Rôle de la communication acoustique dans l'organisation des soins biparentaux chez les oiseaux : étude chez le diamant mandarin (Taeniopygia guttata) et la mésange charbonnière (Parus major)

Ingrid Boucaud

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Membre de UNIVERSITÉ DE LYON

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Par Ingrid Boucaud

Rôle de la communication acoustique dans l'organisation des soins biparentaux chez les oiseaux

Etude chez le diamant mandarin (*Taeniopygia guttata*) et la mésange charbonnière (*Parus major*)

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INTRODUCTION GENERALE

I. INTRODUCTION GENERALE

I.1. Les soins parentaux et biparentaux

I.1.a. Quel est l'intérêt des soins parentaux ?

Chez beaucoup d'espèces animales, les parents ne prodiguent aucun soin à leurs jeunes, mais chez certaines les parents vont augmenter les chances de survie de leur progéniture par différents comportements. Ils vont par exemple pouvoir les protéger des prédateurs, parasites et pathogènes, parfois en construisant des terriers ou des nids, mais aussi des risques environnementaux tels que le froid ou la dessiccation, les approvisionner en nourriture etc. (Royle, Smiseth, & Kolliker, 2012). Si les effets de ces soins à court terme sont évidents, les jeunes pourront également profiter d'effets à long terme après leur indépendance. Par exemple, en nourrissant leurs jeunes, les parents vont influer sur le bon développement de leur cerveau ce qui va avoir un impact sur leurs capacités perceptuelles, cognitives et d'apprentissage futures (exemples : Meaney, 2001; Arnold *et al.*, 2007; Law *et al.*, 2009). Ainsi, de nombreuses études chez les oiseaux ont montré qu'un stress alimentaire pendant le développement des oisillons avait un impact sur la mise en place de leur chant (synthèse par Nowicki, Peters, & Podos, 1998). Chez les espèces avec des soins parentaux prolongés, les jeunes pourront également apprendre certaines compétences qui leurs seront utiles par la suite (Brown & Laland, 2001; Hoppitt *et al.*, 2008) comme trouver de la nourriture (Thornton & McAuliffe, 2006) ou réagir face aux prédateurs (Curio, 1993; Mateo & Holmes, 1997).

I.1.b. Le père et la mère n'ont pas toujours les mêmes rôles

Les femelles pourraient avoir plus tendance à prodiguer des soins parentaux car elles investissent plus dans leurs gamètes que les mâles (anisogamie), et n'augmentent pas leur succès reproducteur en multipliant les partenaires (Bateman, 1948; Trivers, 1972). Cependant, un investissement passé ne peut pas à lui seul expliquer l'investissement futur d'un individu et d'autres forces doivent donc également jouer dans le rôle des sexes dans les soins parentaux (Dawkins & Carlisle, 1976; Kokko & Jennions, 2008). Diverses explications écologiques et sociales ont été proposées pour expliquer qui des mâles ou des femelles devait s'investir le plus, par exemple l'incertitude d'être le père, la sélection sexuelle ou le sexe ratio chez les adultes reproducteurs (Royle *et al.*, 2012). De plus, s'il semble que ce sont les femelles qui prodiguent le plus de soins parentaux, il n'existe pas moins une grande diversité dans le partage de l'effort parental entre mâles et femelles (Royle *et al.*, 2012). Notre vision est souvent biaisée par le fait que ce sont les femelles qui font le plus de soins parentaux chez la plupart des espèces de mammifères (Royle *et al.*, 2012). Cette tendance est également vraie chez les arthropodes et les reptiles qui produisent des soins parentaux (Royle *et al.*, 2012). Cependant, chez les anoures on trouve autant d'espèces chez lesquelles l'essentiel des soins est dispensé par les mâles que par les femelles (Reynolds, Goodwin, & Freckleton, 2002; Summers, Sea McKeon, & Heying, 2006) et chez les poisons téléostéens ce sont les mâles qui produisent l'essentiel des soins parentaux (Reynolds *et al.*, 2002). Enfin, chez les oiseaux, mâles et femelles participent en général ensemble aux soins parentaux (Cockburn, 2006).

I.1.c. Les soins biparentaux sont très répandus chez les oiseaux mais les sexes peuvent avoir des rôles différents

Alors que les soins biparentaux sont peu fréquents chez les autres taxons, ils sont présents chez 81% des espèces d'oiseaux, tandis que la femelle seule prodigue des soins chez 8% des espèces et le mâle seul chez 1% (Cockburn, 2006). Enfin, 9% des espèces pratiquent la reproduction coopérative et 1% échappe aux soins parentaux en pratiquant le parasitisme de nichée ou en utilisant la géothermie pour incuber les œufs (Cockburn, 2006). Chez les oiseaux, les soins parentaux consistent principalement à construire un nid, incuber les œufs, puis défendre et nourrir les jeunes. Les jeunes oiseaux demandent beaucoup de soins. Tout d'abord, les œufs doivent être incubés pour que les embryons survivent et se développent (Webb, 1987). Puis, après l'éclosion, les oisillons sont incapables de se débrouiller seuls et doivent à la fois être couvés et nourris. C'est sans doute cette demande importante qui nécessite que les deux parents s'investissent dans les soins aux jeunes. En effet, les soins biparentaux sont considérés comme évolutivement stables lorsqu'un parent ne peut pas élever sa descendance seul (Maynard Smith, 1977).

Chez les espèces à soins biparentaux, la contribution de chaque sexe varie d'une espèce à une autre (Skutch, 1957; Webb *et al.*, 2010). Certaines activités peuvent être réalisées uniquement par un sexe ou être partagées par le mâle et la femelle. Les femelles passent en proportion plus de temps à construire le nid et à incuber les œufs (Møller & Cuervo, 2000; Schwagmeyer, Mock, & Parker, 2002), cependant, les mâles peuvent alors participer aux soins parentaux de façon indirecte en s'occupant de la défense du territoire ou en nourrissant la femelle. En effet, chez les espèces où la femelle s'occupe seule de l'incubation, cette dernière peut être nourrie en partie ou complètement par son mâle (Kendeigh, 1952; Lyon & Montgomerie, 1985; Lifjeld, Slagsvold, & Stenmark, 1987; Moreno *et al.*, 2011) et ces nourrissages peuvent avoir un impact indirect sur l'incubation en permettant à la femelle de passer plus de temps sur les œufs (Lyon & Montgomerie, 1985; Lifjeld & Slagsvold, 1986; Hałupka, 1994; Klatt, Stutchbury, & Evans, 2008; Lloyd *et al.*, 2009; Stein, Oh, & Badyaev, 2009; Matysioková, Cockburn, & Remeš, 2011; Moore & Rohwer, 2012; Matysiokova & Remes, 2014).

