitness outcomes and thus questioned the origin of these patterns.

Hypotheses on the origin of patterns

Assortative pairing has been mainly studied as a pattern resulting from sexual selection through either mate choice or from the post-pairing behavioural convergence of the partners (Schuett et al. 2010, Jiang et al. 2013, Laubu et al. 2016). For example, in zebra finch, highly exploratory females choose more exploratory than less exploratory males and they produce chicks in better body condition when they paired with exploratory males (Schuett et al. 2011a,

b). Sexual selection would favour a positive assortative pairing if there were fitness benefits to be paired with a similar partner or converging to behavioural similarity. However, in the present study, the two positive assortative pairing patterns for boldness and plasticity did not seem to provide fitness benefits at least in the number of fledglings. Assortative pairing may also result from ecological mechanisms due to the non-random distribution of phenotypes within the environment (Ferrer and Penteriani 2003, Flockhart and Wiebe 2007). This non-random distribution of phenotypes may either be due to a temporal or geographic segregation of phenotypes. Indeed, in our study area, Montagu's harrier arrivals from wintering areas are spread over the breeding period (Arroyo et al. 2004). This could arise from different reasons such as winter conditions or migration pathway for instance (Limiñana et al. 2012, 2013) but also from their behaviour. Personality could be linked to migration behaviour as shown in blue tits (*Cyanistes caeruleus*); migratory individuals are bolder than resident individuals (van Noordwijk et al. 2006, Nilsson et al. 2010, 2014). Montagu's harrier migration timing could thus depend on boldness; bold individuals migrating first, thus pairing first with available individuals, e.g. other bold individuals. The second and not-exclusive hypothesis is the spatial segregation of phenotypes depending on boldness. There are growing evidences for personality-matching habitat choice hypothesis (Edelaar et al. 2008, Carrete and Tella 2010, Cote et al. 2010, Wolf and Weissing 2012, Seltmann et al. 2014, Jacob et al. 2015, Zhao et al. 2016, Holtmann et al. 2017, Sprau and Dingemanse 2017). Animals would select a suitable habitat depending on their personality (Holtmann et al. 2017, Sprau and Dingemanse 2017). For example, in dunnocks (*Prunella modularis*), shy individuals settle in areas with lower human frequentation than bold individuals (Holtmann et al. 2017). Consequently, pairing could occur according to the spatial availability of phenotypes. In our study population, differences in distribution of Montagu's harriers in space could occur depending on their boldness and environmental conditions such as human activities. This later hypothesis is currently investigated, and first results suggest that Montagu's harrier may nest accordingly to human activities depending on their own personality (Chapter IV).

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Chapitre 3

Does parents' boldness influence food provisioning and nestling quality in Montagu's harrier?

Résultats préliminaires, les analyses de la troisième saison de reproduction
(2019) seront rajoutées ultérieurement



Does parents' boldness influence food provisioning and nestling quality in Montagu's harrier?

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Preliminary results

Abstract

Body and physiological nestling conditions depend on the parental investment through their food provisioning. Individual personality and plasticity could influence its investment in food provisioning during reproduction. However, to our knowledges, the links between parent personality or plasticity and nestling physiological condition has never been investigated. Based on a previous study establishing a positive assortative pairing pattern in boldness and its plasticity in a Montagu's harrier (*Circus pygargus*) population, the present study evaluated the links between parents' behaviour and i) their food provisioning, ii) their fledgling quality (measured by body condition, haematocrit and plasma carotenoid concentration). Four indices were calculated to characterise the behavioural association within pairs: pair boldness, boldness similarity within pairs, pair plasticity, plasticity similarity within pairs. Less similar pairs in plasticity provided a higher proportion of micromammals and more generally, greater food biomass than more similar pairs that delivered more insects to their nestlings. In addition, the higher proportion of insects supplied by not-plastic pairs seemed to explain the greater carotenoid concentration of their nestlings compared to nestlings from plastic pairs. However, no other link was found between parents' behaviour and nestling body and physiological conditions. These results also provide support to previous findings suggesting that sexual selection is probably not the major driver of the assortative pairing pattern observed in this population of Montagu's harriers.

Keywords: assortative pairing, behavioural similarity, carotenoids, parental investment, personality, plasticity.

Introduction

The developmental phase before independence is a critical period because it influences life-history trajectory of an organism (Metcalf and Monaghan 2001, Monaghan 2007). Environmental factors such as temperature, parasite infestation or particularly food availability affect nestling condition (Kitaysky et al. 1999, Bańbura et al. 2013, Sumasgutner et al. 2018). In altricial birds, nestlings depend on their parents until fledging, for their food provisioning (Martin 1987, Royle et al. 2012). Consequently, nestling body condition and physiological state including stress level, homeostasis and immune system, are largely determined by the quantity and quality of food provisioned by the parents (Martin 1987, Bańbura et al. 2008, Klug and Bonsall 2014, van Oers et al. 2015, Sumasgutner et al. 2018). For instance, through experimental manipulations of parental effort, an increased brood size induces a decrease in nestling body condition in Eurasian kestrel (*Falco tinnunculus*, Korpimäki and Rita 1996) and blue-footed booby (*Sula nebouxii*, Velando and Alonso-Alvarez 2003). Moreover, nestling body condition may vary depending on the food quality delivered by the parents. For example, in house sparrows (*Passer domesticus*), high delivery rate of large prey increases fledging mass and thus, their probability to survive up to the recruitment (Schwagmeyer and Mock 2008). At the physiological level, a decrease in food intake may reduce erythropoiesis thus resulting in anaemia (reviewed in Fair et al. 2007). Anaemia may have detrimental consequences for organisms such as decreasing oxygen transfer to organs and tissues resulting in hypoxia (Fair et al. 2007). A decline in food quality and/or quantity may also reduce the acquisition of certain elements that cannot be synthesized by the organisms such as carotenoid pigments (Møller et al. 2000). Carotenoids are even important to acquire in the food as they are involved in several physiological processes such as immunity and oxidative stress prevention (Møller et al. 2000). Additionally, they are involved in coloration-based sexual characters (Møller et al. 2000, Hill et al. 2002) often used as honest signal of quality in several species, for example in common blackbird (*Turdus merula*, Faivre et al. 2003). The plasma carotenoid concentration may thus depend on the food quality and quantity provided by the parents (Sternalski et al. 2010). Indeed, prey vary in nutritional value and in their carotenoid concentration, insects for instance are richer in carotenoids than micromammals (Sumasgutner et al. 2018). For example, carotenoid concentration in the

micromammal Savi's pine vole (*Microtus savii*) is ca. 0.35 µg/g whereas Coleopteran and Lepidopteran insects ranged from 12.4 to 28.5 µg/g (Casagrande et al. 2006, Eeva et al. 2010). Nestlings supplemented with carotenoid-rich diet have higher plasma carotenoid concentration than control in Eurasian kestrel, great tits (*Parus major*), Montagu's harrier (*Circus pygargus*) and marsh harrier (*Circus aeruginosus*) (Casagrande et al. 2007, Costantini et al. 2007, Eeva et al. 2008, Sternalski et al. 2010, 2012). Therefore, this carotenoid-rich diet may provide positive effects on both immune function through immuno-stimulation and immune-regulation functions (Favre et al. 2003, Biard et al. 2006, Strandin et al. 2018). Some parents could be specialised more on a prey category that is more or less rich in carotenoids, than other parents and thus, the quantity and the quality of food provisioning could differ depending on parents' investment quality (Schwagmeyer and Mock 2003).

Some individuals may provide higher food quantity and/or quality than others thus resulting in contrasted parental investment (Nakagawa et al. 2007, Westneat et al. 2011, Burtka and Grindstaff 2015). These differences in parental investment among individuals could be linked to their personality following the pace-of-life syndrome hypothesis (Schwagmeyer and Mock 2003, Réale et al. 2010, Mutzel et al. 2013). For example, in house wrens (*Troglodytes aedon*) and blue tits (*Cyanistes Caeruleus*), aggressive males have a lower feeding rate than less aggressive males (Barnett et al. 2012, Mutzel et al. 2013). The parents' personality is thus expected to influence the body and physiological conditions of offspring through their food provisioning. Moreover, in biparental care species, nestling quality may vary depending on the combination of personality and plasticity from both parents (Both et al. 2005, Schuett et al. 2011, Harris and Siefferman 2014). In great tits (*Parus major*) or zebra finches (*Taeniopygia guttata*), similar pairs in aggressiveness or exploration have chicks in better body condition than dissimilar pairs (Both et al. 2005, Schuett et al. 2011). Indeed, the similarity within pairs may reduce sexual conflicts and provide better behavioural compatibility and coordination and thus, a greater parental investment (Spoon et al. 2006, Royle et al. 2010, Schuett et al. 2010, 2011, Gabriel and Black 2012, Harris and Siefferman 2014, Burtka and Grindstaff 2015). For instance, in great tits, similar pairs in exploration have a greater food provisioning rate than dissimilar pairs, possibly because they have a better behavioural synchronisation (David et al. 2015). However, the link between nestling quality and behavioural combination from parents has only been assessed in species with similar roles

in parental care between partners (Both et al. 2005, Schuett et al. 2011, Harris and Siefferman 2014) but to our knowledges, not in species with sex-specific care. Moreover, no study has investigated the consequences of behavioural association within pairs on the physiological condition of nestlings.

The Montagu's harrier is a species with biparental care but specific to the sexes: the female incubates the eggs and the male is responsible for food provisioning for the female and chicks (see the review by Arroyo et al. 2004). Near the fledging, the female may also hunt for additional prey. The major prey of this raptor species is the common vole (*Microtus arvalis*), an energy-rich prey but poor in carotenoids (Millon et al. 2008, Sternalski et al. 2010). Montagu's harrier diet may also be completed with invertebrates such as crickets or grasshoppers, which are carotenoid-rich prey but poorer in caloric intake, and more rarely with passerine birds, also carotenoid-rich prey (Salamolard et al. 2000, Sternalski et al. 2010). Different food provisioning strategies may occur among pairs and they could be linked to the behavioural association within pairs. Previous studies have established differences in boldness in Montagu's harrier population (Arroyo et al. 2017) and individuals also display some degree of plasticity in their boldness (Chapter II). Moreover, individuals have been shown to be positively paired for boldness and plasticity but similar pairs did not seem to have a better reproductive success (Chapter II). However, how pairing pattern impacts food provisioning and consequently, fledgling body and physiological conditions has never been investigated in this species. In the present study, we used pair boldness and plasticity measured by both their level within the population and the similarity within the pair, thus resulting in four behavioural indices for each pair: pair boldness, boldness similarity within pairs, pair plasticity, plasticity similarity within pairs. Then, we aimed at testing whether these behavioural characteristics of the pairs may be linked to their parental investment through food provisioning strategies and their consequences on nestling body and physiological conditions. First, the parents' behaviour and the link to their food provisioning were investigated. Similarity within pairs providing a better behavioural coordination, similar pairs in boldness and plasticity should have a higher food provisioning (biomass intake). The effect of parents' behaviour on the prey category (micromammals and/or insects) they forage was also investigated. Second, nestling body and physiological conditions were studied in relation with behavioural association within pairs for boldness and plasticity through the four indices. Similar pairs for boldness and

plasticity should have chicks in better body and physiological conditions with higher haematocrit at fledging than less similar pairs.

Material & Methods

Study site and model species

Data were collected within the LTSER (Long-Term Socio-Ecological Research) Zone Atelier Plaine et Val de Sèvre (ZAPVS, western France, 46° 110 N, 0° 280 W), covering ca. 435 km² of farmland and where the Montagu's harrier population has been exhaustively monitored since 1995 (Bretagnolle et al. 2018). The Montagu's harrier nests on the ground in heathland and marshland (Arroyo et al. 2004). However, through the agricultural intensification and the decline in natural habitats, this raptor species now nests mainly in cereal crops (Arroyo et al. 2002, 2004). Despite his protected status, the population is decreasing throughout France potentially because of the intensification of agricultural practices in the last decades (Butet and Leroux 2001, Santangeli et al. 2015). Females lay up to six eggs around the end of April and early May for the earliest pairs on the ZAPVS (Millon et al. 2008). The incubation and the rearing period last around 29 and 30-35 days respectively (García and Arroyo 2001, Arroyo et al. 2007)

Nest monitoring – overall design

Montagu's harrier nests were localized in the ZAPVS during three breeding seasons and the experimental design was the same each season (2017: 43 nests; 2018: 40 nests; 2019: 40 nests). Once nests have been discovered, the boldness and its plasticity of males and females were estimated with repeated measures of flight initiation distance (FID) until fledging (see below and Chapter II). Depending on the brood size and differences in hatching dates within brood, nests were visited twice during the incubation and four or five times during the chick rearing period (from hatching to fledging; lag between visits: 7 ± 2 days). At the last nest visit before fledging (26 ± 2 days old chicks), the parental investment through food provisioning

was recorded as well as morphological metrics on nestlings to calculate body condition, and blood sampling for haematocrit measure and carotenoid titration (see below for details).

Parents' boldness and plasticity

Two experimental designs allowed measuring male and female FID (Chapter II). Once a nest was discovered, a pole was positioned close to the nest along a path or a road, allowing the male to perch. Every morning, males standing on a pole were approached by the experimenter from a starting distance of ca. 200 m depending on landscape configuration. Male FID was measured at least two times over the breeding season with an interval of some days between repeats (mean \pm standard deviation: 7.87 ± 5.45 days, $N = 107$ observations). FID was measured for 13 males in 2017 (36 observations; 2.77 ± 0.83 measures per individual), 13 males in 2018 (33 observations; 2.54 ± 0.52 measures per individual) and 13 males in 2019 (38 observations; 2.92 ± 0.86 measures per individual). The starting distance and the rank of approaches that may influence FID, were recorded at each approach. As females rarely perched on pole during incubation, female FID was measured during nest visits, when the experimenter approached the nest. At least two measures per female were performed over the breeding season for 27 females in 2017 (79 observations, 2.93 ± 0.78 measures per individual), 16 in 2018 (42 observations, 2.63 ± 0.80 measures per individual) and 23 in 2019 (60 observations, 2.63 ± 0.80 measures per individual). The rank of approaches was also recorded at each approach.

Individual boldness and plasticity for males and females were estimated from Linear Mixed-effects Models (LMMs) following the *Within-subject centering* (WSC) method proposed by van de Pol and Wright (2009). For males, LMM analysed the relationship of FID on the starting distance and the rank of approaches. For females, LMM analysed the relationship between FID and the rank of approaches. For both sexes, intercept and slope from LMMs were used respectively to characterize individual boldness and plasticity (see details in Chapter II). Two positive assortative pairing patterns were established between male and female boldness and plasticity ($n = 30$ pairs all years pooled; Chapter II). Four behavioural indices were calculated for each pair using two principal component analyses (PCA; Chapter II), the first one on female and male boldness, and the second one on female and male plasticity. From each

PCA, the first axis represented the pair boldness or plasticity and the absolute values of the second axis represented the similarity of the partners within pairs in boldness or plasticity. Consequently, four indices described the behavioural association within pairs: i) pair boldness (low values: bold pairs; high values: shy pairs), ii) boldness similarity within pairs (low values close to 0: more similar pairs; high values: less similar pairs), iii) pair plasticity (low values: not-plastic pairs; high values: plastic pairs;), iv) plasticity similarity within pairs (low values close to 0: more similar pairs; high values: less similar pairs).