I.1.d. Les soins biparentaux peuvent être une source de conflit mais aussi de coopération entre les sexes

La notion de conflit entre les sexes peut être définie comme « un conflit entre les intérêts évolutifs des individus des deux sexes » (Parker, 1979). Dans le cas des soins biparentaux, mâle et femelle doivent ajuster la quantité de soins que chacun va apporter. Ce partage peut être vu comme un conflit d'intérêt entre les sexes car chaque parent bénéficie de l'investissement de son partenaire mais paie un coût, en termes de survie et de fécondité, en prodiguant lui-même une partie des soins (Trivers, 1972). En effet, l'effort parental d'un individu va permettre d'améliorer son succès de reproduction immédiat mais au détriment de ses reproductions futures (Trivers, 1972). Parce que chaque parent va fournir moins d'efforts si c'est l'autre qui s'investit le plus, les modèles prédisent que chacun va diminuer ses efforts de façon à optimiser sa fitness totale (Houston & Davies, 1985; Westneat & Sargent, 1996; McNamara, Gasson, & Houston, 1999; McNamara *et al.*, 2003; Houston, Szekely, & McNamara, 2005; Lessells, 2012). Pour que les soins biparentaux soient évolutivement stables, le modèle de Houston-Davies (1985) prédit qu'un parent va compenser seulement en partie une diminution de l'effort de son partenaire. En effet, une compensation complète autoriserait un des parents à exploiter l'autre rendant les soins biparentaux évolutivement instables. Dans ce

modèle, cet ajustement entre les parents ne se fait pas en temps réel mais est obtenu à l'échelle de temps de l'évolution (on parle alors de 'sealed bid'). Plus tard, les modèles ont intégré la notion que les parents négocient en temps réel la quantité de soins prodiguée par chacun (McNamara *et al.*, 1999, 2003; Johnstone & Hinde, 2006). On parle alors de modèle de 'négociation' par opposition au 'sealed bid' de Houston-Davies (1985). McNamara et *al*. (1999, 2003) font la même prédiction que Houston et Davies (1985) mais non plus à l'échelle de l'évolution : cette fois c'est en temps réel qu'un parent va compenser partiellement une diminution de l'investissement de son partenaire en augmentant son propre investissement. Le modèle de Jones, Ruxton, & Monaghan (2002) va quant à lui prédire qu'un parent peut compenser totalement ou déserter face à une diminution de l'investissement de son partenaire chez les espèces pour lesquelles la reproduction peut échouer complètement suite à une diminution des soins parentaux. Enfin, le modèle de Johnstone & Hinde (2006) prédit que les parents peuvent utiliser l'investissement parental de leur partenaire comme un indice des soins à apporter et tendre à produire le même effort (« matching »), ce qui est l'inverse de la compensation.

Ces modèles ont été testés empiriquement par de nombreuses études qui ont modifié la quantité de soins parentaux apportée par un des parents et mesuré la réponse de son partenaire. Ces études ont utilisé différentes méthodes telle que retirer un des deux parents (Mrowka, 1982; Whittingham, Dunn, & Robertson, 1994; Rauter & Moore, 2004; Suzuki & Nagano, 2009), handicaper un parent, généralement en coupant des plumes ou en faisant porter des poids chez les oiseaux, (Wright & Cuthill, 1989; Slagsvold & Lifjeld, 1990; Whittingham *et al.*, 1994; Markman, Yom-Tov, & Wright, 1995; Lozano & Lemon, 1996; Moreno *et al.*, 1999; Sanz, Kranenbarg, & Tinbergen, 2000; Schwagmeyer *et al.*, 2002), modifier le taux de testostérone du mâle (Saino & Møller, 1995; Hunt, Hahn, & Wingfield, 1999; Stoehr & Hill, 2000), ou bien émettre des cris de quémande de poussins à un parent en particulier (Hinde, 2006). Une méta-analyse réalisée sur 54 études faites chez les oiseaux a montré que les parents réagissent en général aux efforts de leur partenaire par une compensation partielle (Harrison *et al.*, 2009). Cette conclusion rejoint bien les prédictions des modèles de (Houston & Davies, 1985; McNamara *et al.*, 1999, 2003), cependant, la réponse des oiseaux dépend en partie du type d'expérimentation et n'est pas toujours la même pour les mâles et les femelles (Harrison *et al.*, 2009). De plus, comme prédit par les modèles de (Jones *et al.*, 2002) et de (Johnstone & Hinde, 2006), d'autres alternatives à la compensation partielle existent et ont été observées chez certaines espèces. Les parents peuvent ne pas compenser du tout (Slagsvold & Lifjeld, 1990; Lozano & Lemon, 1996; Moreno *et al.*, 1999; Schwagmeyer *et al.*, 2002; Rauter & Moore, 2004; Suzuki & Nagano, 2009), compenser complètement (Mrowka, 1982; Hunt *et al.*, 1999; Stoehr & Hill, 2000), ou même surcompenser (Griggio & Pilastro, 2007). Enfin, les parents peuvent produire un effort qui tend à être le même que celui de leur partenaire (« matching » ; Hinde, 2006; Hinde & Kilner, 2007; Meade *et al.*, 2011).

Le conflit entre les sexes est considéré comme inévitable à moins que les intérêts des deux sexes ne soient parfaitement conciliables. Ce ne peut-être que le cas d'une monogamie vraie, c'est-à-dire d'une monogamie dans laquelle mâles et femelles n'ont qu'un seul partenaire sexuel qui ne sera pas remplacé s'il vient à mourir (Lessells, 2006). Cette possibilité est plutôt considérée comme un point de référence théorique que comme une réalité (Lessells, 2006). Cependant, la monogamie existe chez de nombreuses espèces, et bien qu'elle ne soit pas parfaite, et que le conflit entre les sexes ne puisse donc pas être totalement écarté, elle pourrait également être vue comme une forme de coopération entre les partenaires (Black, 1996). . Chez les espèces qui se reproduisent plusieurs fois avec le même partenaire ou qui présentent des soins parentaux particulièrement longs, un parent qui s'investit excessivement par rapport à son partenaire risque effectivement de ne pas

pouvoir maintenir son effort par la suite et donc de réduire son investissement. Ce comportement serait alors préjudiciable pour les deux parents. Dans cette situation le couple pourrait donc être vu comme une unité de coopération (Black, 1996), et l'intérêt des partenaires serait de minimiser le coût des soins parentaux pour le couple dans son ensemble plutôt que pour eux-mêmes.

I.1.e. Comment les parents coordonnent les soins biparentaux ?

Si de nombreuses études se sont intéressées à comment un parent réagit face à une diminution ou à une augmentation de la quantité de soins parentaux dispensée par son partenaire, peu se sont intéressées aux mécanismes qui permettent cet ajustement. Les parents pourraient utiliser des informations directes sur la quantité de soins nécessaires comme l'intensité des cris de quémande des jeunes qui est considérée comme un signal honnête de leurs besoins (Kilner & Johnstone, 1997; Horn & Leonard, 2005). Ils pourraient également utiliser des informations venant de leur partenaire. C'est ce que propose par exemple le modèle de (Johnstone & Hinde, 2006) qui prédit qu'une augmentation de l'effort d'un parent va être un indicateur pour l'autre des besoins de la nichée. Ce modèle prédit un « matching » de la quantité de soins parentaux apportés par le mâle et la femelle. Un mécanisme possible qui permettrait aux parents de fournir chacun la même quantité de soins parentaux pourrait être de synchroniser leurs activités. C'est ce qu'on observe par exemple chez certaines espèces où les deux parents se rendent ensemble au nid pour nourrir les jeunes (Meade *et al.*, 2011; Mariette & Griffith, 2012, 2015). Les parents pourraient aussi se synchroniser en ne faisant pas les soins ensemble mais de façon alternée. C'est ce que propose le modèle de (Johnstone *et al.*, 2014) qui a été vérifié chez la mésange charbonnière (*Parus major* ; Johnstone *et al.*, 2014). En effet, chez cette espèce, mâle et femelle alternent leurs visites au nid pour nourrir les poussins. Enfin les parents pourraient communiquer sur les soins parentaux à apporter. Lorsque les mâles font des soins parentaux indirects pendant l'incubation en nourrissant la femelle, cette dernière peut communiquer ses besoins en utilisant des cris de quémande (Tobias & Seddon, 2002; Otter, Atherton, & van Oort, 2007; Moore & Rohwer, 2012; Cantarero *et al.*, 2014). Mais aucune étude n'a encore montré que la communication pouvait servir aux parents à se coordonner lorsqu'ils partagent les mêmes tâches comme l'incubation et le nourrissage des poussins.

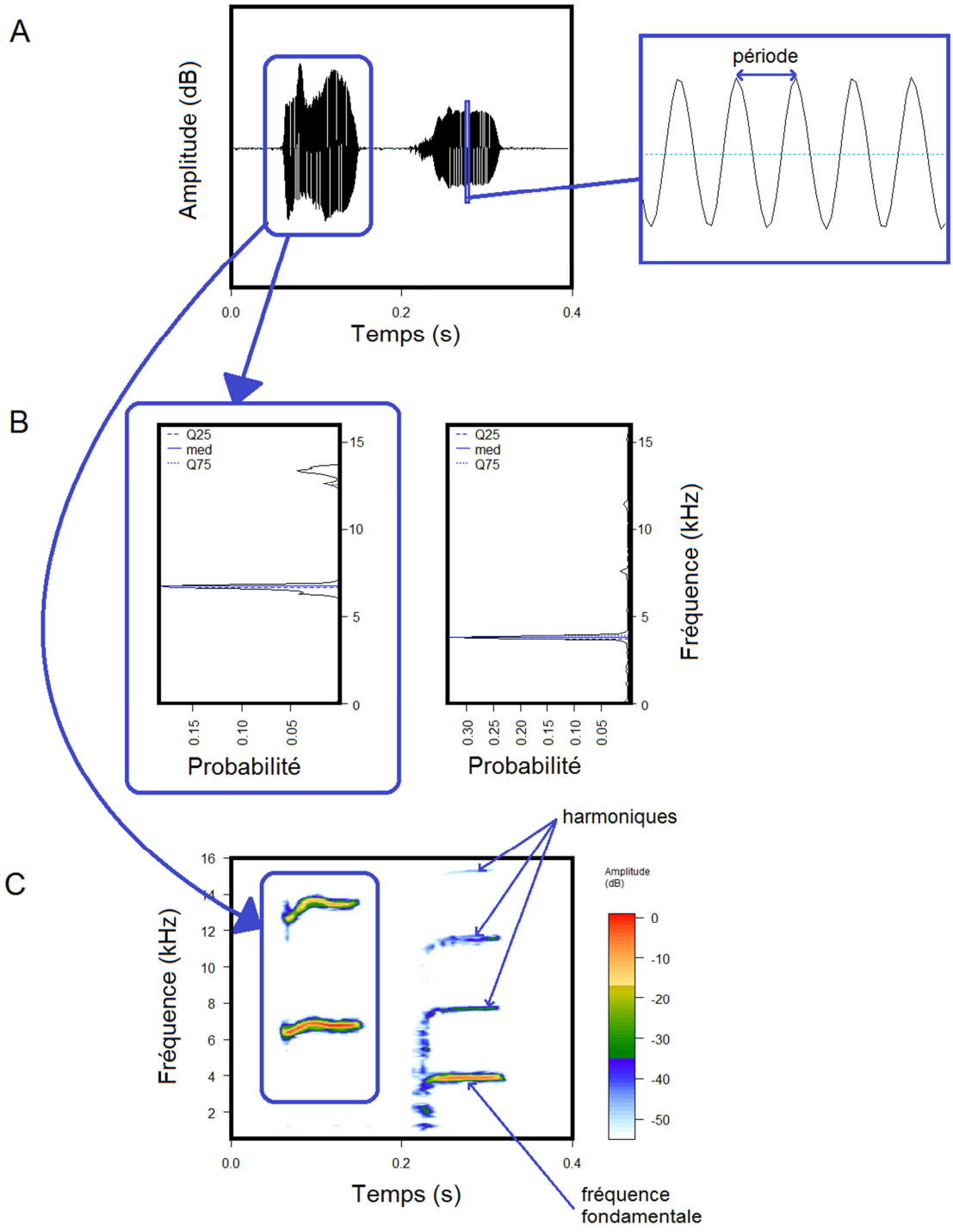

Figure 1. Différentes représentations du son : A) oscillogramme, B) spectre fréquentiel et C) sonagramme (ou spectrogramme).

I.2. La communication

I.2.a. Qu'est-ce que la communication ?