Food provisioning assessment

To measure food provisioning by parents, each nest was video monitored once using a small camera (MUVI™ K-series) hidden in the crop closed to the nest to limit potential disturbance. Cameras were positioned during the pre-fledging visit either in the morning or in the end of afternoon which correspond to picks in food provisioning for Montagu's harrier (Clarke 1996). Records of food provisioned by parents were obtained for 32 nests and lasted ca. 2h36 min. \pm 31 min. (mean \pm standard deviation, s.d.; n = 2017: 13 nests; 2018: 19 nests). Videos were analysed by C.M. From the records, prey were divided into three categories: micromammal, bird or insect and the number of each item was noted. Food provisioning was thus expressed as the number of items per hour and per nestling for each prey category. Because both parents can hunt prey for nestlings at the end of the rearing period, food provisioning represented the parental investment for the pair. The proportion of each prey category was calculated by dividing the number of prey/hour/nestling divided by the total number of prey (all categories pooled)/hour/nestling. Moreover, a biomass value was assigned for each prey category: 50 g for bird, 30 g for micromammal and 5 g for insect following Arroyo et al. (1997). The total biomass of prey per hour and per nestling was calculated for each nest and named thereafter 'food biomass'. Montagu's harrier pairs with available data on both food provisioning parameters and parents' boldness and plasticity were 10 pairs.

Body and physiological conditions of nestlings

At the pre-fledging visit, the right and left tarsus lengths of the nestlings were measured using a digital calliper (accuracy: ± 0.1 mm). The right and left wings chord lengths were also measured with a ruler (accuracy: ± 1 mm). Nestlings were weighed using a spring scale (Pesola® 500 g, accuracy: ± 5 g) and the body condition was estimated by the scale mass index proposed by Peig and Green (2009) as follows:

$$SMI_i = Mass_i \times \left(\frac{\text{Mean tarsus length of the group}}{Tarsus length_i} \right)^{b_{sma}}$$

where b_{sma} is the slope of the major axis regression of $\log(\text{body mass})$ on $\log(\text{mean of both left and right tarsus lengths})$ following the standard major axis method for each chick i (using 'smatr' package, Warton et al. 2010).

A blood sampling (ca. 300 μ L) was performed on nestlings from the puncture of the brachial vein using heparinized capillaries (Hirschmann Laborgeräte) and blood samples were kept refrigerated (0-5°C) for two to three hours. Then, in order to obtain plasma samples, blood samples were centrifuged (10 min. at 9000 rpm, Bio Lion XC-LED12K). Plasma samples were then stored at -20°C until carotenoid titration by spectrophotometry (following Biard et al. 2010). The carotenoid titration was performed for plasma samples from 2017 and 2018 breeding seasons. An additional sample of blood was taken in a 10 μ L microcapillary tube (Hirschmann Laborgeräte) and kept also refrigerated (0-5°C) for five hours until centrifugation (8 min. at 9000 rpm) to measure the haematocrit. The haematocrit was calculated by the relative amount of red blood cells, i.e., the coat layer of red blood cells divided by the total layer of the tube filled with blood (Biard et al. 2006). The haematocrit was measured for 186 chicks and the plasma carotenoid concentration was titrated for 95 chicks. The haematocrit was measured for three breeding seasons (2017 to 2019) whereas the plasma carotenoid concentration was titrated for the two first breeding seasons (2017 and 2018).

Statistical analyses

Linking food provisioning to boldness and plasticity within pairs

One Principal component analysis (PCA) was performed to get a synthetic variable reflecting parental food provisioning strategy depending on the prey category. A singular value decomposition of the centred and scaled data matrix was used (Crawley 2012). The PCA included the proportions of micromammals, birds and insects (number of items delivered per hour and per nestling). The first axis of the PCA was retained to reflect the food provisioning strategy, named thereafter “food strategy” as the eigenvalue was above 1 and accounted for 52 % of the total variance. The links between behavioural indices of the pairs (pair boldness, boldness similarity within pairs, pair plasticity, plasticity similarity within pairs) and i/ food strategy and ii/ food biomass, were analysed using Spearman’s rank correlation tests (n = 10 pairs). A ρ correlation coefficient of 0.1, 0.3 or 0.5 were considered as “small”, “medium” or “large” effect respectively, as proposed by Cohen et al. (1988).

Effects of parents’ behaviour on nestling quality

To study the effects of the parents’ behaviour on nestling haematocrit (62 chicks and 24 nests), carotenoid concentration (31 chicks and 13 nests) and body condition (64 chicks and 24 nests), LMMs were performed (using ‘lmerTest’ package, Kuznetsova et al. 2014). For each nestling parameter, two separate LMMs were performed: one included the pair boldness and boldness similarity within pairs as fixed effects and the second included pair plasticity and plasticity similarity within pairs. For all LMMs, the sex of nestlings and the clutch size were also included as fixed effects and the sampling year and the nest identity were included as random effects. For each LMM, the statistical significance of each parameter was analysed using model comparison with likelihood ratio-based χ^2 -statistics (using ‘car’ package, Fox and Weisberg 2011, Fox et al. 2012).

All statistics were performed with R software (v. 3.5.1, R Development Core Team 2018).

Ethics statement

Bird manipulation was allowed by a permit of the CRBPO (Centre de Recherches sur la Biologie des Populations d'Oiseaux - Museum National d'Histoire Naturelle, licence #1308). The methods used for capture, handling, banding and blood sampling comply with French guidelines for ethical use of animals in research.

Results

Linking food provisioning to boldness and plasticity within pairs

A summary of prey categories and food biomass depending on the years is given in Table 1. The first axis of the PCA, representing food strategy, was positively correlated with the proportion of insects (Spearman's rank correlation test: $\rho = 0.72$; CI 95% = [0.46; 0.87]) and birds ($\rho = 0.47$; CI 95% = [0.18; 0.74]) and negatively correlated with micromammals provisioning ($\rho = -0.96$; CI 95% = [-1.0; -0.87]; Fig. 1). High positive values of food strategy characterized food provisioning including more insects, and high negative values characterized food provisioning including more micromammals.

Table 1. Mean \pm standard deviation (range) of prey category (number of prey items / hour / nestling) and food biomass (g) for Montagu's harrier pairs for each year.

Food provisioning parameters	2017 N = 13	2018 N = 19
Micromammal	0.37 \pm 0.46 (0-1.79)	0.34 \pm 0.18 (0-0.60)
Bird	0.06 \pm 0.08 (0-0.15)	0.02 \pm 0.04 (0-0.14)
Insect	0.07 \pm 0.18 (0-0.61)	0.09 \pm 0.16 (0-0.48)
Food biomass	14.36 \pm 13.75 (0-53.79)	11.43 \pm 5.60 (0-19.96)

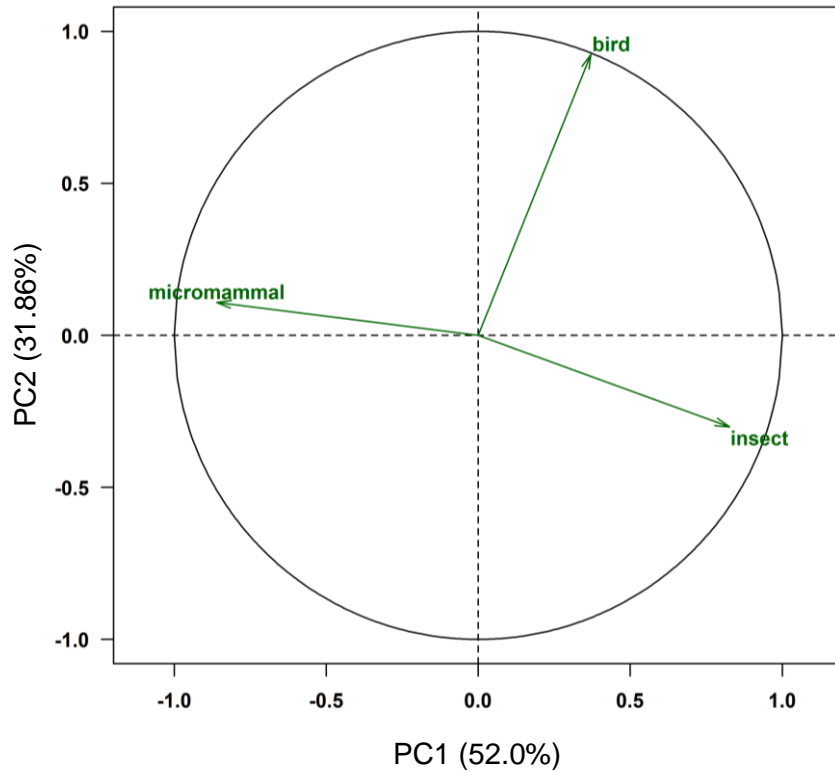


Figure 1. Principal component analysis (PCA) on the proportions of insects, birds and micromammals delivered to nestlings. Arrows represented the correlation of initial variables to the principal component.

Due to our low sample size ($n = 10$ pairs), results should be carefully considered. Indeed, even if effect sizes suggest the existence of medium to strong correlation (according to Cohen 1988), all correlations but one (plasticity similarity within pairs and food strategy) are not statistically significant (95% CI overlapped 0).

Pair boldness was not correlated to food strategy ($\rho = 0.01$; 95% CI = [-0.67; 0.71]) but with food biomass ($\rho = -0.33$; 95% CI = [-0.85; 0.50]), bold pairs delivering a higher food biomass to their nestlings than shy pairs. Boldness similarity within pairs was neither correlated to food strategy ($\rho = 0.08$; 95% CI = [-0.66; 0.59]) nor to food biomass ($\rho = 0.04$; 95% CI = [-0.69; 0.75]). Pair plasticity was correlated to food strategy ($\rho = -0.38$; 95% CI = [-0.87; 0.27]) and to food biomass ($\rho = 0.31$; 95% CI = [-0.41; 0.88]; Fig. 2a, b). Plastic pairs delivered more micromammals and a higher food biomass to their nestlings than not-plastic pairs (Fig. 2a, b). Plasticity similarity within pairs was correlated to food strategy ($\rho = -0.32$; 95% CI = [-0.67; 0.71]) and with food biomass ($\rho = 0.67$; 95% CI = [-0.07; 1.0]; Fig. 2c, d). More similar pairs in

plasticity provided more insects and a lower food biomass while less similar pairs delivered more micromammals and a higher food biomass (Fig. 2c, d).

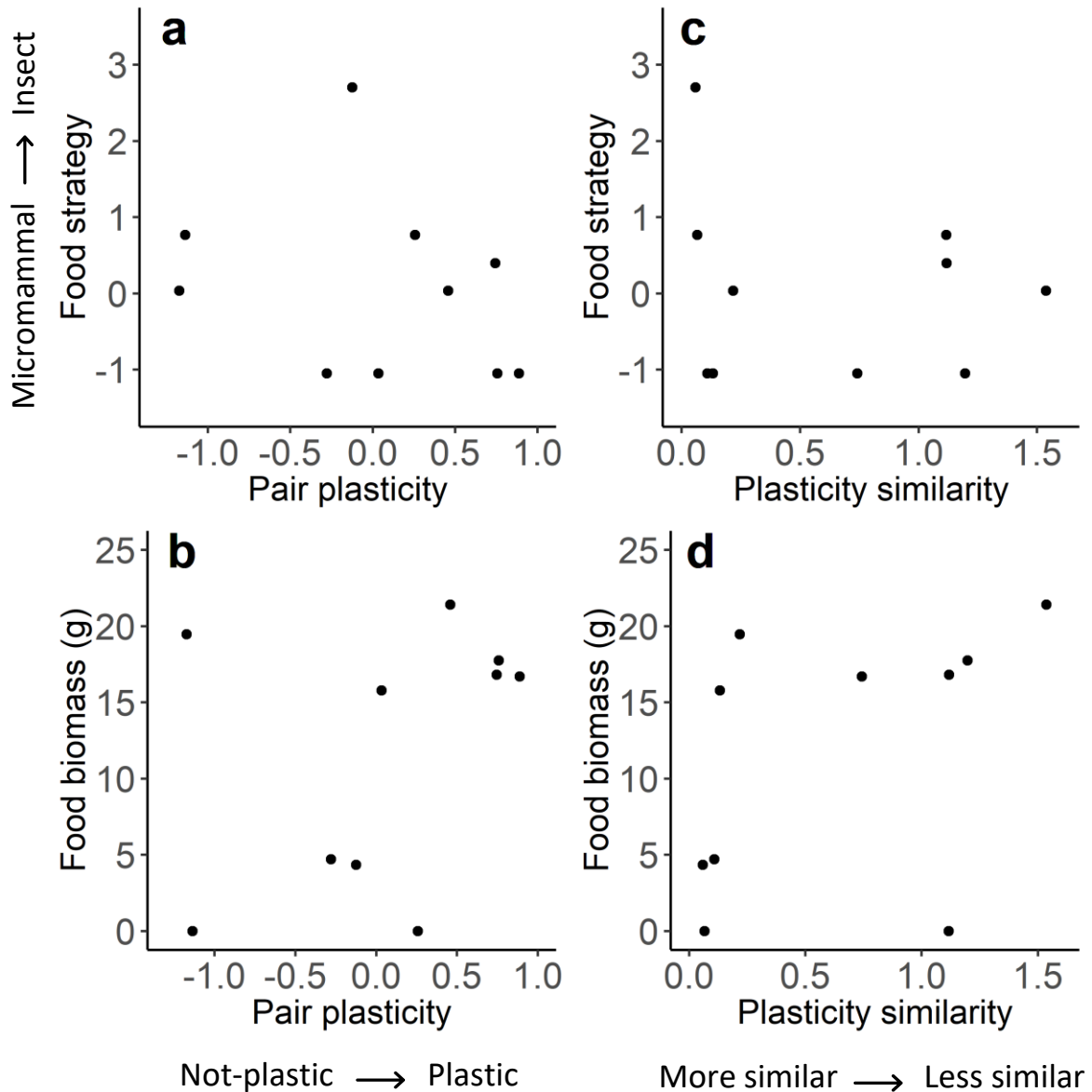


Figure 2. Relationship between a) food strategy and pair plasticity, b) food biomass and pair plasticity, c) food strategy and plasticity similarity within pairs, and d) food biomass and plasticity similarity within pairs (n = 10 pairs).

Effects of boldness and plasticity patterns within pairs on nestling quality

A summary of the nestling quality parameters depending on the years are given in Table 2. Pair boldness and boldness similarity within pairs did not have any effect on nestling body condition, haematocrit and carotenoid concentration at fledging (Table 3). Pair plasticity and plasticity similarity within pairs did not have any effect on nestling body condition and haematocrit (Table 4). However, pair plasticity influenced the plasma carotenoid concentration of nestlings (Table 4). Not-plastic pairs had nestlings with a higher carotenoid concentration than plastic pairs (Fig. 3). The sex of nestlings and the clutch size did not have any effect on nestling body condition and haematocrit (Tables 3 and 4). The plasma carotenoid concentration of nestlings increased with the clutch size (Table 4).

Table 2. Mean \pm standard deviation (range, N=sample size) of body condition (g), haematocrit, plasma carotenoid concentration (ng/ μ L) for Montagu's harrier fledglings, for each year.