La communication est un processus durant lequel un émetteur encode des informations dans un signal qui se propage dans l'environnement pour atteindre un récepteur. Le récepteur doit alors être capable de décoder l'information pour pouvoir éventuellement y répondre. Les rôles pourront s'inverser et le récepteur deviendra alors émetteur tandis que l'émetteur deviendra récepteur (Rendall, Owren, & Ryan, 2009). La communication animale est extrêmement variée. Le signal peut être transmis à travers différents canaux de communication : chimique, visuel, mécanique ou acoustique. Le canal utilisé va dépendre des capacités des individus émetteurs et récepteurs à produire et recevoir le signal mais aussi du type d'information à transmettre et du milieu de propagation. Chaque canal présente en effet des avantages et des inconvénients en termes de portée, de vitesse de changement, de passage d'obstacles, de localisation et de coût énergétique (Davies, Krebs, & West, 2012). Par exemple, le canal acoustique peut être utilisé pour de la communication longue portée, même en présence d'obstacles et permet des modifications rapides du signal. Cependant la localisation du signal est moins bonne que pour les signaux visuels et tactiles (Davies *et al.*, 2012). Parfois, plusieurs canaux de communication peuvent être utilisés en même temps, on parle alors de communication multimodale (Partan, 2004).

I.2.b. Le son peut être représenté de différentes manières

Un son est une onde mécanique qui met en vibration un milieu de propagation. Dans l'air, le son se déplace sous forme d'une variation de pression (enchaînement de compressions et de dépressions) crée par la source sonore. Enregistrer un son consiste donc à mesurer des variations de pression à un point donné (Bradbury & Vehrencamp, 2011b). Il existe trois principales représentations du son (Fig. 1). L'oscillogramme (Fig. 1A) représente les variations de pression en fonction du temps. Son amplitude est une mesure de la déviation de la pression mesurée par rapport à la pression ambiante. Plus cette amplitude est grande, plus le son est fort. La période, quant à elle, correspond à l'inverse de la fréquence, ainsi, plus la période est petite et plus le son est aigu. A part dans le cas des sons purs, un son n'est pas composé d'une seule fréquence à un temps donné. La transformée de Fourrier permet de calculer et de représenter la distribution en fréquence d'un son dans un intervalle de temps donné. Cette distribution est appelée spectre fréquentiel (Fig. 1B). En analyse acoustique, elle peut être décrite par les mesures statistiques classiques décrivant les distributions telles que la moyenne, la médiane, le mode, les quartiles etc. (voir figure 1B pour quelques exemples). Enfin, la transformée de fourrier calculée sur des intervalles de temps successifs permet de représenter des variations dans le temps de la distribution en fréquence du son, c'est le sonagramme (ou spectrogramme; Fig. 1C). Cette représentation va permettre de faire différentes mesures, notamment sur la fréquence fondamentale (fréquence la plus basse) et la fréquence dominante (fréquence de plus forte amplitude, elle correspond à la fréquence fondamentale sur la figure 1C). Le sonagramme est la représentation du son la plus commune dans les études sur la communication animale.

I.2.c. De nombreuses informations peuvent être codées dans les signaux acoustiques

Un signal acoustique est défini par trois paramètres : fréquence, amplitude et temps. Les variations de ces trois paramètres les uns par rapport aux autres vont permettre de coder de nombreuses informations. Les signaux acoustiques peuvent tout d'abord renseigner sur l'identité d'un individu. Chez les oiseaux par exemple, les chants permettent de distinguer les espèces y compris certaines qui sont visuellement très proches (Payne, 1986). Ces différences jouent un rôle important dans le choix d'un partenaire (Clayton, 1990) et dans la défense d'un territoire face aux conspécifiques (Becker; Brémond, 1968, 1976) ou aux espèces qui possèdent la même niche écologique (Emlen, Rising, & Thompson, 1975; Catchpole, 1978; Prescott, 1987). Au sein d'une espèce, les signaux acoustiques peuvent différencier les sexes (Rendall *et al.*, 2004; Vignal & Kelley, 2007; Aubin *et al.*, 2007) et même les individus. Dans ce dernier cas on parle de signature individuelle. Cette signature joue un rôle important dans de nombreuses interactions comme dans le lien parents/jeunes (Jouventin, Aubin, & Lengagne, 1999; Sèbe *et al.*, 2007; Catchpole & Slater, 2008; Briefer, Torre, & McElligott, 2012) ou entre les membres d'un couple (Catchpole & Slater, 2008). La signature acoustique individuelle peut également permettre aux individus d'établir une hiérarchie pour éviter les conflits, ou bien de reconnaitre leurs voisins pour éviter de dépenser de l'énergie inutilement dans la défense d'un territoire (« effet cher ennemi » ; Fisher, 1954).

Les signaux acoustiques peuvent également donner de nombreuses informations temporaires sur un individu. Ils peuvent par exemple indiquer une émotion (Manser, 2010; Briefer, 2012), ou un état physiologique comme la réceptivité sexuelle (Langmore *et al.*, 1996; Semple & McComb, 2000) ou la faim dans le cas de jeunes qui quémandent à leur parents (Kilner & Johnstone, 1997). Enfin, les signaux acoustiques peuvent donner des informations sur l'environnement comme la présence de nourriture ou de prédateurs (Bradbury & Vehrencamp, 2011a).

Enfin, parce que la communication peut être un processus interactif entre deux individus ou plus, la façon dont un animal répond à un autre peut aussi coder des informations. Par exemple, lors d'interactions territoriales chez les oiseaux, les mâles peuvent chanter par-dessus le chant d'un rival ou bien adopter le même type de chant que ce dernier (Bradbury & Vehrencamp, 2011a). Ces comportements sont généralement considérés comme des signaux agressifs bien qu'ils puissent avoir des significations différentes selon les espèces (Searcy & Beecher, 2009; Bradbury & Vehrencamp, 2011a).

I.3. La communication acoustique chez les oiseaux chanteurs

I.3.a. Les sons produit par les oiseaux peuvent être distingués en cris et en chants

Les oiseaux produisent une très grande variété de sons. Les oiseaux chanteurs (ou oscines) ont été distingués des autres Passeriformes car ils sont capables de produire des vocalisations extrêmement élaborées qualifiées de chants, qui se distinguent des cris, plus simples. Ces performances sont possibles grâce à un organe particulier aux oiseaux : la syrinx qui est particulièrement complexe chez les oiseaux chanteurs (During *et al.*, 2013).

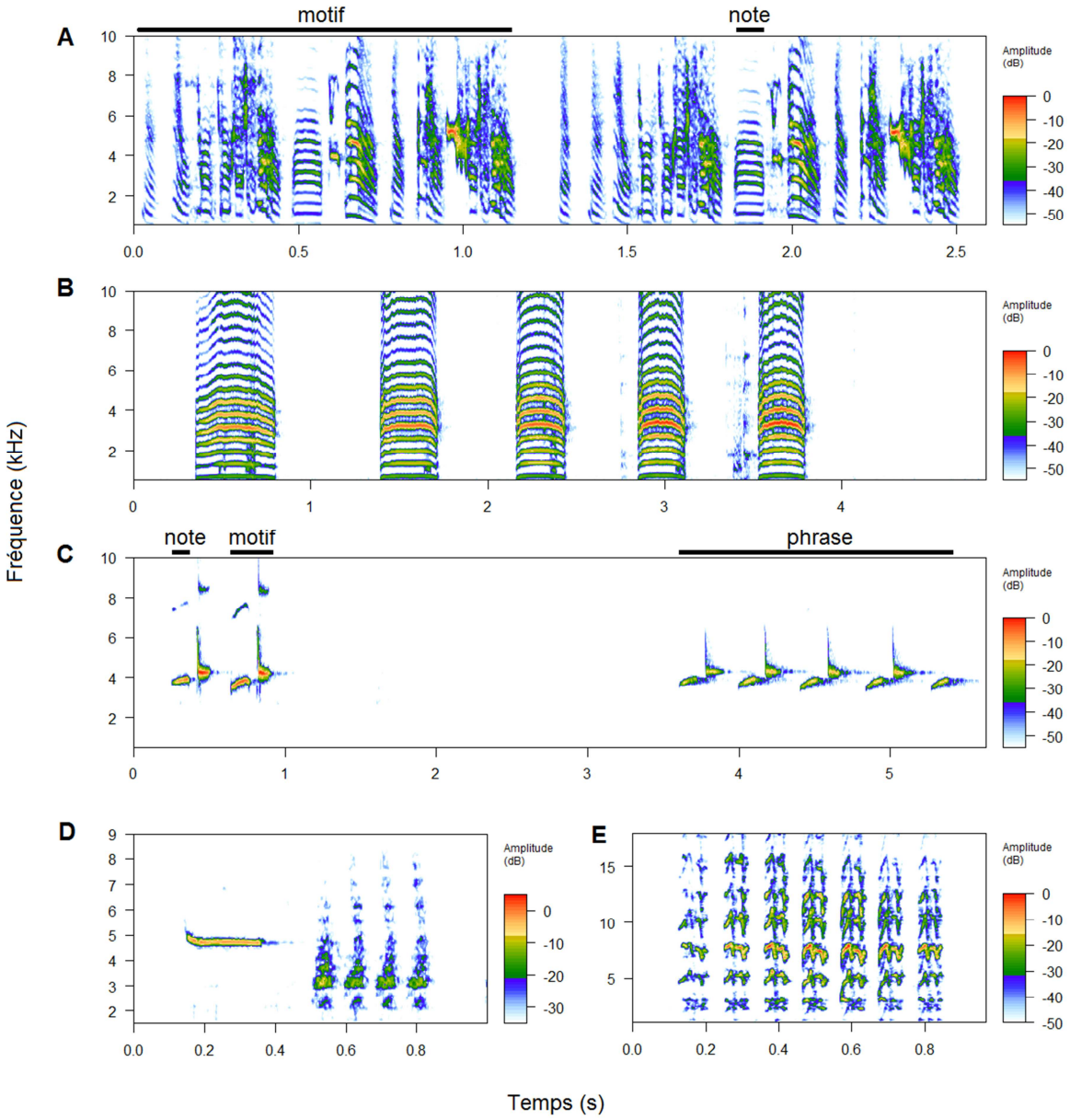

Figure 2. Exemple de chants et de cris chez le diamant mandarin et la mésange charbonnière : A) chant de diamant mandarin mâle ; B) cris de distance de diamant mandarin femelle ; C) chant de mésange charbonnière ; D) cri d'alarme de mésange charbonnière ; E) « chattering » : cris émis par une femelle mésange charbonnière depuis l'intérieur de son nid. On note ici que les cris d'alarme de mésange charbonnière (D) présentent une structure composée de deux types de note et qu'ils ne sont donc pas beaucoup moins complexes que le chant (C).

Les chants ont à l'origine été définis comme des vocalisations longues et complexes produites par les mâles pendant la saison de reproduction (Catchpole & Slater, 2008). Ils sont composés de notes ou syllabes (traces continues sur le sonagramme, séparées par des silences), ordonnées en motifs qui sont eux-mêmes assemblés en phrases (Fig. 2A et 2C). En comparaison, les cris sont des vocalisations plus courtes, et plus simples qui sont produites par les deux sexes tout au long de l'année (Fig. 2B, 2D et 2E). Il existe cependant de nombreuses limites à ces définitions (Catchpole & Slater, 2008). Tout d'abord, cette ancienne idée que seuls les mâles chantent vient de l'observation des espèces des zones tempérées chez qui les femelles ne chantent pas ou moins fréquemment. Mais chez la plupart des espèces tropicales les femelles chantent (Langmore, 1998). De plus, il a récemment été montré que la présence de chants de femelles était bien plus répandue que ce qui était habituellement admis, y compris chez les espèces des zones tempérées, et qu'il pourrait même s'agir d'un caractère ancestral (Garamszegi *et al.*, 2007; Odom *et al.*, 2014). Une autre limite à la définition de chant est que de nombreuses espèces les utilisent en dehors de la période de reproduction (Langmore, 1998). Enfin, il est parfois difficile de dire si un son peut être qualifié de complexe ou non et il existe un recoupement entre les chants simples et les cris complexes (Fig. 2 ; Catchpole & Slater, 2008). Bien que la distinction entre chants et cris soit donc parfois arbitraire, ces termes peuvent s'avérer utiles et restent très employés. Je les utiliserai donc dans la suite de ce manuscrit.

I.3.b. Les chants sont des signaux complexes et ont des fonctions dans la reproduction

Chez les oiseaux, les chants sont très étudiés. En plus d'être des vocalisations facilement détectables et donc faciles à enregistrer, ce sont des signaux acoustiques très élaborés. Ils sont très structurés et donnent lieu à des enchainements d'activité motrice stables et extrêmement complexes. De plus, les chants sont appris d'un ou de plusieurs tuteurs sociaux ce qui peut donner lieu à des dialectes d'une population à une autre (Catchpole & Slater, 2008). Ces raisons font qu'ils sont un sujet d'étude particulièrement intéressant en neuroscience, ou pour étudier les mécanismes de l'apprentissage et de la transmission culturelle.

Chez la plupart des espèces des zones tempérées, le chant des mâles coïncide avec la saison de reproduction et ses fonctions principales sont la défense du territoire et l'attraction de partenaires (Slagsvold, 1977; Catchpole & Slater, 2008). Comme dit plus haut, la présence de chants chez les femelles a été sous-estimée, et de ce fait, moins d'études s'y sont intéressées. Il est suggéré que les chants de femelles pourraient avoir des fonctions similaires à celles des chants de mâles (Beletsky, 1983a,b; Hoelzel, 1986; Langmore & Davies, 1997; Illes & Yunes-Jimenez, 2009; DeVries, Winters, & Jawor, 2014).