Nestling quality parameters	2017	2018	2019
Body condition	314.48 \pm 29.58 (277.59-399.08, N=14)	308.13 \pm 28.96 (269.23-363.46, N=19)	296.37 \pm 38.0 (195.07-399.71, N=31)
Haematocrit	41.96 \pm 4.11 (35.70-49.70, N=14)	35.69 \pm 5.44 (28.61-45.31, N=19)	38.92 \pm 4.89 (26.56-48.06, N=29)
Carotenoid concentration	41.56 \pm 4.24 (35.70-49.70, N=12)	35.69 \pm 5.44 (28.61-45.31, N=19)	Not yet analysed

Table 3. Results from LMMs assessing the effects of boldness association within pairs on the body condition (64 chicks and 24 nests), the haematocrit (62 chicks and 24 nests) and the plasma carotenoid concentration (31 chicks and 13 nests) of Montagu’s harrier fledglings. Model comparison with likelihood ratio-based χ^2 -statistics was used to test significance of each explanatory variable (including statistic values, degrees of freedom and p values).

Explanatory variables	χ^2	<i>P</i>
<i>Body condition</i>		
Boldness similarity	1.82	0.18
Pair boldness	1.44	0.23
Clutch size	0.08	0.81
Sex	0.33	0.57
<i>Haematocrit</i>		
Boldness similarity	2.06	0.15
Pair boldness	0.94	0.33
Clutch size	2.05	0.15
Sex	1.02	0.31
<i>Carotenoid concentration</i>		
Boldness similarity	0.003	0.96
Pair boldness	1.10	0.30
Clutch size	2.42	0.12
Sex	0.12	0.73

Table 4. Results from LMMs assessing the effects of plasticity association within pairs on the body condition (64 chicks and 24 nests), the haematocrit (62 chicks and 24 nests) and the plasma carotenoid concentration (31 chicks and 13 nests) of Montagu’s harrier fledglings. Model comparison with likelihood ratio-based χ^2 -statistics was used to test significance of each explanatory variable (including statistic values, degrees of freedom and p values). Significant effects are in bold.

Explanatory variables	χ^2	<i>P</i>
<i>Body condition</i>		
Plasticity similarity	0.11	0.74
Pair plasticity	0.03	0.86
Clutch size	0.03	0.87
Sex	0.65	0.42
<i>Haematocrit</i>		
Plasticity similarity	1.10	0.29
Pair plasticity	1.74	0.19
Clutch size	2.82	0.09
Sex	1.03	0.31
<i>Carotenoid concentration</i>		
Plasticity similarity	2.22	0.14
Pair plasticity	7.11	<0.01
Clutch size	4.08	0.04
Sex	0.02	0.88

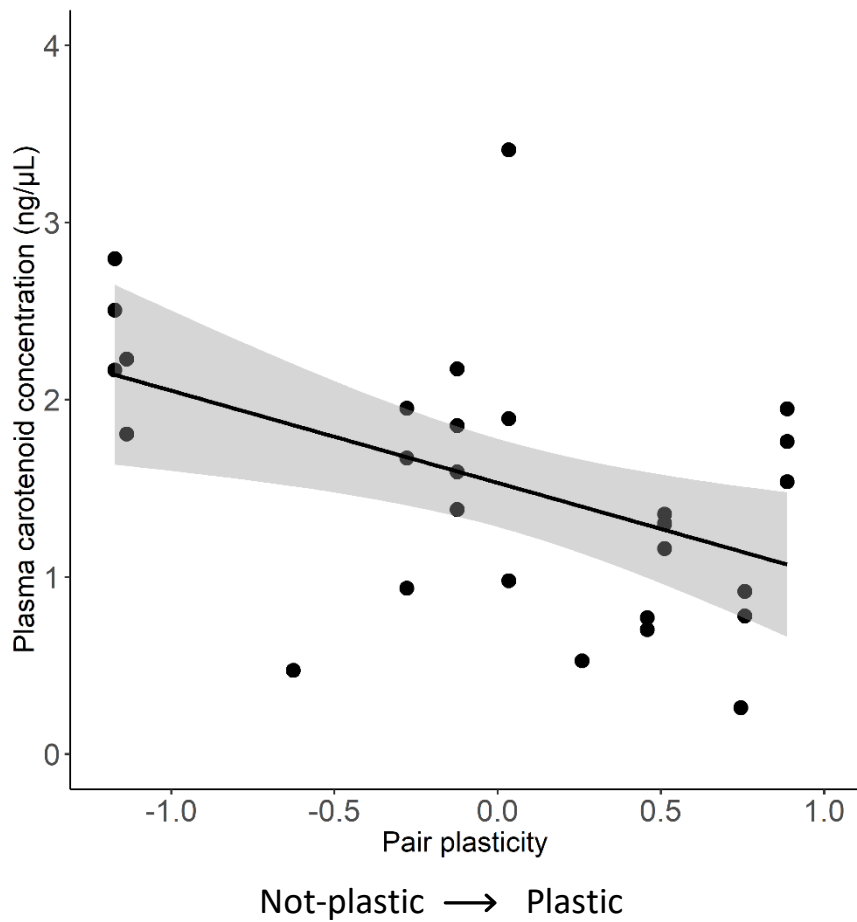


Figure 3. Effects of pair plasticity on plasma carotenoid concentration of fledglings (31 fledglings and 13 nests). Line represented predicted plasma carotenoid concentrations depending on the pair plasticity and shading represented 95% confidence interval.

Discussion

This study is the first to investigate the links between nestling physiological condition and both parents' personality and plasticity. Different food provisioning strategies were found among Montagu's harrier pairs. Bold pairs supplied a higher food biomass to their nestlings compared to shy pairs. Plastic pairs provided more micromammals and a higher food biomass than not-plastic pairs that supplied more insects and a lower food biomass. Less similar pairs in plasticity delivered more micromammals and a higher food biomass while more similar pairs

provided more insects and a lower food biomass. Nestlings from not-plastic pairs had greater carotenoid concentrations than those from plastic pairs. However, no evidence for a relationship between either the body condition or the haematocrit of nestlings and parents' boldness and plasticity was found. Finally, the plasma carotenoid concentration increased with the clutch size as shown in Eurasian kestrels (Laaksonen et al. 2008). The sex of nestlings did not affect nestling quality contrary to previous study by Sternalski et al. (2010).

Effects of parents' boldness and plasticity on their food provisioning

Montagu's harrier pairs showed different food provisioning strategies: some pairs mainly fed their offspring with insects while other pairs fed them with micromammals. This result is in line with previous study on Montagu's harrier, males differing from each other in their diet composition (Terraube et al. 2014) and conforms with increasing evidences of differences in parental food provisioning in other species (Schwagmeyer and Mock 2003, Costantini et al. 2005, Nakagawa et al. 2007, Westneat et al. 2011, Royle et al. 2012, Greggor et al. 2017). However, few studies have investigated the links between personality and food provisioning (Kontinen et al. 2009, Patrick and Browning 2011, Barnett et al. 2012, Mutzel et al. 2013, Greggor et al. 2017). The food strategy and the food biomass varied depending on the pair boldness, pair plasticity and plasticity similarity within pairs. First, bold pairs seemed to deliver a higher food biomass to their nestlings compared to shy pairs. The boldness is often linked to exploration, as proposed by the pace-of life syndrome hypothesis: bold individuals are faster in exploration (Réale et al. 2010, Mazué et al. 2015). Exploration could provide to bold individuals some abilities to forage and hunt a higher prey biomass. Second, plastic pairs provided more micromammals and a greater food biomass to their nestlings while not-plastic pairs delivered more insects and a lower food biomass. Because the plasticity represents the ability of individuals to adjust their behaviour according to changing environmental conditions, plastic pairs could better adjust their foraging and hunting behaviours and thus, deliver a higher food biomass (Dingemanse et al. 2010). In a previous study, the diet diversity decreased with increasing hunting success and the diet composition depended on the habitat foraging (Terraube et al. 2014). The boldness plasticity could provide some advantages to forage and hunt micromammals specifically and/or to adjust habitat foraging depending on

micromammal availability. Further studies are needed to investigate relation between boldness plasticity and foraging abilities. Finally, less similar pairs in plasticity delivered more micromammals and a higher food biomass to their nestlings than more similar pairs that supplied more insects. These results contrasted with those shown in great tits, in which less similar pairs in exploration have a lower food provisioning rate than more similar pairs (David et al. 2015). In our study, less similar pairs could have a greater behavioural compatibility and coordination favouring a greater food biomass delivered to nestlings. The greater food provisioning from less similar pairs in plasticity could explain a higher reproductive success shown in a previous study (Chapter II). Our study provides some elements on the relationship between boldness or plasticity from pair and their food provisioning investment that need further analyses by adding the third breeding season (2019) to increase reliability.

Effects of pair boldness and plasticity on nestling quality

Although Montagu's harrier males and females are assorted for their boldness and plasticity in our study population (Chapter II), no effect of their similarity in boldness and plasticity was found on the nestling quality through their body condition, haematocrit and plasma carotenoid concentration. In other species, such as zebra finch, similar pairs in exploration personality and plasticity have nestlings in better body condition than dissimilar pairs (Schuett et al. 2011). However, the carotenoid concentration of nestlings varied depending on the pair plasticity. Nestlings from not-plastic pairs had a higher carotenoid concentration than those from plastic pairs. The relationship could be explained by the link between pair plasticity and their food strategy. Not-plastic pairs were specialised to hunt and provide insects, a carotenoid-rich prey, resulting in a higher carotenoid concentration for their nestlings. Conversely, the lower carotenoid concentration of nestlings from plastic pairs could be explained by parent's hunting strategy based on micromammals, a carotenoid-poor prey. The higher carotenoid concentration of nestlings from not-plastic pairs could improve their immune competence, reduce free radicals produced by parasite infection and slow down the replication rate of parasites, as shown in other studies (Møller et al. 2000, Mougeot et al. 2007, Baeta et al. 2008).

Conclusions and perspectives

In a previous study, we found a positive assortative pairing for boldness and plasticity in our Montagu's harrier population. However, benefits from this pattern were not obvious on the reproductive success, as well as on the nestling quality through body and physiological conditions (Chapter II). More similar pairs in boldness or plasticity did not have a higher reproductive success and nestlings with better body and physiological conditions. A hypothesis could be that pairing patterns do not result from mate choice (i.e., sexual selection) but instead could result from the spatial and/or temporal availability of similar phenotypes. The spatial distribution of individuals according to their boldness is currently under investigation (Chapter IV) and first results suggest that Montagu's harrier nests would be distributed depending on human infrastructures.

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Chapitre 4

**Human infrastructures shape nest distribution
in the landscape depending on individual personality
in Montagu's harrier**

En préparation pour Ecology Letters



Human infrastructures shape nest distribution in the landscape depending on individual personality in Montagu's harrier

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In preparation for Ecology Letters

Abstract

The personality-matching habitat choice hypothesis suggests a difference in the distribution of individuals across habitats depending on their personality. Individual boldness may favour the tolerance toward humans and may thus make possible living close to human infrastructures and activities. In the present study, the spatial distribution of Montagu's harrier nests and reproductive success related to human infrastructure (building, road, path) proximity and density were investigated depending on female boldness (bold vs. shy) based on a long-term monitoring study of 19 years. Taking into account for female boldness, there was a difference in nest distribution depending on buildings, shy females avoiding more buildings than bold females, while there was no difference for their location according to roads and paths. However, later reproductive females nested further from paths maybe because human frequentation on paths could be more important later in the breeding season (i.e., in late spring). Path density surrounding the nests negatively affected the reproductive success regardless of female boldness. Human activities related to this infrastructure category may thus induce a disturbance and stress for females. Therefore, they may decrease their presence on the nest and their food provisioning, thus resulting in lower reproductive success. Further investigations are needed to understand the mechanisms of disturbance from paths. The different distribution of the females depending on their boldness and the effects on reproductive success may have evolutive implications. These effects may impact the population structure and in the long-term its viability. They are discussed and conservation measures are proposed.

Keywords: *Circus pygargus*, boldness, human disturbance, nest site selection, reproductive success, spatial distribution.

Introduction

Animal breeding site selection is crucial because different environmental components such as abiotic conditions, food resources and predator presence may have direct and indirect effects on the reproductive success (Hall et al. 1997, Jones 2001, Chase 2002, Warner et al. 2002, Barbaro et al. 2008). Consequently, suitable breeding sites are distributed depending on the temporal and spatial availability of these environmental components within the landscape (Clark and Shutler 1999, Stenhouse et al. 2005). Human activities and infrastructures are increasingly present in the environment (McKinney 2002, Balmford et al. 2009) and are others components that could be taken into account in the breeding site selection process (Kolbe and Janzen 2002, Blanc et al. 2006, Remacha and Delgado 2009). Through direct effects on behaviour and physiologic stress, the anthropogenic disturbance often affects negatively the reproductive success of animals (González et al. 2006, Ellenberg et al 2007, Zuberogitia et al. 2008, Strasser and Heath 2013, Hutfluss and Dingemanse 2019). For example, the traffic noise may increase the physiologic stress for individuals, decrease their opportunities to detect prey and increase the time spent to scan predators (Halfwerk et al. 2011, Strasser and Heath 2013). Therefore, the energy invested in reproduction is reduced and thus the clutch size is smaller, as shown in great tit (*Parus major*, Halfwerk et al. 2011). The nest attendance such as food delivering to nestlings and time spent at nest decreases with increasing human presence (e.g., fishermen, passers-by, hunters) and activities (e.g., helicopters, motorbikes) resulting in reduced reproductive success, in marsh harrier (*Circus aeruginosus*) and bearded vulture (*Gypaetus barbatus*) for example (Fernández et Azkona 1993, Arroyo and Razin 2006). Consequently, human activities and infrastructures may interact with animal breeding site selection process and individuals may thus select their breeding site to avoid human disturbances (Yasué and Dearden 2006, Martínez-Abraín et al. 2010, Hanane 2014, Pedrana et al. 2014). For instance, European nightjars (*Caprimulgus europaeus*) and great tits avoid areas with high density of walking and cycling tracks or nest further away from highly frequented tracks (Lowe et al. 2014, Hutfluss and Dingemanse 2019).

Within the same population, anthropogenic disturbances may however affect individuals differently. Some individuals could be more or less tolerant to human activities than others

depending on their personality. Indeed, populations are made of individuals exhibiting different personalities, i.e. individuals that behave consistently in different way from each other across time and contexts (Réale et al. 2007, Biro and Stamps 2008, Dingemanse et al. 2010). Personality traits such as boldness or exploration could be involved in individual abilities to disperse or colonise new habitats, or even in human tolerance (Wolf and Weissing 2012). Boldness may favour the tolerance toward human and may allow individuals living closer to human infrastructures (Carrete and Tella 2010, Evans et al. 2010, Atwell et al. 2012). In burrowing owl (*Athene cunicularia*) for instance, bold individuals nest closer to the roads than shy individuals (Carrete and Tella 2010). Therefore, depending on their personality, individuals are supposed to be selective in the way they use their environment in regard to human activities or infrastructures, shy less tolerant individuals avoiding more human activities and infrastructures than bold ones (Wolf and Weissing 2012, Hotlmann et al. 2017, Sprau and Dingemanse 2017). However, to date, few studies have investigated how human activities shape breeding site spatial distribution depending on personality (Martin and Réale 2008, Carrete and Tella 2010, Hotlmann et al. 2017, Sprau and Dingemanse 2017) and particularly, the consequences on their reproductive success (Zhao et al. 2016) while this may represent a major component to understand population decline and thus provide new insights for population management (McDougall et al. 2006, Smith and Blumstein 2013).

The Montagu's harrier (*Circus pygargus*) is a migratory species that lives in arable land habitat during breeding period and nests on the ground in cereal crops making it vulnerable to human activities (including agricultural practices, activities around village, recreative activities, traffic noise; see the synthesis by Arroyo et al. 2004). This raptor species is semi-colonial, pairs nest either isolated or in loose colonies depending on years and areas (maximum aggregation ca. 500m on our study site, Cornulier and Bretagnolle 2006). Life close to conspecifics could allow higher antipredator defence (Arroyo et al. 2001). Thus, semi-coloniality could allow nesting in higher predation risk areas, thus the number of conspecific nests could influence nest site selection. Egg-laying in the study area ranges from the end of April to June. This difference in egg-laying date possibly results from differences in migratory pattern and could also affect nest site selection depending on the availability of suitable breeding sites. Links between nest site selection and reproductive timing could differ according to the individual boldness. A long-term study highlighted differences in boldness in

Montagu's harrier females within the population and the consequences on their reproductive success (Arroyo et al. 2017). In the present study, we investigated i/ if human infrastructures shaped nest spatial distribution in the landscape depending on Montagu's harrier female boldness, and ii/ if the nest distribution related to infrastructures may influence the reproductive success of females depending on their boldness. The study was carried out in an intensive farmland and was based on a long-term data set from Arroyo et al. (2017) in order to continue with a spatial analysis (from 1995 to 2013). We hypothesized that nest site selection may depend on the proximity and density of infrastructures in the landscape and on female boldness. In addition, we expected that egg-laying date and the presence of conspecific nests could also impact nest site selection. More precisely, we expected shy females to nest in landscapes with low infrastructure density and far from them. However, this could be modulated by egg-laying date which may be linked to habitat availability and phenology. Late egg-laying date may result in less favourable site availability and may constrain to settle in more disturbed landscapes. Also, conspecific presence in the landscape may interact for nest site selection through the security they provide against predation, making less favourable habitats after all suitable for nesting. Lastly, we expected that the proximity and density of infrastructures around nest site have a negative effect on the reproductive success, depending on female boldness. Shy females nesting in sites with lower infrastructure density should have a better reproductive success than shy females nesting in site with higher infrastructure density. For nests settled in site with high infrastructure density around them, shy females should have a lower reproductive success than bold females.

Material & methods

Study site and model species

The study was conducted in the LTSER (Long-Term Socio-Ecological Research) Zone Atelier Plaine et Val de Sèvre (ZAPVS, western France, 46° 110 N, 0° 280 W), an intensive agricultural area mostly dedicated to cereal crop production. ZAPVS covers ca. 435 km² of farmland (Bretagnolle et al. 2018) where land-use in each crop field as well as human infrastructures

such as villages, industrial and agricultural buildings, motorway (one crossed the study site), roads and paths have been monitored since 1995, and mapped onto a Geographical Information System (QUANTUMGIS 2.18) (QGIS Development Team, 2017).

Montagu's harrier (*Circus pygargus*) has been exhaustively monitored since 1995 in the ZAPVS (Bretagnolle et al. 2018), each nest being geo-localized using a Global Positioning System receiver (x, y coordinates) and surveyed over the whole reproductive season from late April (earliest egg-laying) to the end of July (incubation duration: 29 days on average and nestling rearing duration: 30-35 days) (García and Arroyo 2001, Arroyo et al. 2007). Common vole abundance (*Microtus arvalis*), the main prey of this raptor, has been surveyed through its abundance fluctuations among years (Bretagnolle et al. 2018). Common vole abundance has been shown to influence the number of Montagu's harrier breeders and their reproductive success (Millon and Bretagnolle 2008, Arroyo et al. 2017). During years of high abundance in common voles, up to 100 pairs of Montagu's harriers can nest in the ZAPVS (Millon and Bretagnolle 2008).

Montagu's harrier female boldness and reproduction monitoring

Once nests were localized, they were visited by experimenters almost twice during incubation and once a week during the rearing period. At each nest visit, the fleeing probability of the female was recorded: 1 = the female stayed around the nest during the visit or 0 = disappeared from sight. The male presence (0 = absent; 1 = present) during each nest visit was also recorded and the visit date transformed, thereafter, into an estimate of nesting stage (relative to the laying onset from each female = day 0). The fleeing probability allowed estimating the female boldness (estimated by Arroyo et al. 2017). Female boldness was estimated from a generalized linear mixed-effects model fitted to a binomial-error distribution with a logit link function (BGLMM) analysing the fleeing probability in relation to the nesting stage estimate (its quadratic term) and the male presence, and including nest identity as random effect. Female boldness represented random intercept extracted from the BGLMM. Boldness was categorized into two groups: bold females with a low fleeing probability = 0; shy females with a high fleeing probability = 1.

During incubation, nest visits allowed recording the final clutch size and measuring eggs. Egg length and width were measured using a calliper (accuracy: ± 0.1 mm) and egg mass using Pesola® electronic scale (accuracy: ± 0.1 g) in order to estimate the laying date following Arroyo et al. (2017). At the end of the rearing period, a last visit has been done to check fledging success. The reproductive success was characterized by the proportion: number of fledglings / clutch size. Two factors, the relative laying date and the visit frequency, from Arroyo et al. (2017), were used in the present study because they are known to influence the reproductive success. The relative laying date represented for a given nest, the difference between its laying date and the mean laying date from all nests for that particular year. Negative values including zero characterized early reproductive females and positive values characterized late reproductive ones. For each nest, the visit frequency represented the number of visits divided by total length of monitoring.

Vole abundance

In April and June, live-trapping was conducted in five different crop types in order to estimate the vole abundance (vole captures/100 traps) (Arroyo et al. 2017, Bretagnolle et al. 2018). The annual vole abundance from Arroyo et al. (2017) was used in the present study. Each trapping session (April and June) represented the average abundance from the different crop types and then the annual vole abundance was the average from the two trapping seasons per year at the scale of the whole ZAPVS (Barraquand et al. 2014, Arroyo et al. 2017).

Nest distribution in the landscape

Three different human infrastructure types were considered to study their effect on nest location in the landscape: buildings whatever their use (i.e., habitations, industrial and agricultural buildings), roads (motorway, national, departmental and communal roads) and paths (agricultural and bike paths). In our GIS database, buildings were quantified by their surface while linear elements (roads and paths) were quantified by their length. Then, two categories of metrics were extracted from the GIS database for each focus nest. First, the distance from the nest to the nearest infrastructure of each type (building, road, path) was

calculated: $Dist_{build}$, $Dist_{road}$, $Dist_{path}$. Secondly, to calculate the density of each infrastructure type around nest, we decided to take twice the median of $Dist_{build}$ (median = 530 m) to define the buffer size, thus we chose a 1000 m radius buffer. We calculated for each 1000 m radius from nest, the density of its surface covered with buildings, thereafter named $Dens_{build}$. For roads and paths, we calculated their density in the 1000 m radius landscapes as their length per km^2 : $Dens_{road}$ and $Dens_{path}$. Following Cornulier and Bretagnolle (2006), the maximum aggregation value for Montagu's harrier nests in our study site was ca. 500 m. Therefore, we extracted the number of conspecific nests within 500 m radius landscape (including nests without information on female boldness) for each nest from the GIS database: named thereafter $N_{neighbour}$.

Landscape metrics were calculated using R software (v. 3.5.1, R Development Core Team 2018) and 'rgdal', 'rgeos' and 'raster' packages (Bivand et al. 2015, 2019, Hijmans et al. 2015, respectively).

Statistical analyses

The effect of human infrastructures on nest distribution were characterized by modelling i/ the distance from the nest to the nearest infrastructure and ii/ the infrastructure density within 1000 m radius landscapes around each nest, in both cases, depending on female boldness and other factors which may interact. These analyses were performed for 791 nests. Then, the consequences of infrastructure proximity and density on the reproductive success of females were investigated depending on female boldness and were assessed for 753 nests, because information on reproductive success was not available for 38 nests.

Nest distribution: effects of human infrastructures and female boldness

Linear mixed-effects models (LMMs) were used to model the distances from the nests to the nearest different infrastructures ($Dist_{build}$, $Dist_{road}$, $Dist_{path}$), and the density of the different infrastructures in the landscapes around the nests ($Dens_{build}$, $Dens_{road}$, $Dens_{path}$; using

'lmerTest' package, Kuznetsova et al. 2014). $Dist_{road}$ and $Dist_{path}$ were log-transformed and $Dens_{build}$ was square root transformed to meet normality and homoscedasticity assumptions. Thus, six separate models were tested including female boldness, $N_{neighbour}$, the relative laying date and their interactions as fixed effects. The relative laying date and $N_{neighbour}$ were centred and scaled and we checked that fixed effects were not correlated. There was no collinearity between fixed effects as the proportion of the variance in the dependent variable that is explained by the independent variable (R^2) was low: $R^2 = 25\%$ for boldness explained by relative laying date; $R^2 = 22\%$ for boldness explained by $N_{neighbour}$; $R^2 = 3\%$ for relative laying date explained by $N_{neighbour}$.

Reproductive success

The reproductive success (number of fledglings / clutch size) of females was assessed in six separate BGLMMs, each testing the effect of the six calculated metrics characterizing nest site features ($Dist_{build}$, $Dist_{road}$, $Dist_{path}$, $Dens_{build}$, $Dens_{road}$ and $Dens_{path}$). Female boldness, relative laying date and vole abundance in the ZAPVS were included as fixed effects in the models. In addition, the visit frequency was included in the models to take into account for additional human disturbance due to research activities. Two interaction terms were added in the models: the interaction between female boldness and human infrastructure metrics and the interaction between female boldness and visit frequency. Each continuous variable was centred and scaled. Not all females were tagged so it is almost impossible to control for female identity. Therefore, we choose to include the year as random effect in all LMMs and BGLMMs. For all models (LMMs and BGLMMs), the statistical significance of each parameter was assessed by model comparison with likelihood ratio-based χ^2 -statistics (Fox and Weisberg 2011). Significant effects of the interactions were then tested using post hoc tests based on least-squares means associated to Benjamini–Hochberg's correction for multiple comparisons to control for false discovery rate (using 'multcomp' and 'lsmeans' packages, Hothorn et al. 2008, Lenth 2016, respectively, Benjamini and Hochberg 1995). If an interaction was not significant, it was excluded from the model.

Statistics were performed with R software (v. 3.5.1, R Development Core Team 2018).

Results

Nest distribution: effects of human infrastructures and female boldness

A summary of the infrastructure metrics depending on female boldness are given in Table 1. The $Dist_{build}$ only varied depending on the $N_{neighbour}$ (Table 2). It increased with increasing $N_{neighbour}$ (Fig. 1). The $Dist_{build}$ was not explained by female boldness nor the relative laying date. The $Dist_{road}$, was only explained by $N_{neighbour}$ (Table 2). It decreased with increasing $N_{neighbour}$ (Table 2; Supplementary material Appendix 1 Table A1). No other factor impacted $Dist_{road}$ (Table 2). The $Dist_{path}$ increased with increasing relative laying date (Table 2; Fig. 2). The $Dist_{path}$ did not vary depending on female boldness nor $N_{neighbour}$ (Table 2). The $Dens_{build}$, was lower around nests from shy females than around nests from bold females (Table 2; Fig. 3). No other factor impacted $Dens_{build}$ (Table 2). The $Dens_{path}$ tended to decrease with increasing relative laying date (Table 2; Supplementary material Appendix 1 Table A1). Female boldness and the relative laying date did not influence the $Dens_{path}$ (Table 2). Female boldness, relative laying date, $N_{neighbour}$ and their interactions did not impact $Dens_{road}$ (Table 2).

Table 1. Mean and [95% confidence interval] of $Dist_{infrastructure}$ (meters) and $Dens_{infrastructure}$ metrics depending on female boldness (N = 590 bold females and 201 shy females).

Infrastructure metrics	Bold	Shy
$Dist_{build}$	560.35 [540.37; 580.34]	598.38 [556.37; 640.39]
$Dist_{road}$	176.93 [166.43; 187.42]	179.57 [160.83; 198.31]
$Dist_{path}$	204.68 [192.76; 216.60]	222.15 [201.06; 243.25]
$Dens_{build}$	0.0044 [0.0040; 0.0049]	0.0038 [0.0030; 0.0045]
$Dens_{road}$	0.0193 [0.019; 0.020]	0.0188 [0.0176; 0.020]
$Dens_{path}$	0.0192 [0.0186; 0.0197]	0.0187 [0.0176; 0.0197]

Table 2. Summary of LMMs testing the effects of the boldness, the relative laying date, the $N_{\text{neighbour}}$ and its interaction terms on the $\text{Dist}_{\text{infrastructure}}$ and the $\text{Dens}_{\text{infrastructure}}$ metrics around the nest with random intercept on years. The model comparison with likelihood ratio-based χ^2 -statistics allowed to test the significance of each explanatory variable (including statistic values, degrees of freedom and p-values). Significant effects are in bold. If the interaction terms were significant, simple effects cannot be tested and if they were not significant, they were excluded from the model.