I.3.c. Les duos sont une forme de communication interactive chez les couples

S'il existe des espèces chez lesquelles le mâle comme la femelle peuvent chanter, chez certaines, mâle et femelle peuvent même chanter ensemble formant ainsi un duo. Les duos chez les oiseaux sont définis comme des productions de sons vocaux ou non entre deux individus, généralement appariés, qui montrent une certaine coordination temporelle (Farabaugh, 1982; Hall, 2004). Ils ont surtout été étudiés chez des espèces tropicales chez lesquelles mâle et femelle chantent ensemble (Farabaugh, 1982; Hall, 2004). Les duos peuvent avoir différentes fonctions comme maintenir le lien du couple (Hall, 2000; Benedict, 2010), maintenir le contact et permettre la

reconnaissance entre les deux individus (Mays, Yao, & Yuan, 2006; Logue, 2007; Benedict, 2010), synchroniser l'effort de reproduction (Todt & Hultsch, 1982; Weng *et al.*, 2012), limiter les copulations extra-couple (Levin, 1996; Seddon, Butchart, & Odling-Smee, 2002; Grafe & Bitz, 2004; Seddon & Tobias, 2006; Marshall-Ball, Mann, & Slater, 2006; Rogers, Langmore, & Mulder, 2007; Koloff & Mennill, 2011; Weng *et al.*, 2012) et défendre le territoire (Hall, 2000; Grafe & Bitz, 2004; Hale, 2006; Marshall-Ball *et al.*, 2006; Molles & Waas, 2006; Hall & Peters, 2008; Bradley & Mennill, 2009; Benedict, 2010; Koloff & Mennill, 2011; Templeton *et al.*, 2011; Dahlin & Wright, 2012; Weng *et al.*, 2012). Plusieurs études ont montré que ces fonctions n'étaient pas exclusives (Hall, 2000, 2004; Grafe & Bitz, 2004; Marshall-Ball *et al.*, 2006; Benedict, 2010; Koloff & Mennill, 2011; Weng *et al.*, 2012). L'implication des duos n'a cependant encore jamais été envisagée dans l'organisation des soins biparentaux.

En plus de montrer une grande diversité au niveau de leurs fonctions, les duos sont également très diversifiés en termes de structure. 37% des espèces produisant des duos montrent de la variabilité dans la façon dont les éléments sont organisés (Dahlin & Benedict, 2013) et la variabilité inter-espèce est très importante. Même chez des espèces apparentées, les duos peuvent varier depuis des échanges de vocalisations plus ou moins précis jusqu'à des performances très complexes en terme de coordination entre les deux individus (Mann *et al.*, 2009). Dans les duos où les oiseaux alternent des éléments de chant, certains ont une coordination telle qu'il semble n'y avoir qu'un seul oiseau qui chante. Cette coordination exceptionnelle requiert des processus de décision rapide de la part des deux partenaires (Templeton *et al.*, 2013).

Malgré leur grande diversité, les duos n'ont été observés que chez 3 à 4.3% des espèces d'oiseaux (Farabaugh, 1982; Hall, 2009), et bien qu'ils soient généralement associés avec la monogamie à long terme (Farabaugh, 1982; Malacarne, Cucco, & Camanni, 1991; Benedict, 2008; Dahlin & Benedict, 2013), d'autres formes de communication entre les partenaires pourraient exister chez les espèces monogames. La communication interactive des couples pourraient impliquer des vocalisations plus simples comme les cris (Lamprecht *et al.*, 1985; Wright & Dahlin, 2007) ou des vocalisations de faible amplitude (Todt, Hultsch, & Duvall, 1981; Morton & Derrickson, 1996). En particulier, les sons produits par les femelles à l'intérieur du nid et utilisés pour communiquer avec les mâles pourraient être plus répandus que ce que l'on pensait (Ritchison, 1983; Beletsky & Orians, 1985; Yasukawa, 1989; McDonald & Greenberg, 1991; Halkin, 1997).

I.3.d. Les cris sont des signaux souvent discrets mais d'une grande diversité

En communication acoustique, la recherche s'intéresse depuis longtemps aux signaux les plus ostensibles. Ces signaux, généralement de forte amplitude, peuvent se propager jusqu'à de multiples récepteurs et jouent des fonctions importantes dans la territorialité, l'attraction d'un partenaire et le signalement d'un danger (Anderson & Reichard, 2015). Comparativement, peu d'études se sont intéressées aux signaux plus discrets produits par de nombreuses espèces dans les interactions de proximité (Anderson & Reichard, 2015). Dabelsteen et al. (1998) pointent ce fait pour la première fois chez les oiseaux et démontrent en s'intéressant à différentes espèces que les signaux discrets pourraient être répandus. Les oiseaux pourraient en effet avoir besoin de discrétion lors de certains comportements pour éviter par exemple d'attirer des prédateurs ou d'attirer des compétiteurs potentiels lors des parades nuptiales. La communication acoustique à courte distance commence maintenant à être plus étudiée et se retrouve aussi bien chez des amphibiens, des poissons, des oiseaux, des insectes et des mammifères (Reichard & Anderson, 2015). Elle est utilisée dans des contextes multiples comme les parades nuptiales, la communication parents/jeunes, pour garder le

contact avec un partenaire, ou pour signaler un prédateur sans ce faire localiser par ce dernier (synthétisé dans Reichard & Anderson, 2015).

Chez les oiseaux, les cris généralement plus discrets, sont moins étudiés que les chants. Pourtant, certains peuvent être appris de la même façon que ces derniers (Mundinger, 1970, 1979; Marler & Mundinger, 1975; Riebel & Slater, 1998) et il existe une grande diversité dans leurs types et leurs fonctions (Marler, 2004b). La taille du répertoire de cris d'une espèce est difficile à estimer car il est nécessaire d'observer les individus tout au long de l'année, dans différents contextes, et de pouvoir faire des catégories de cris. En effet, il peut s'avérer difficile de faire la distinction entre deux types de cris lorsqu'ils ne sont pas clairement séparés mais qu'il existe un gradient de l'un à autre. Hors il a été montré que les caractéristiques de ces signaux gradés pouvaient coder différentes informations pour les animaux et que la distinction était donc importante (Vergne *et al.*, 2011; Price & Fischer, 2013) Malgré tout, lorsque le répertoire vocal d'une espèce est défini, on observe souvent au moins une dizaine de cris distincts (Gompertz, 1961; Ficken, Ficken, & Witkin, 1978; Saunders, 1983; Collias, 1987; Zann, 1996), même si certaines espèces peuvent avoir des répertoires plus restreints (Marler, 1956). Ces différents cris peuvent être utilisés dans de nombreux contextes comme les agressions, pour signaler la présence d'un prédateur, garder le contact avec des partenaires sociaux lors des déplacements, signaler un endroit où passer la nuit, signaler un endroit où trouver de la nourriture, ou quémander de la nourriture (Marler, 2004). On retrouve également différents contextes d'émission de cris dans la communication des couples. Les cris peuvent par exemple permettre de reconnaitre et de garder le contact avec son partenaire (Robertson, 1996; Vignal, Mathevon, & Mottin, 2008; Eda-Fujiwara *et al.*, 2011; Digby, Bell, & Teal, 2013), de signaler la présence de nourriture (Gyger & Marler, 1988; Evans & Marler, 1994), ou de prédateurs (Krams, Krama, & Igaune, 2006; Colombelli-Négrel, Robertson, & Kleindorfer, 2010). Chez certaines espèces, les femelles font des cris lorsqu'elles quittent le nid. Ces cris peuvent indiquer à leur partenaire qu'elles sont prêtes à copuler ou bien induire une augmentation de la vigilance des mâles face à de potentiel prédateur qui pourraient s'en prendre à la nichée (Beletsky & Orians, 1985; McDonald & Greenberg, 1991; Grunst, Grunst, & Rotenberry, 2014). Enfin, si les cris varient selon les contextes, différents types de cris peuvent également être utilisés dans une même situation, permettant ainsi une communication très fine. Par exemple, chez la mésange charbonnière japonaise (*Parus major minor*) il existe différents type de cris d'alarme chez les adultes qui suscitent des réponses comportementales différentes de la part des poussins mais aussi des autres adultes présents (Suzuki, 2011, 2012, 2014).

I.4. Démarche : comparaison de la communication acoustique des couples de deux espèces pendant la reproduction

Les soins biparentaux sont très étudiés dans le cadre de la théorie du confit entre les sexes (voir partie I.1.d). Alors que les études s'attachent à comprendre comment un parent réagit face à une augmentation ou à une diminution des soins parentaux prodigués par son partenaire, très peu se sont intéressées aux mécanismes qui permettaient aux parents d'ajuster leur comportement l'un en fonction de l'autre et en particulier à la communication entre le mâle et la femelle (voir partie I.1.e). Chez les oiseaux, il a été montré que la communication acoustique jouait un rôle dans certains processus de négociation. Chez la chouette effraie (*Tyto alba*), les poussins communiquent leur niveau de faim en l'absence des parents et laissent l'individu le plus affamé récupérer la première proie qui va être apportée au nid (Roulin & Dreiss, 2012). Chez le cratérope bicolore (*Turdoides bicolor*), les individus se partagent le rôle de sentinelle pour le groupe en communiquant sur leur état

de satiété (Bell *et al.*, 2010). La communication acoustique pourrait également jouer un rôle dans la négociation autour des soins biparentaux. Cependant, peu de choses sont encore connues sur la communication des couples pendant la reproduction. En particulier la communication acoustique pourrait être sous-estimée du fait de sa discrétion. Le diamant mandarin et la mésange charbonnière en sont de bons exemples. Bien que ces deux espèces soient parmi les plus étudiées dans le monde et que leurs comportements aient été décrit en détail (Hinde, 1952; Perrins, 1979; Cramp & Perrins, 1993; Zann, 1996; Griffith & Buchanan, 2010), le fait que les couples fassent des échanges vocaux autour du nid n'a été décrit que très tardivement (Gorissen, Eens, & Nelson, 2004; Gorissen & Eens, 2005; Elie *et al.*, 2010; Halfwerk *et al.*, 2011). Ces observations montrent qu'il est probable que ce type de communication soit présent chez de nombreuses espèces d'oiseaux. Hors, s'il est difficile de suivre et d'enregistrer des oiseaux qui se déplacent, il est en revanche relativement aisé de le faire au niveau du nid et des études futures pourraient permettre de mieux comprendre comment les oiseaux communiquent pour organiser les soins biparentaux.

Dans le cadre de ma thèse, j'ai étudié la communication au nid chez les couples de diamants mandarins et de mésanges charbonnières pendant la reproduction, en particulier pendant l'incubation. Cette période est particulièrement intéressante car la communication à lieu seulement entre le mâle et la femelle alors que les poussins y entreront plus tard en communiquant notamment sur leurs besoins. De plus, à cette période, mâles et femelles peuvent avoir des rôles différents selon les espèces : chez certaines l'incubation va être partagée par les deux parents alors que chez d'autres la femelle seule incube mais peut être nourrie par son mâle (Kendeigh, 1952; Skutch, 1957). Si les oiseaux communiquent effectivement autour de l'incubation, on peut donc s'attendre à une communication ayant une forme et des fonctions différentes entre les deux types d'espèces. C'est pourquoi j'ai choisi d'étudier à la fois une espèce chez laquelle l'incubation est partagée : le diamant mandarin, et une espèce chez laquelle la femelle incube seule : la mésange charbonnière.

I.5. Le diamant mandarin

I.5.a. Les partenaires du couple sont très liés et partagent les mêmes tâches pendant la reproduction

Le diamant mandarin (*Taeniopygia guttata*) est un petit passereau qui vit dans les milieux ouverts de la plupart des zones arides et semi-arides d'Australie (Fig. 3 ; Zann, 1996). Il est nomade, hautement sociable et niche en colonies (Zann, 1996). Les oiseaux se déplacent en groupes pour trouver les graines de graminées qu'ils consomment toute l'année. Ces groupes peuvent aller de moins de 10 individus pendant la saison de reproduction, jusqu'à 150 à 300 oiseaux hors saison (Fig. 3C ; Zann, 1996).