	Explanatory variables	χ_1^2	<i>P</i>
<i>Dist_{build}</i>			
	Boldness	1.67	0.20
	Relative laying date	0.02	0.90
	$N_{\text{neighbour}}$	15.29	<0.0001
<i>Dist_{road}^a</i>			
	Boldness	0.72	0.40
	Relative laying date	0.96	0.33
	$N_{\text{neighbour}}$	7.79	<0.01
<i>Dist_{path}^a</i>			
	Boldness	0.73	0.39
	Relative laying date	5.91	0.02
	$N_{\text{neighbour}}$	2.04	0.15
<i>Dens_{build}^b</i>			
	Boldness	5.43	0.02
	Relative laying date	1.16	0.28
	$N_{\text{neighbour}}$	0.03	0.86
<i>Dens_{road}</i>			
	Boldness	0.87	0.35
	Relative laying date	0.66	0.42
	$N_{\text{neighbour}}$	1.46	0.23
<i>Dens_{path}</i>			
	Boldness	0.03	0.86
	Relative laying date	3.89	0.05
	$N_{\text{neighbour}}$	2.28	0.13

^a log transformation; ^b square root transformation

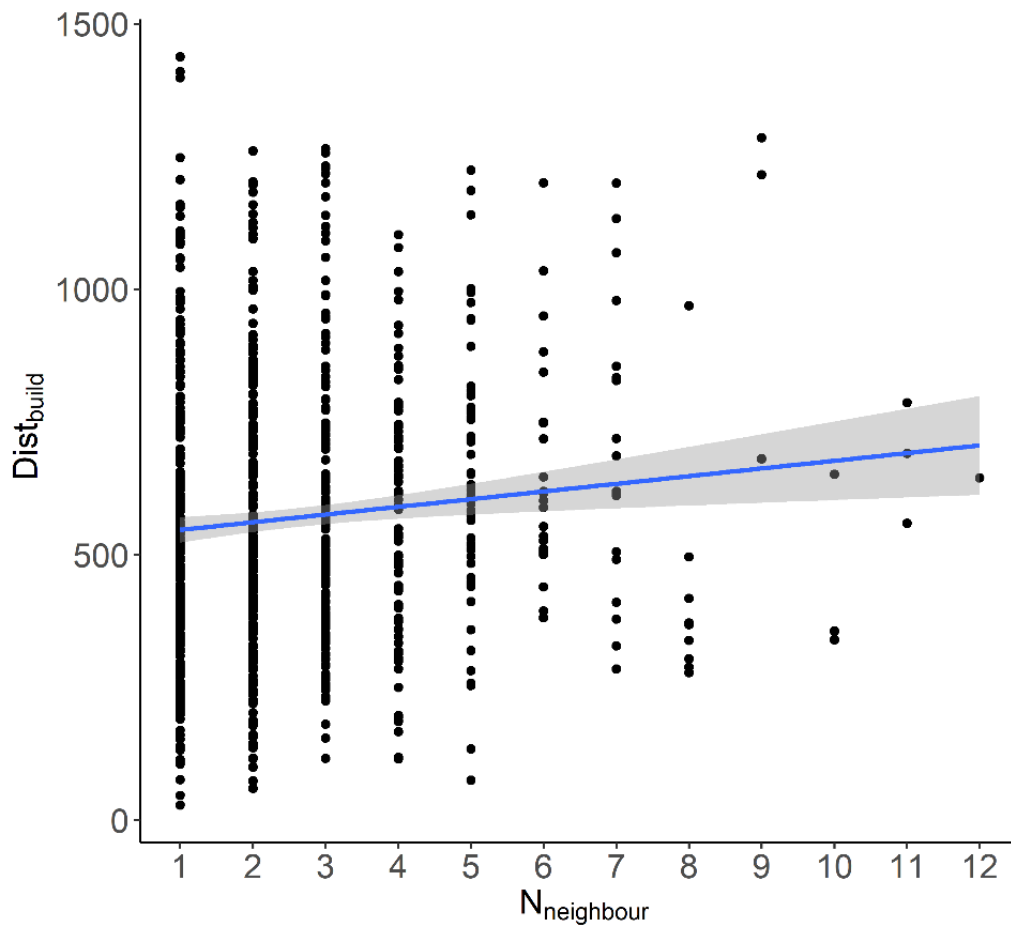


Figure 1. Effects of the $N_{\text{neighbour}}$ within 500m radius around nest on the $\text{Dist}_{\text{build}}$ (meters). Points represented the observed values of $\text{Dist}_{\text{build}}$ depending on the $N_{\text{neighbour}}$. Blue line represented predicted $\text{Dist}_{\text{build}}$ depending on the $N_{\text{neighbour}}$ and shading represented 95% confidence interval.

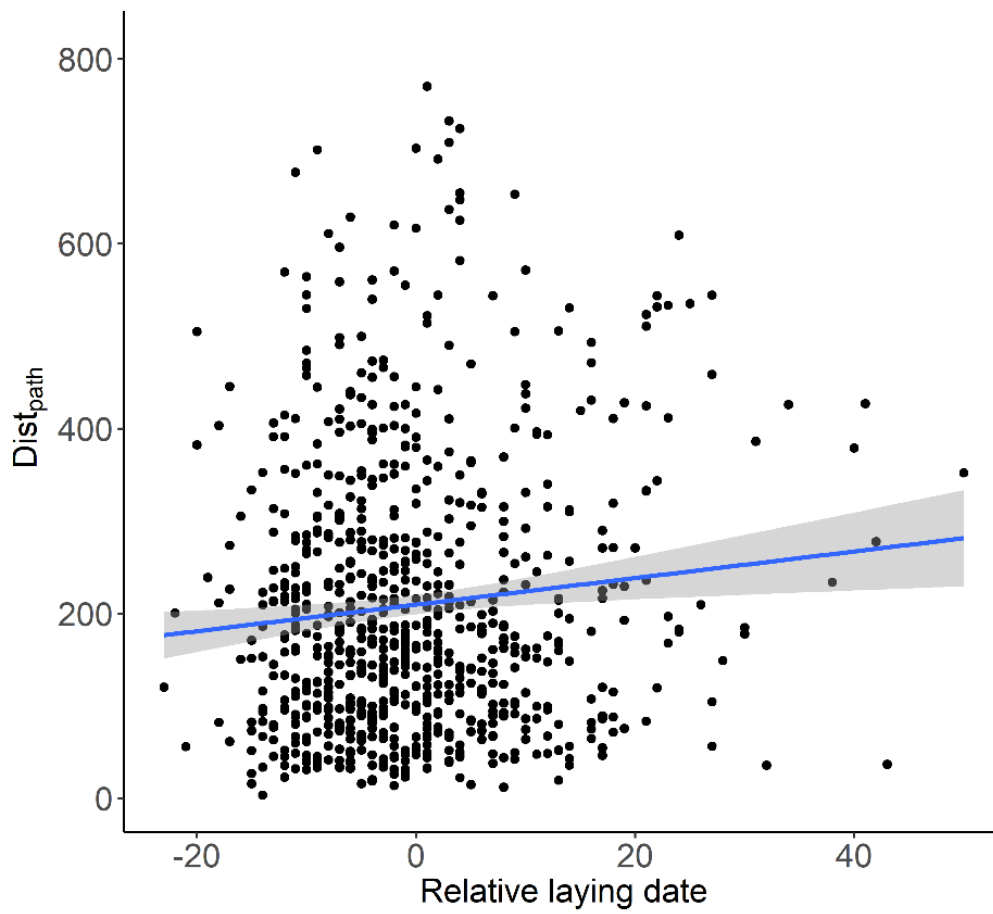


Figure 2. Effects of the relative laying date on the $\text{Dist}_{\text{path}}$ (meters). Points represented the observed values of $\text{Dist}_{\text{path}}$ depending on the relative laying date. Blue line represented predicted $\text{Dist}_{\text{path}}$ depending on the relative laying date and shading represented 95% confidence interval.

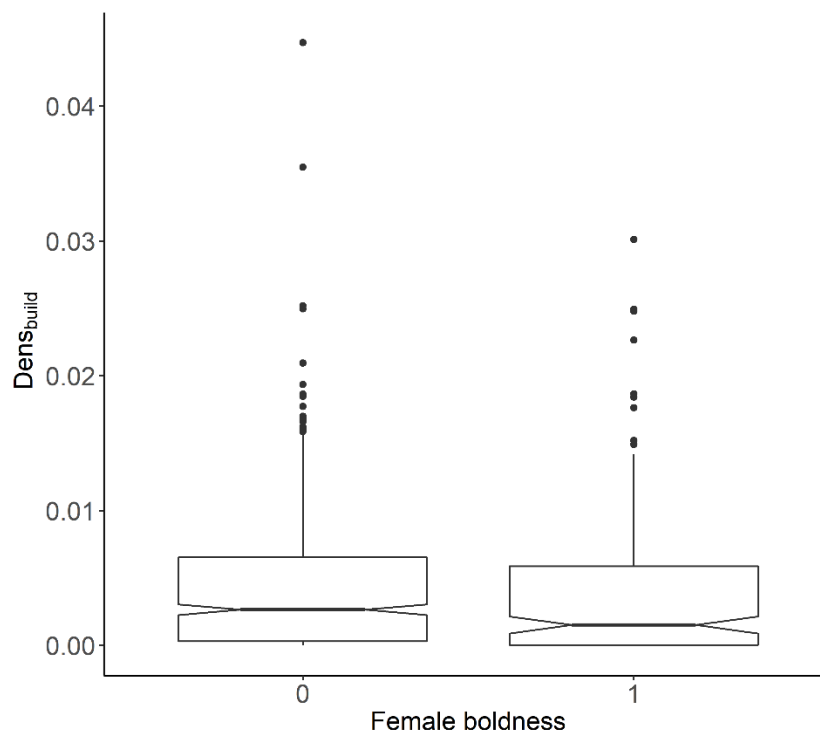


Figure 3. $Dens_{build}$ around nests depending on female boldness (0 = bold; 1 = shy). Box plot represented the median (heavy lines within boxes), 25% and 75% quartiles (boxes), 1.5 times the interquartile range (whiskers) and data points outside the whiskers, for the $Dens_{build}$ depending on female boldness.

Reproductive success: effects of human infrastructures and female boldness

The mean reproductive success was 0.47 (95%CI = [0.44; 0.50]) ranging from 0.13 in 2003 to 0.79 in 2002. The reproductive success was lower for both shy and bold females with increasing path density, $Dens_{path}$ (Table 3; Fig. 4). Other human infrastructures in the landscapes surrounding the nests did not have any effect on the reproductive success (Table 3). The reproductive success was lower with increasing visit frequency and this relationship was more marked for shy females (Table 3; Supplementary material Appendix 1 Table A2). The relative laying date influenced significantly the reproductive success: females that reproduced later in the season had a lower reproductive success than early reproductive females (Table 3; Supplementary material Appendix 1 Table A2). The reproductive success tended to be

higher with increasing vole abundance ($P = 0.06$) (Table 3; Supplementary material Appendix 1 Table A2).

Table 3. Summary of BGLMMs testing the effects of the boldness and its interactions with the $Dist_{\text{infrastructure}}$ and the $Dens_{\text{infrastructure}}$ metrics, the vole abundance, the relative laying date, the interaction between visit frequency and boldness, and all single effects on the reproductive success with random intercept on years. The model comparison with likelihood ratio-based χ^2 -statistics allowed to test the significance of each explanatory variable (including statistic values, degrees of freedom and p-values). Significant effects are in bold. If the interaction terms were significant, simple effects cannot be tested and if they were not significant, they were excluded from the model.

	Explanatory variables	χ_1^2	P
<i>Model $Dist_{\text{build}}$</i>			
	Relative laying date	31.29	<0.0001
	Vole abundance	3.50	0.06
	Visit frequency	37.52	<0.0001
	$Dist_{\text{build}}$	0.71	0.40
	Boldness	1.58	0.21
	Boldness x Visit frequency	14.44	<0.001
<i>Model $Dist_{\text{road}}$</i>			
	Relative laying date	31.46	<0.0001
	Vole abundance	3.52	0.06
	Visit frequency	36.90	<0.0001
	$Dist_{\text{road}}$	2.63	0.10
	Boldness	1.31	0.25
	Boldness x Visit frequency	15.07	<0.001
<i>Model $Dist_{\text{path}}$</i>			
	Relative laying date	32.48	<0.0001
	Vole abundance	3.53	0.06
	Visit frequency	36.60	<0.0001
	$Dist_{\text{path}}$	3.15	0.08
	Boldness	1.68	0.20

	Boldness x Visit frequency	14.65	<0.001
<i>Model Dens_{build}</i>			
	Relative laying date	31.27	<0.0001
	Vole abundance	3.50	0.06
	Visit frequency	37.36	<0.0001
	Dens _{build}	0.21	0.64
	Boldness	1.55	0.21
	Boldness x Visit frequency	14.75	<0.001
<i>Model Dens_{road}</i>			
	Relative laying date	32.01	<0.0001
	Vole abundance	3.51	0.06
	Visit frequency	36.29	<0.0001
	Dens _{road}	2.27	0.51
	Boldness	1.42	0.22
	Boldness x Visit frequency	14.78	<0.001
<i>Model Dens_{path}</i>			
	Relative laying date	32.36	<0.0001
	Vole abundance	3.57	0.06
	Visit frequency	36.02	<0.0001
	Dens_{path}	5.51	0.02
	Boldness	1.69	0.19
	Boldness x Visit frequency	15.43	<0.0001

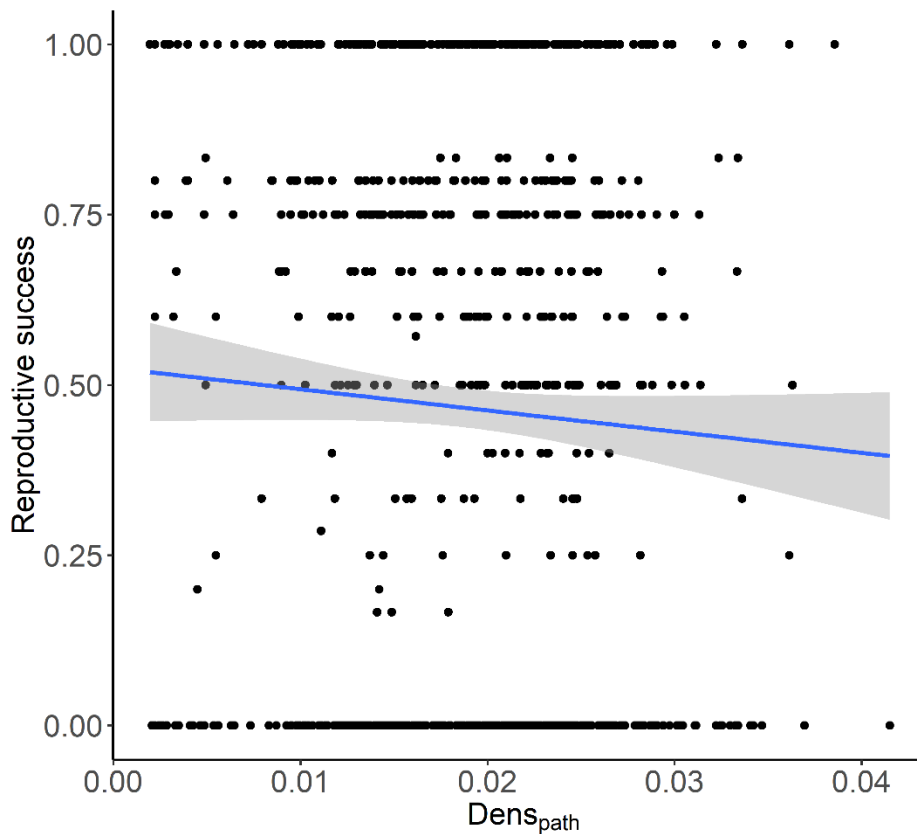


Figure 4. Effects of the $Dens_{path}$ (km/km^2) around the nest on the reproductive success (fledglings / clutch size proportion). Points represented the observed values of reproductive success for each female depending on $Dens_{path}$. Blue line represented predicted reproductive success depending on $Dens_{path}$ and shading represented 95% confidence interval.

Discussion

In this study, the spatial distribution of Montagu's harrier nests according to human infrastructures was examined depending on female boldness, their timing in reproduction and the number of conspecific neighbours surrounding the nest. Buildings shaped nest distribution depending on female boldness, building density being lower around shy female nests than bold female nests. The distance from the nest to the nearest building increased with increasing number of conspecifics around the nest. Later reproductive females nested further from paths than early females. Path density in the landscape around the nest had a negative effect on the reproductive success regardless of female boldness. The relative laying date, the vole

abundance and the interaction between the female boldness and the visit frequency also influenced the reproductive success in the same way as shown in Arroyo et al. (2017).

A different distribution of boldness types in the landscape

Montagu's harrier nests were distributed in the ZAPVS according to female boldness and the building density in the landscape surrounding nests. Shy females seemed to avoid more buildings than bold females, since the building density around their nest was lower. A first explanation could be that the different distribution of boldness types according to infrastructures could be the result of a habituation process, as shown in king penguin (*Aptenodytes patagonicus*, Viblanc et al. 2012, see the synthesis by Blumstein 2016). Females nesting in area with higher building density could habituate to human disturbance through the season and become increasingly bold. However, boldness was measured once they began nesting, thus few times after their return from wintering sites, leaving little time to habituate. Moreover, females did not show a habituation in their fleeing probability (boldness estimate) over the breeding season (Arroyo et al. 2017). Habituation process was a less evident hypothesis than the personality-matching habitat choice hypothesis as suggested by other studies (Edelaar et al. 2008, Carrete and Tella 2010, 2011, Cote et al. 2010, Jacob et al. 2015, Holtmann et al. 2017, Sprau and Dingemanse 2017). Consequently, in regard to their own personality, Montagu's harrier females could choose their nest site depending on the building density. Buildings involve human frequentation and anthropogenic noise (McKinney 2002, Barber et al. 2010) and shy females could be less tolerant to this disturbance. Our results are in accordance with another study in which shy dunnocks (*Prunella modularis*) nested in areas with lower human frequentation (Carrete and Tella 2010, Holtmann et al. 2017). In addition, Montagu's harrier colonies seemed to be established further from buildings since the distance from the nest to the nearest building increased with increasing number of conspecifics around nests. There is a general trend in Montagu's harrier population to avoid villages probably resulting from old intense persecution of raptors and/or from the need to have sufficient feeding areas around nest (Cornulier and Bretagnolle 2006). Finally, female boldness did not impact nest distribution according to roads and paths. Roads involve less human frequentation but car traffic and paths do not systematically involve human frequentation or

more ephemeral (agricultural vehicles, walkers, joggers, cyclists). Paths may involve less human frequentation when most females chose their nest site (April-May) than later in the breeding season. It could explain variations in the distance from the nest to the nearest path depending on the relative laying date. The increase of human frequentation on paths later in the season could be used as indices by later reproductive females through their nest site selection process. Therefore, later females avoid paths. Anthropogenic indices used in the nest site selection should be directly measurable. Not continuous or ephemeral disturbance could be more difficult to measure and to integrate into the nest site selection process.

Consequences of human infrastructures and female boldness on reproductive success

Buildings that influenced the nest site selection in Montagu's harrier did not affect the reproductive success. The hypothesis of personality-matching habitat choice suggests that individuals select suitable habitat for their personality (Chalfoun and Schmidt 2012, Holtmann et al. 2017). It involves a decrease in individual stress and a reduction of fitness costs (Holtmann et al. 2017). Through a nest site selection related to buildings, the effects of building density on reproductive success could be limited. However, the path density affected negatively the reproductive success of Montagu's harrier. Two hypotheses may explain this result. The first explanation could be that nests with higher path density around them could be more exposed to predation because predators may follow paths through their movements, resulting in lower reproductive success. Another explanation could be that paths may involve human frequentation by recreative (e.g. walkers with or without dog, joggers, cyclists) and agricultural activities that were maybe more frequent and intense in June and July during the rearing period. Agricultural and/or recreative activities on paths could cause a disturbance and stress for Montagu's harriers. In addition, harvesting is increasingly earlier in the season and a majority of chicks are not fledged before harvesting. Nests are protected and almost 4 m² of crop around nest are not harvested. Consequently, nests become more visible from paths after harvesting and human disturbance may increase. Stressed females could spend more time fleeing, flying or being vigilant and decrease their food provisioning and/or nest protection against adverse weather and predation under human disturbance, as shown in marsh harrier and bearded vulture (Fernández and Azkona 1993, Arroyo and Razin 2006).

Consequently, offspring could be more prone to starvation and/or predation thus resulting in lower reproductive success. Next investigations are needed to identify the disturbance nature related to paths in order to limit them.

Evolutionary implications for the population

The different distribution of boldness types caused by buildings may have evolutionary implications for the Montagu's harrier population on the ZAPVS. In a previous study, a positive assortative pairing according to boldness was highlighted in the same population (Chapter II). However, no fitness benefit was detected from this pairing pattern suggesting that this pattern probably results from ecological constraints rather than sexual selection. Similarly to Spanish imperial eagles, (*Aquila adalberti*) which show an age-dependent spatial distribution leading to a positive age-assortative pairing (Ferrer and Penteriani 2003), the distribution of Montagu's harrier females depending on their boldness may partly explain the boldness-dependant assortative pairing. In addition, the Montagu's harrier population could display different migratory patterns among individuals explaining variations in egg-laying date and they could be linked to individual boldness, as shown in blue tits *Cyanistes caeruleus* (Nilsson et al. 2010). Differences in migratory patterns related to boldness could involve boldness-dependant temporal distribution and therefore, it may also contribute to the pairing pattern. Next investigations are needed to precise the effects of human infrastructures and activities on the population structure and explore the hypothesis of boldness-dependant temporal distribution. Finally, Montagu's harrier is a protected species and its populations are decreasing in France. Therefore, the negative effects of path density around nests on the reproductive success are matter of concern for the conservation of this species. The disturbance nature from paths should be identified using focal observations and cameras, and new conservation measures could be established. Human frequentation on paths could be sensitised using regulatory signs respecting wildlife and positioned along paths. These conservation measures could be concentrated on Montagu's harrier colonies and in the Special Protected Area (Natura 2000) on our study site, where farmers are sensitive to agro-environmental measures. New agro-environmental measures could include a delay in harvesting until the fledging of chicks as already suggested by Arroyo et al. (2002). These new

conservation measures could reduce the negative effect of path density on reproductive success in Montagu's harrier and also benefit to a large number of species nesting in crops.

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Electronic supplementary material

Appendix 1

Table A1. Summary of LMMs testing the effects of the boldness, the relative laying date, the $N_{\text{neighbour}}$ and its interaction terms on the $\text{Dist}_{\text{infrastructure}}$ and the $\text{Dens}_{\text{infrastructure}}$ around the nest with random intercept on years, including the parameter estimate \pm standard error (s.e.), statistic values and degrees of freedom. Significant effects are in bold. If the interaction terms were significant, simple effects cannot be tested and if they were not significant, they were excluded from the model.

Explanatory variables	Parameter estimate \pm s.e.	df	<i>t</i>	<i>P</i>
<i>Dist_{build}</i>				
Intercept	572.87 \pm 16.56	16.14	34.60	<0.0001
Shy	28.63 \pm 22.15	747.77	1.29	0.20
Relative laying date	-1.14 \pm 9.29	772.49	-0.12	0.90
$N_{\text{neighbour}}$	39.16 \pm 10.01	701.05	3.91	<0.001
<i>Dist_{road}^a</i>				
Intercept	4.92 \pm 0.03	21.03	151.82	<0.0001
Shy	0.04 \pm 0.06	558.51	0.63	0.53
Relative laying date	-0.03 \pm 0.03	778.70	-0.98	0.33
$N_{\text{neighbour}}$	-0.07 \pm 0.03	366.48	-2.79	<0.01
<i>Dist_{path}^a</i>				
Intercept	5.0 \pm 0.06	80.13	88.59	<0.0001
Shy	0.06 \pm 0.07	655.70	0.85	0.40
Relative laying date	0.07 \pm 0.03	778.70	2.43	0.02
$N_{\text{neighbour}}$	0.02 \pm 0.02	503.0	1.43	0.15
<i>Dens_{build}^b</i>				
Intercept	0.05 \pm 0.002	17.96	22.54	<0.0001
Shy	-0.01 \pm 0.003	718.80	-2.33	0.02

	Relative laying date	0.002 ± 0.002	775.0	1.08	0.28
	N _{neighbour}	-0.0003 ± 0.002	635.40	-0.18	0.86
<i>Dens_{road}</i>					
	Intercept	0.02 ± 0.0004	21.0	54.45	<0.0001
	Shy	-0.0006 ± 0.0006	592.50	-0.93	0.35
	Relative laying date	0.0002 ± 0.0003	779.40	0.81	0.42
	N _{neighbour}	-0.0004 ± 0.0003	397.30	-1.21	0.23
<i>Dens_{path}</i>					
	Intercept	0.02 ± 0.0004	23.66	51.89	<0.0001
	Shy	-0.0001 ± 0.0006	685.40	-0.18	0.86
	Relative laying date	-0.0005 ± 0.0003	779.0	-1.97	0.05
	N _{neighbour}	-0.0004 ± 0.0003	559.30	-1.51	0.13

^a log transformation; ^b square root transformation

Table A2. Summary of GLMMs testing the effects of the boldness and its interactions with the $Dist_{\text{infrastructure}}$ and $Dens_{\text{infrastructure}}$, the vole abundance, the relative laying date, the interaction between visit frequency and boldness, and all single effects on the reproductive success with random intercept on years, including the parameter estimate \pm standard error (s.e.), statistic values and degrees of freedom. Significant effects are in bold. If the interaction terms were significant, simple effects cannot be tested and if they were not significant, they were excluded from the model.

Explanatory variables	Parameter estimate \pm s.e.	z	P
<i>Model $Dist_{\text{build}}$</i>			
Intercept	-0.03 \pm 0.14	-0.22	0.82
Relative laying date	-0.24 \pm 0.04	-5.59	<0.0001
Vole abundance	0.27 \pm 0.15	1.87	0.06
Visit frequency	-0.34 \pm 0.06	-6.13	<0.0001
$Dist_{\text{build}}$	0.03 \pm 0.04	0.85	0.40
Shy	-0.12 \pm 0.10	-1.26	0.21
Shy x Visit frequency	-0.44 \pm 0.12	-3.80	<0.001
<i>Model $Dist_{\text{road}}$</i>			
Intercept	-0.02 \pm 0.14	-0.24	0.81
Relative laying date	-0.24 \pm 0.04	-5.61	<0.0001
Vole abundance	0.27 \pm 0.15	1.88	0.06
Visit frequency	-0.34 \pm 0.06	-6.08	<0.0001
$Dist_{\text{road}}$	-0.06 \pm 0.04	-1.62	0.11
Shy	-0.11 \pm 0.10	-1.15	0.25
Shy x Visit frequency	-0.45 \pm 0.12	-3.88	<0.001
<i>Model $Dist_{\text{path}}$</i>			
Intercept	-0.03 \pm 0.14	-0.20	0.85
Relative laying date	-0.24 \pm 0.04	-5.70	<0.0001
Vole abundance	0.28 \pm 0.15	1.88	0.06
Visit frequency	-0.34 \pm 0.06	-6.05	<0.0001
$Dist_{\text{path}}$	0.07 \pm 0.04	1.78	0.08
Shy	-0.13 \pm 0.10	-1.30	0.20

	Shy x Visit frequency	-0.45 ± 0.12	-3.83	<0.001
<i>Model Dens_{build}</i>				
	Intercept	-0.03 ± 0.14	-0.22	0.83
	Relative laying date	-0.24 ± 0.04	-5.59	<0.0001
	Vole abundance	0.27 ± 0.15	1.87	0.06
	Visit frequency	-0.34 ± 0.06	-6.11	<0.0001
	Dens _{build}	-0.02 ± 0.04	-0.46	0.65
	Shy	-0.12 ± 0.10	-1.24	0.21
	Shy x Visit frequency	-0.45 ± 0.12	-3.84	<0.001
<i>Model Dens_{road}</i>				
	Intercept	-0.03 ± 0.14	-0.21	0.83
	Relative laying date	-0.24 ± 0.04	-5.66	<0.0001
	Vole abundance	0.27 ± 0.15	1.87	0.06
	Visit frequency	-0.34 ± 0.06	-6.02	<0.0001
	Dens _{road}	0.07 ± 0.05	1.51	0.51
	Shy	-0.45 ± 0.12	-1.19	0.22
	Shy x Visit frequency	-0.45 ± 0.12	-3.85	<0.001
<i>Model Dens_{path}</i>				
	Intercept	-0.02 ± 0.13	-0.17	0.87
	Relative laying date	-0.24 ± 0.04	-5.69	<0.0001
	Vole abundance	0.27 ± 0.14	1.89	0.06
	Visit frequency	-0.36 ± 0.06	-6.0	<0.0001
	Dens_{path}	-0.10 ± 0.04	-2.35	0.02
	Shy	-0.13 ± 0.10	-1.30	0.19
	Shy x Visit frequency	-0.46 ± 0.12	-3.93	<0.0001

Discussion générale et perspectives



Au cours de mes travaux de thèse, j'ai examiné les effets du dérangement anthropique sur le comportement, la physiologie et les traits d'histoire de vie d'une espèce patrimoniale, le busard cendré. Ces effets ont été évalués en conditions naturelles lors de manipulations et expositions successives à l'Homme et en examinant les différences comportementales inter-individuelles ainsi que leurs implications écologiques et évolutives dans la population, en réponse à la présence d'infrastructures et activités humaines. La discussion générale de mon travail rappellera dans un premier temps les principaux résultats acquis, puis discutera de notre contribution à la compréhension des impacts anthropiques sur les animaux, pour finalement proposer des pistes de réflexions permettant d'améliorer le suivi et la gestion des populations sauvages.

Synthèse des principaux résultats

Grâce à des approches expérimentales et corrélationnelles, plusieurs effets du dérangement anthropique sur le comportement, la physiologie et le succès reproducteur des busards cendrés ont été établis et des implications écologiques et évolutives des différences inter-individuelles ont été mises en évidence. Au cours des visites successives des nids, les tentatives de fuite, l'agressivité et les concentrations de corticostérone en stress-induit des poussins augmentaient pour les deux groupes de nids (visités par les mêmes ou différents expérimentateurs). Alors que nous prédisions un phénomène d'habituation pour les poussins visités et manipulés par les mêmes expérimentateurs, nous avons observé à l'inverse une sensibilisation des poussins. Nous avons interprété cette différence entre les deux groupes comme étant associée à une prise de sang. Les poussins associeraient cet événement aversif aux expérimentateurs et seraient sensibilisés au cours des visites. Cette sensibilisation des poussins durant la période de développement post-natal pourrait avoir des conséquences sur leur physiologie mais également l'expression de leur phénotype à l'âge adulte et notamment leur témérité. Ce trait de personnalité et sa plasticité semblent, par ailleurs, être impliqués dans le fonctionnement de la population de busard cendré. En effet, les couples de busard cendré étaient appariés positivement pour leur témérité et leur plasticité comportementale. Les indices calculés de similarité et de type de témérité et plasticité des couples semblent être liés aux stratégies parentales d'approvisionnement en proies. Les couples moins similaires

dans la plasticité délivraient une plus grande proportion de micromammifères et de biomasse en proies que les couples les plus similaires. Ce meilleur approvisionnement pourrait expliquer un meilleur succès reproducteur pour ces couples moins similaires. La plus grande proportion d'insectes délivrée par les couples moins plastiques semblerait procurer de meilleures concentrations en caroténoïdes aux poussins par rapport à ceux issus des couples plus plastiques. Cependant, le patron d'appariement ne semblait pas procurer plus d'avantages sur leur succès reproducteur et la condition corporelle et physiologique des poussins pour les partenaires les plus similaires dans la témérité et la plasticité. Ceci pose question sur l'origine du patron d'appariement qui pourrait ne pas être uniquement le résultat d'un processus de sélection sexuelle mais également d'un processus écologique comme une disponibilité spatiale et temporelle différente des individus en fonction de leur témérité. Des différences dans la répartition spatiale des nids de busard cendré par rapport au bâti et en fonction de la témérité des femelles ont, par ailleurs, été mises en évidence grâce aux données collectées sur le long terme (1995-2013). Les femelles timides semblaient s'éloigner plus du bâti que les femelles téméraires en nichant sur des sites avec une plus faible densité de bâtis. Enfin, la densité de chemins autour des nids impactait négativement le succès reproducteur, indépendamment de la personnalité des femelles. La fréquentation humaine que peuvent induire les chemins, pourrait créer un dérangement et expliquer ce succès reproducteur réduit. Ces effets pourraient à long terme affecter la dynamique de la population de busard cendré. Ainsi, l'ensemble des résultats obtenus permettent de mettre en évidence différents niveaux d'implication du comportement en réponse aux perturbations anthropiques notamment en termes de gestion des populations sauvages dans un but de conservation mais aussi de recherche.

Impacts du dérangement anthropique

L'ensemble de mes travaux a révélé des effets à la fois directs et indirects des activités et infrastructures humaines sur le comportement, la physiologie et les traits d'histoire de vie des busards cendrés. Ces effets peuvent avoir à plus ou moins long terme, des conséquences sur le phénotype des individus, sur la dynamique des populations directement en affectant le

succès reproducteur ou de manière indirecte en influençant la structure et la composition phénotypique des populations.

Le dérangement anthropique peut impacter directement l'intensité des réponses comportementales et physiologiques des animaux et entraîner un phénomène de sensibilisation. Les captures et manipulations successives réalisées par les mêmes expérimentateurs peuvent conduire à ces effets comme nous l'avons montré chez les poussins de busard cendré durant le suivi des nids. Une exposition directe et répétée des animaux à l'Homme se produit souvent dans les activités de recherche et de conservation et ce sur une grande diversité d'espèces mais également de taxons (Romero & Wikelski 2002, van Oers & Carere 2007, Martin & Réale 2008, Almasi et al. 2015). Ce phénomène de sensibilisation peut ainsi s'avérer fréquent et peut non seulement être associé à un stress à court terme pour les animaux, mais pourrait également affecter leur phénotype, notamment leur personnalité à l'âge adulte. En effet, les traits de personnalité sont à la fois déterminés génétiquement et mais aussi influencés par l'environnement dans lequel évolue l'individu (Stamps & Groothuis 2010, van Oers & Sinn 2011). Des facteurs environnementaux intervenant pendant les phases pré- et post-natales peuvent donc affecter le phénotype des individus à l'âge adulte (Carere et al. 2005, Roulin et al. 2010, Naguib et al. 2011, Boogert et al. 2014, van Oers et al. 2015). Une exposition à un stress pendant le développement aurait des effets à long terme sur le comportement des individus (Spencer & Verhulst 2007, Schoech et al. 2011, Zimmer et al. 2013). Par exemple, des diamants mandarins (*Taeniopygia guttata*) ayant reçu une dose de corticostérone pendant la phase post-natale, avaient une néophobie (i.e., la peur de la nouveauté) réduite par rapport aux individus contrôles (Spencer & Verhulst 2007). De même, des cailles du Japon (*Coturnix japonica*) exposées à un stress pendant les phases pré- et post-natales avaient des comportements plus exploratoires et prenaient plus de risques à l'âge adulte que les individus non soumis à un stress pendant ces deux phases du développement (Zimmer et al. 2013). Dans le cas de notre population d'étude, les individus sensibilisés pourraient prendre plus de risques à l'âge adulte que les individus non sensibilisés pendant le développement post-natal. En affectant le phénotype des individus, ce phénomène de sensibilisation pourrait à long terme participer à une modification de la composition phénotypique de la population.

En provoquant un stress avec des effets directs sur le comportement et la physiologie, les activités et infrastructures humaines peuvent impacter la répartition spatiale des animaux et leur succès reproducteur et par conséquent, affecter la dynamique des populations. Une tendance globale d'évitement des villages par les busards cendrés a été mise en évidence dans la population étudiée, avec les plus grosses colonies nichant plus loin du bâti (Cornulier & Bretagnolle 2006). Le dérangement anthropique peut ainsi entraîner une diminution de la densité d'individus dans les zones anthropisées comme chez l'alouette lulu (*Lullula arborea*) dont la densité d'individus est réduite sur des zones avec une plus forte fréquentation humaine liée aux activités récréatives (Mallord et al. 2007). A long terme, un développement de l'habitat urbain pourrait entraîner une diminution de la taille de la population de busard cendré sur la zone d'étude. De plus, la fréquentation humaine que peuvent induire les chemins (promeneurs avec ou sans chien, joggeurs, cyclistes et passage de tracteurs) pourrait expliquer l'effet négatif de la densité de chemins sur le succès reproducteur et avoir un impact non négligeable sur la dynamique de la population. Des effets négatifs à long terme de perturbations humaines ont été mis en évidence sur la dynamique des populations animales à travers différents taxons (Iverson et al. 2006, Liley & Sutherland 2007, Kerbiriou et al. 2009, Coetzee & Chown 2016). Par exemple, la présence humaine *via* les activités touristiques diminue le succès reproducteur des otaries de Californie (*Zalophus californianus*) ce qui entraîne à long terme une réduction de la croissance de la population (French et al. 2011). Les effets du dérangement anthropique sur la répartition spatiale et le succès reproducteur peuvent avoir un part non négligeable dans le déclin des populations. Il est donc important, dans de futures études, d'identifier la nature du dérangement dans l'effet des chemins sur le succès reproducteur des busards cendrés et d'évaluer son effet sur la dynamique de la population à l'aide de modélisation, par exemple, avec les modèles individu-centré qui permettent de prédire les effets spatio-temporels du dérangement anthropique sur les populations animales (Stillman et al. 2007, Bennett et al. 2009).

D'autres effets du dérangement anthropique peuvent être plus subtils sur les populations puisqu'ils peuvent influencer indirectement leur structure en affectant la répartition spatiale des phénotypes. Des différences dans la tolérance au dérangement produit par le bâti en fonction de la personnalité des individus semblaient conduire à une répartition spatiale différente des phénotypes dans la population de busard cendré. Or, des variations dans la

disponibilité spatiale des phénotypes peuvent affecter le patron d'appariement d'une population (Ferrer & Penteriani 2003, Ingley & Johnson 2014, Taborsky et al. 2014). Très peu d'études ont mis en évidence des patrons d'appariement qui se produisent en fonction de la disponibilité spatiale du partenaire et non par un choix du partenaire issu du processus de sélection sexuelle (Ferrer & Penteriani 2003, Snowberg & Bolnick 2012, Taborsky et al. 2014). Dans la population étudiée de busard cendré, le patron d'appariement en fonction de la témérité des individus ne semblait pas procurer plus de bénéfices en termes d'aptitude phénotypique pour les couples les plus similaires. Le patron d'appariement ne serait pas le résultat d'un processus de sélection sexuelle seul mais pourrait également être induit par la différence de répartition spatiale des busards en fonction de leur témérité. Une distribution temporelle différente des busards en fonction de leur témérité à travers des variations dans le comportement de migration (phénologie, durée), pourrait s'ajouter, favorisant également le patron d'appariement observé. Par exemple, chez le pic flamboyant (*Colaptes auratus*), différents comportements de migration entre les deux sous-espèces semblent expliquer un appariement assortatif (Flockhart & Wiebe 2007). D'autres études sont nécessaires pour examiner ces deux hypothèses sur le patron d'appariement. Grâce aux suivis d'individus marqués ou équipés de balises GPS, des informations plus précises sur leur date d'arrivée sur la ZAPVS, leur déplacement et leur occupation de l'habitat permettraient d'explorer plus précisément ces deux hypothèses. L'étude de la date d'arrivée des busards sur la ZAPVS au retour de migration en fonction de leur témérité, nous renseignerait sur une possible ségrégation temporelle dépendante de la témérité. Les individus téméraires pourraient arriver en premier sur la zone d'étude et s'apparier avec les phénotypes disponibles, i.e., des individus téméraires. L'analyse de l'occupation de l'habitat des individus par rapport aux infrastructures, particulièrement au retour de migration, permettrait de savoir si les différences dans la répartition spatiale des phénotypes peuvent être à l'origine du patron d'appariement. Des données sont déjà disponibles pour des mâles équipés de balises GPS en 2017 et 2018, mais de nouveaux programmes pourraient intégrer l'équipement de femelles busards cendrés sur la ZAPVS. Les études évaluant les effets des activités humaines sur l'appariement des individus et leurs conséquences évolutives sont rares (Tarlow & Blumstein 2007). Une étude sur les cichlidés dans le Lac Victoria a mis en évidence les effets de l'eutrophisation du milieu liée aux activités humaines, sur le choix du partenaire (Seehausen et al. 1997). La turbidité de l'eau réduit la discrimination des couleurs sur

lesquelles est basé le choix du partenaire conduisant à une diminution de la diversité phénotypique (Seehausen et al. 1997). L'étude du patron d'appariement et de son origine se révèle importante pour comprendre toute la complexité des effets des activités humaines sur la dynamique des populations.

Enfin, la différence d'effets du dérangement anthropique sur les individus en fonction de leur personnalité et les effets d'une sensibilisation sur leur phénotype, cités plus haut, peuvent entraîner à long terme une modification de la composition phénotypique des populations. Les impacts négatifs des activités et infrastructures humaines plus marqués pour les individus timides, dans la sélection du site de nidification et sur le succès reproducteur (Arroyo et al. 2017), pourraient conduire à une raréfaction de ce phénotype dans la population. L'effet du phénomène de sensibilisation pouvant orienter le phénotype des individus vers une personnalité plus téméraire, pourrait augmenter la proportion d'individus téméraires dans la population. Cette tendance a d'ailleurs été mise en évidence dans la population étudiée de busard cendré, la composition phénotypique de la population sur la ZAPVS ayant changé avec une diminution de la proportion de femelles timides et une augmentation de la proportion de femelles téméraires (Arroyo et al. 2017). D'autres études ont montré des différences de composition phénotypique entre des populations de bruant chanteur (*Melospiza melodia*) et de Junco ardoisé (*Junco hyemalis hyemalis*) urbaines et rurales, l'habitat urbain n'étant pas favorable à la colonisation par des individus timides (Evans et al. 2010, Atwell et al. 2012). Cependant, une réduction de la diversité phénotypique d'une population peut avoir d'importantes conséquences sur sa viabilité (Smith & Blumstein 2013). En effet, les traits de personnalité comme l'agressivité ou la témérité peuvent être associés aux traits d'histoire de vie des individus, en augmentant le succès reproducteur, par exemple, mais ces liens varient en fonction du contexte (Réale et al. 2007, Biro & Stamps 2008, Smith & Blumstein 2008, Réale et al. 2010, Wolf & Weissing 2012). Un type de personnalité serait alors plus performant dans une certaine condition environnementale alors qu'un autre type de personnalité le serait dans une autre condition (Réale & Festa-Bianchet 2003, Dingemanse et al. 2004, Quinn et al. 2009, Wolf & Weissing 2010). Par exemple, chez la mésange charbonnière (*Parus major*), dans les années de faibles abondances en nourriture, les femelles qui explorent rapidement l'environnement ont une meilleure survie que celles qui explorent lentement, alors que l'effet inverse se produit sous de fortes abondances alimentaires

(Dingemanse et al. 2004). Si la proportion d'un type de personnalité dans la population diminue plus qu'un autre, la population pourrait être vulnérable à un rapide changement des conditions environnementales (Wolf & Weissing 2012). Ceci pourrait se produire dans le cas où le type de personnalité majoritairement représenté dans la population serait moins bien adapté à la nouvelle situation environnementale (McDougall et al. 2006). Par conséquent, l'effet combiné d'une réduction de la diversité phénotypique d'une population et un changement rapide des conditions environnementales, pourrait être un facteur supplémentaire aggravant le déclin des populations animales et notamment aviaires particulièrement en milieu rural (Wretenberg et al. 2006, Gregory et al. 2007, Voříšek et al. 2010, Inger et al. 2015, Maxwell et al. 2016).

Le dérangement anthropique peut ainsi avoir de multiples effets à court et long termes conduisant à un affaiblissement non négligeable des populations animales. L'évaluation de ces effets plus ou moins complexes et discrets est primordiale pour proposer des mesures de protection et de conservation adaptées et limiter ces effets.

Conséquences pour la gestion et la conservation des populations

La mise en évidence des effets directs et indirects du dérangement anthropique et de ses possibles conséquences à long terme aux niveaux individuel et populationnel, permet d'apporter de nouveaux éléments de réflexion pour améliorer le suivi et la protection des populations sauvages. Ils permettent notamment de souligner l'importance de prendre en compte la variabilité comportementale inter-individuelle dans la biologie de la conservation. En effet, le comportement d'un individu est à l'interface entre l'environnement et la physiologie de l'individu et constitue donc la première ligne de réponse face aux perturbations. Il est donc indispensable de le prendre en considération.

Le phénomène de sensibilisation au cours de captures successives, mais surtout l'importance du facteur expérimentateur dans ce phénomène, amènent à s'interroger sur le dogme prônant l'utilisation du même expérimentateur pour effectuer des mesures répétées sur les individus. Certes, cette méthode permet d'éviter les biais de mesures entre expérimentateurs mais elle peut provoquer une sensibilisation et un stress supplémentaire

chez les animaux, qui peuvent interagir avec les observations et mesures réalisées. Suivant la question, la méthode à privilégier (changement ou non des expérimentateurs) dans le suivi des individus, ne serait pas la même. Les paramètres mesurés (comportement, hormone de stress) dans les études sur la personnalité (Carere & Maestriperi 2013) ou l'évolution d'impacts anthropiques (Almasi et al. 2015) par exemple, pourraient être biaisés si l'identité de l'expérimentateur n'est pas standardisée. Dans ce cas, le non-changement d'expérimentateur serait à privilégier en vérifiant, toutefois, les effets d'une sensibilisation, i.e., augmentation de la sécrétion de glucocorticoïdes, sur la condition corporelle et physiologique des individus (voir Müller et al. 2009, Virgin & Rosvall 2018). D'autres études sont nécessaires pour évaluer quels éléments favorisent un phénomène de sensibilisation. En fonction des capacités cognitives des espèces, la reconnaissance de l'expérimentateur par l'animal pourrait conduire à sa sensibilisation et nécessiterait plus de recherches en évaluant également quels indices (visage, odeur, vêtement, façon de manipuler) seraient utilisés par les animaux pour cette reconnaissance. Par exemple, cette hypothèse de discrimination d'expérimentateurs pourrait être évaluée en utilisant des masques comme indiqué par Marzluff et al. (2010) pour tester l'utilisation d'indices du visage par les animaux. Le nombre d'expérimentateurs serait également un paramètre à standardiser puisqu'il peut faire varier les réponses comportementales des individus comme nous l'avons suspecté chez les femelles busards cendrés. Enfin, dans les activités de conservation, le nombre d'expérimentateurs serait à limiter pour réduire l'intensité du stress provoqué et le changement d'expérimentateurs serait à privilégier pour éviter une sensibilisation des animaux. Notre étude est l'une des premières à mettre en évidence un phénomène de sensibilisation au cours des activités de recherche et conservation, mais avant tout à révéler l'importance du facteur expérimentateur qui est à ajuster. La méthode du changement d'expérimentateurs serait bien évidemment applicable au suivi des nids de busard en milieu associatif mais pourrait surtout être testée chez un grand nombre d'espèces bénéficiant de statut de protection et/ ou de programmes de conservation, comme ceux réalisés pour la crécerelle d'Amérique (*Falco sparverius*) et l'océanite tempête (*Hydrobates pelagicus*) (Katzner et al. 2005, Libois et al. 2012).

De nouvelles mesures de protection des nids de busard cendré pourraient s'ajouter aux mesures actuelles afin de limiter les effets du dérangement anthropique sur le succès

reproducteur. Depuis 1994 sur la ZAPVS, tous les nids trouvés de busard cendré mais aussi de busard Saint-Martin et busard des roseaux sont protégés avant la moisson des cultures de céréales (Bretagnolle et al. 2018). Une mesure de protection également utilisée en milieu associatif et dans d'autres pays, consiste à poser un grillage autour du nid avant la moisson afin d'éviter la dispersion des poussins dans la culture et leur destruction par les machines agricoles (Figure 13a). Cette mesure de protection est permise grâce à la collaboration avec les exploitants agricoles (Bretagnolle et al. 2018). L'installation du grillage permet également de limiter une prédation terrestre après la moisson, les nids étant plus facilement détectables (Figure 13b). Cette mesure de protection est indispensable puisque sans celle-ci, 60 % des poussins pourraient mourir lors des moissons des cultures agricoles (Arroyo et al. 2002b). Cependant, cette mesure ne permet pas de limiter le dérangement anthropique provoqué par la fréquentation humaine sur les chemins et par les pratiques agricoles. De nouvelles mesures de protection et conservation consisteraient à identifier précisément la nature du dérangement généré par les chemins sur le succès reproducteur en mesurant directement la fréquentation humaine et les activités autour des nids à l'aide de focales et/ou de caméras. Elles évalueraient également si la réduction du succès reproducteur est plus prononcée après les moissons. Ces dernières années, les moissons sont de plus en plus précoces, probablement à cause du changement climatique. Par conséquent, une grande majorité des nids de busard cendré sont encore actifs avec des poussins voire des œufs au moment des moissons. Après les moissons, les nids protégés deviennent alors très visibles depuis les chemins et les routes, suscitant probablement la curiosité des promeneurs (Figure 13b).

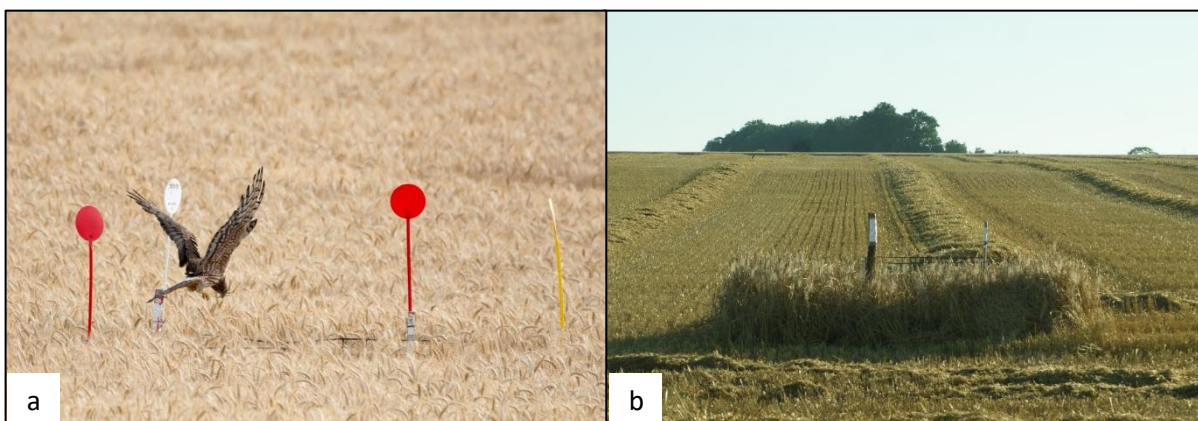


Figure 13 : Nid protégé de busard cendré a) avant la moisson et b) après la moisson de la culture de céréales sur la parcelle (© C. Ingrand et C. Mangelinck).

Si la baisse du succès reproducteur s'avère être reliée directement à la fréquentation humaine sur les chemins et aux comportements des promeneurs (e.g., chien non tenu en laisse, visite des nids), des mesures de prévention pourraient être mises en place. Des panneaux rappelant les règles de bonne conduite envers la faune sauvage (e.g., chien tenu en laisse, interdiction de pénétrer dans les parcelles de cultures) pourraient être disposés en bordure de chemins. Par exemple, sur les sites de reproduction du Gorfou de Schlegel (*Eudypetes schlegeli*), le respect des recommandations d'une distance d'approche minimale permet d'éviter la fuite des individus et limite le dérangement (Holmes et al. 2005). Pour le busard cendré, les mesures de conservation et protection se concentreraient sur les sites de colonies présentes dans la Zone de Protection Spéciale (ZPS, Natura 2000) sur notre site d'étude mais également dans d'autres ZPS en France. Les contrats de mesures agro-environnementales avec les exploitants agricoles sur cette ZPS permettraient de favoriser l'installation et la reproduction de cette espèce patrimoniale (Arroyo et al. 2002b). Ces mesures apportent une aide financière pour les exploitants agricoles qui s'engagent volontairement à préserver l'environnement en fonction d'un cahier des charges. Certaines d'entre elles, établies dans le programme de conservation de l'outarde canepetière (*Tetrax tetrax*), consistent à développer les espaces de prairies augmentant ainsi les ressources alimentaires pour un grand nombre d'espèces dont le busard cendré. De nouvelles mesures pourraient retarder les dates de moisson jusqu'à l'envol des poussins ou appliquer une plus grande surface de culture délimitée autour du nid et non moissonnée (Arroyo et al. 2002b). Les pratiques agricoles sur les parcelles peuvent être plus ou moins fréquentes en fonction du type de culture et d'agriculture (biologique ou conventionnel) et pourraient également provoquer un dérangement. A l'aide des informations fournies par les enquêtes réalisées auprès des agriculteurs chaque année, l'effet de la fréquence et de l'intensité des pratiques agricoles dans le paysage autour des nids sur le succès reproducteur pourrait être testé. Si ces pratiques s'avèrent avoir un effet, d'autres mesures agro-environnementales pourraient limiter plus activement les pratiques agricoles spécifiquement sur les parcelles accueillant des colonies de busard cendré. Ces mesures agro-environnementales nécessiteraient une évaluation financière de façon à créer un cahier des charges acceptable pour l'agriculteur et en fonction des aides financières qui peuvent lui être attribuées pour compenser les coûts sur sa récolte. Enfin, des mesures de conservation à l'échelle du paysage pourraient adapter les plans locaux d'urbanisme de façon à limiter le développement urbain à proximité des colonies

de busard cendré. Toutes ces nouvelles mesures agro-environnementales, de conservation et de sensibilisation des promeneurs à l'égard de la faune sauvage pourraient profiter à un grand nombre d'espèces inféodées aux paysages agricoles, comme l'outarde canepetière, l'œdicnème criard (*Burhinus oedicanus*) et la perdrix grise (*Perdix perdix*).

Enfin, la variation des effets du dérangement anthropique en fonction de la personnalité des individus se révèle être un élément important à prendre en considération pour la conservation des populations animales. En effet, les conséquences possibles sur la structuration et la composition phénotypique des populations seraient à suivre sur le long terme et à examiner étroitement en fonction de la dynamique des populations. Les variations de diversité phénotypique pourraient être étudiées en fonction des conditions environnementales comme les abondances en nourriture et en prédateurs mais également en fonction des mesures de gestion et protection présentées au-dessus afin d'appréhender leur efficacité. Le suivi de la diversité comportementale des populations serait un élément important à intégrer dans la gestion et la conservation des espèces *in situ* mais également dans les programmes de réintroduction (McDougall et al. 2006, Merrick & Koprowski 2017). Le succès de réintroduction d'une espèce pourrait être optimisé avec une plus grande diversité comportementale des individus relâchés (McDougall et al. 2006, Powell & Gartner 2011, Merrick & Koprowski 2017). Par exemple, deux programmes de relâché sont en cours sur notre site d'étude pour la perdrix grise et pour l'outarde canepetière en partenariat avec la Fédération de chasse, l'Office National de la Chasse et de la Faune Sauvage, le parc zoologique Zoodyssée et le Centre d'Etudes Biologiques de Chizé. Une prochaine étude pourrait identifier les différences comportementales inter-individuelles à travers différents traits de personnalité (agressivité, exploration, témérité) au sein des individus à relâcher pour chaque espèce et réaliser un suivi post-relâché *in natura* des individus grâce à des balises GPS. Ceci permettrait de mieux appréhender l'importance de la diversité comportementale dans la réintroduction et la conservation des populations, et d'en améliorer son succès.

Conclusions

Mes travaux de thèse apportent des éléments de réponses sur les effets directs et indirects du dérangement anthropique sur le comportement, la physiologie et les traits d'histoire de vie des animaux. Ils permettent de remettre en question certaines pratiques dans le suivi des populations et de trouver des solutions adaptées à chaque situation. Les conséquences possibles du dérangement anthropique sur la structure et la composition des populations soulignent l'importance d'évaluer les effets du dérangement non uniquement au niveau global de la population mais également en fonction des différences comportementales inter-individuelles au sein de la population. En biologie de la conservation, la diversité des personnalités au sein des populations serait un facteur à prendre en compte pour suivre la dynamique des populations. D'autres facteurs anthropiques exercent de fortes pressions de sélection négatives sur les populations animales en plaine céréalière. L'impact des produits phytosanitaires serait une cause majeure du déclin des populations à étudier. Cette thèse a également été l'occasion de développer un protocole pour doser l'activité d'une enzyme, l'acétylcholinestérase, qui intervient dans le système nerveux et qui est inhibée par certaines molécules de pesticides. Le développement de ce protocole ouvre de nouvelles perspectives d'après-thèse dans l'exploration des effets d'une exposition aux pesticides sur le comportement et les traits d'histoire de vie des individus.

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Résumé

Le déclin des populations de nombreuses espèces animales depuis un siècle est associé aux activités anthropiques. La surexploitation des espèces ou encore la fragmentation des habitats en sont les principales causes mais d'autres activités humaines ont des effets plus subtils sur les animaux et impactent indirectement leur survie et leur reproduction. Le dérangement anthropique représente toutes les perturbations générées par la présence humaine, des objets ou des bruits anthropiques qui provoquent un stress chez l'animal. Le comportement et la physiologie des animaux en lien avec leurs traits d'histoire de vie peuvent être affectés par ce dérangement, mais de manière différente entre les individus d'une même population en fonction de leur personnalité. De cette façon, le dérangement anthropique pourrait contribuer à long terme au déclin des populations. Le busard cendré (*Circus pygargus*) est une espèce patrimoniale qui niche au sol dans les cultures céréalières, ce qui l'expose aux activités agricoles, récréatives et bruits anthropiques. De plus, sur le site d'étude, la Zone Atelier Plaine et Val de Sèvre, le suivi de sa population implique plusieurs visites au nid et des mesures répétées sur les poussins pouvant générer un dérangement. Mes travaux de thèse se sont intéressés aux impacts des activités et des infrastructures humaines sur le comportement et les traits d'histoire de vie du busard cendré et comment ces effets peuvent varier en fonction des différences comportementales inter-individuelles au sein de la population. Grâce à une approche expérimentale, un phénomène de sensibilisation a été mis en évidence chez les poussins manipulés par les mêmes expérimentateurs au cours de visites successives au nid. Par la suite, la témérité (personnalité et plasticité) des mâles et femelles adultes a été caractérisée. Un patron d'appariement positif à travers la témérité des individus a été montré au sein de la population. Cependant, ce patron d'appariement ne procurerait pas de bénéfices en termes de succès reproducteur, ce qui questionne sur son origine. Ce résultat suggère que d'autres processus que la sélection sexuelle, pourraient induire ce patron d'appariement. L'association parentale dans la témérité a ensuite été reliée à l'approvisionnement en proies des couples, puis aux conditions corporelle et physiologique des poussins. Les couples moins plastiques dans leur témérité délivraient une plus grande proportion d'insectes à leur progéniture, ce qui expliquerait des concentrations plus élevées en caroténoïdes trouvées chez leurs poussins. Aucun effet de la similarité comportementale des parents n'a été montré sur la qualité des poussins. Enfin, les nids de busard cendré étaient distribués différemment par rapport aux infrastructures en fonction de la témérité des femelles. Les femelles timides évitaient plus le bâti dans leur choix de site de nidification que les femelles téméraires. Cette différence de répartition spatiale des phénotypes pourrait être à l'origine du patron d'appariement. La densité de chemins autour du nid affectait négativement le succès reproducteur des femelles quelle que soit leur témérité. Ce résultat nécessite de futures recherches pour identifier la nature du dérangement et limiter ses effets. Ces travaux de thèse apportent des éléments de réponses sur les effets directs et indirects du dérangement anthropique et permettent de remettre en question certaines pratiques dans le suivi des populations afin de les améliorer. Enfin, évaluer les effets du dérangement en fonction des différences inter-individuelles apparait essentiel pour mieux comprendre ses conséquences sur la dynamique des populations.

Mots clés : appariement assortatif, approvisionnement, caroténoïdes, choix du site de nidification, corticostérone, dérangement anthropique, personnalité, plasticité, sensibilisation.

Abstract

The decline of populations for many animal species in the last century has been linked to anthropogenic activities. The species overexploitation or even habitat fragmentation are the major causes but other human activities may have more subtle effects on animals and indirectly may impact their survival and reproduction. Anthropogenic disturbance represents any disruption due to human presence, anthropogenic objects or noise that induce stress for animals. Animal behaviour and physiology linked to life-history traits could be affected by this disturbance but differently among individuals from a same population depending on their personality. In this way, the anthropogenic disturbance may contribute to the population decline. The Montagu's harrier (*Circus pygargus*) is a flagship species that nests on the ground in cereal crops exposing it to agricultural practices, recreative activities and anthropogenic noise. Moreover, the population monitoring on the study site, the Zone Atelier Plaine et Val de Sèvre, involves several nest visits and repeated measures on chicks that may induce a disturbance. My thesis work has been focused on the impacts of human activities and infrastructures on the behaviour and life-history traits of Montagu's harriers and how these impacts may vary depending on behavioural inter-individual differences within the population. Through an experimental approach, a sensitization phenomenon has been highlighted in chicks handled by the same experimenters over repeated nest visits. Thereafter, male and female boldness (personality and plasticity) was characterized. A positive assortative pairing pattern for boldness was highlighted within the population. However, this pattern did not provide fitness benefits which questions about its origin. This result suggests that other process than sexual selection could explain the pairing pattern. Then, the parental association in boldness has been linked to prey provisioning from pairs, and to nestling body and physiological conditions. Less plastic pairs in boldness provided a higher proportion of insects to their offspring that could explain greater carotenoids concentrations found for their chicks. Any effect of behavioural similarity within pairs has been shown on nestling quality. Finally, Montagu's harrier nests were differently distributed in relation to infrastructures depending on female boldness. Shy females would avoid more buildings through nest site selection than bold females. This difference in spatial distribution of phenotypes could result in the positive pairing pattern. The path density around nest negatively affected the reproductive success regardless of female boldness. This result needs further investigations to identify the disturbance nature and limit its effects. This thesis work provides some answers on the direct and indirect effects of anthropogenic disturbance and allows questioning certain practices in the population monitoring to improve them. Finally, the assessment of disturbance effects depending on inter-individual differences is essential to better understand the consequences on population dynamic.

Keywords: anthropogenic disturbance, assortative pairing, carotenoids, corticosterone, nest site selection, personality, plasticity, provisioning, sensitization.