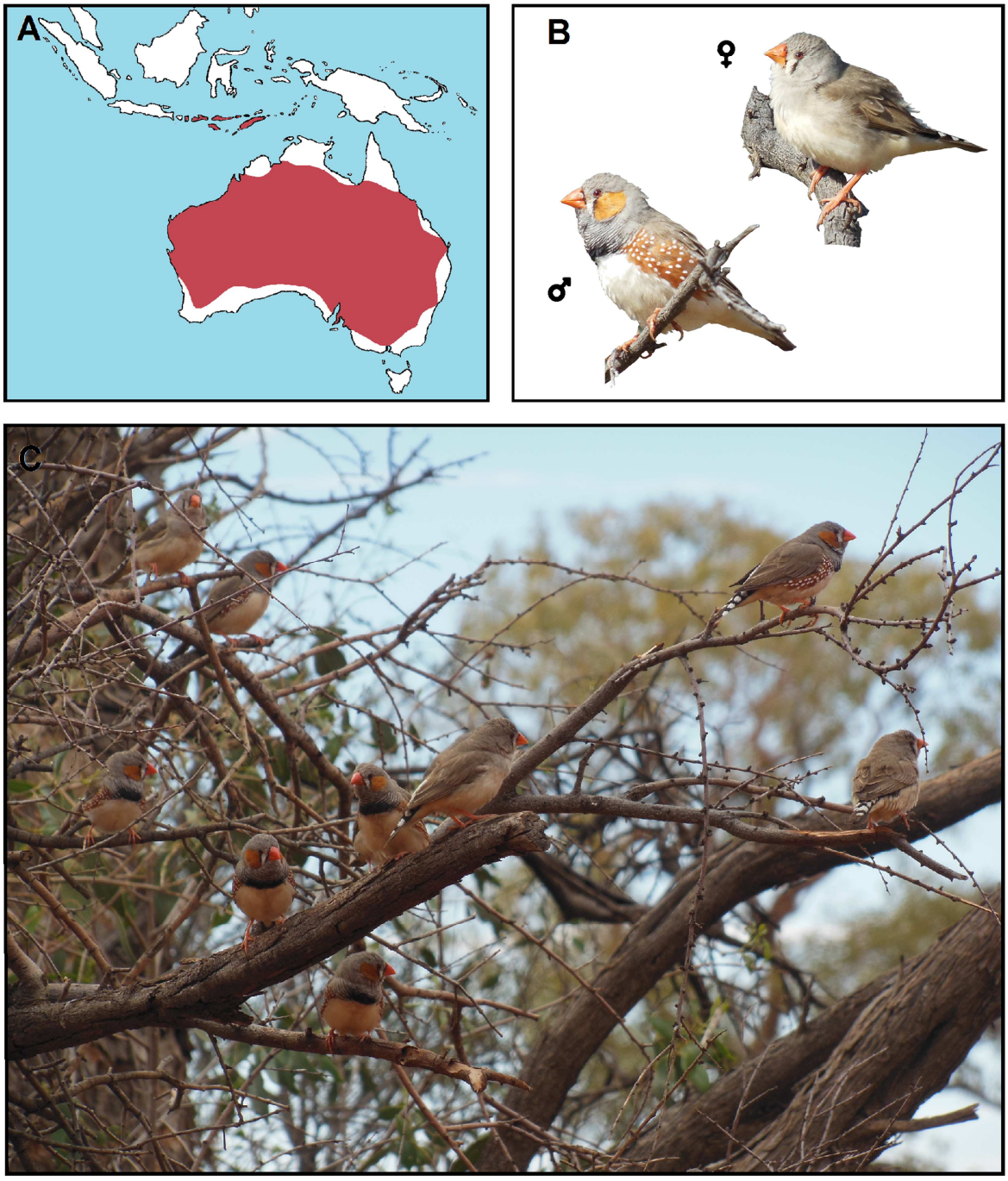

Figure 3. A) Aire de répartition du diamant mandarin, B) mâle et femelle adultes, et C) groupe d'oiseaux dans son environnement.

Les diamants mandarin sont monogames. Ils restent avec le même partenaire toute leur vie (à moins que celui-ci ne meure) et leur taux de paternité extra-couple en milieu naturel (2.4%) est l'un des plus faibles observés chez des passereaux (Zann, 1996; Griffith *et al.*, 2010). Les couples sont parfaitement identifiables par certains comportements spécifiques comme le fait de se percher l'un contre l'autre (« clumping » ; Fig. 4A) ou de se toiletter mutuellement (« allopreening » ; Fig. 4B ; Zann 1996). Les membres d'un couple sont inséparables toute l'année et restent toujours très près l'un de l'autre, excepté lorsqu'un oiseau doit rester dans le nid pendant la reproduction (Zann, 1996).

Le diamant mandarin se reproduit de façon opportuniste lorsque les conditions sont favorables (en général après des pluies qui vont permettre une augmentation de la quantité de graines de graminées dont il se nourrit (Zann, 1996; Perfito *et al.*, 2008). Une saison de reproduction dure 9 à 20 semaines pendant lesquelles un couple peut faire plusieurs nichées (R. A. Zann, 1996). Les soins parentaux sont partagés de façon remarquablement équitable par le mâle et la femelle qui participent tous les deux à la construction du nid, à l'incubation, au nourrissage des jeunes et à la défense du nid (Zann, 1996). Lors de la construction du nid, le mâle apporte les matériaux tandis que la femelle les réarrange pour leur donner leur position finale. Certaines femelles peuvent également aller chercher les petits matériaux qui vont garnir le fond du nid (Zann, 1996). La femelle pond de 2 à 7 œufs à raison de 1 par jour, en général au lever du soleil. L'incubation dure ensuite 12 à 16 jours en milieu naturel (Zann, 1996). La femelle est la seule à développer une plaque incubatrice et à incuber les œufs la nuit (Zann, 1996). Mais durant la journée, le mâle et la femelle se partagent équitablement le temps d'incubation (El-Wailly, 1966; Delesalle, 1986; Zann & Rossetto, 1991; Gorman, Arnold, & Nager, 2005; Gilby, Mainwaring, & Griffith, 2013). Après l'éclosion mâle et femelle se rendent ensemble au nid pour nourrir les poussins dans près de 80% des cas et font autant de visites l'un que l'autre (Mariette & Griffith, 2012). Les poussins s'envolent entre 16 et 18 jours et continuent à être nourris par les deux parents jusqu'à l'âge de 40 jours (Zann, 1996).

I.5.b. Le diamant mandarin a une communication acoustique complexe et est un modèle important dans les études de laboratoire

Chez le diamant mandarin, seul le mâle chante (Fig. 2A). Le chant chez cette espèce représente un modèle pour de nombreuses études sur son développement et sur ses bases neurobiologiques (Griffith & Buchanan, 2010). Le chant du diamant mandarin est appris par imitation d'un tuteur (Immelmann, 1965, 1967, 1969) et nécessite donc la présence de mâles adultes dans l'environnement du jeune (Eales, 1985; Slater, Eales, & Clayton, 1988). De plus, il a été montré qu'un stress alimentaire subi pendant le développement a un impact sur l'apprentissage du chant ('nutritional stress hypothesis' ; Nowicki *et al.*, 1998; Nowicki S., Searcy W., & Peters S., 2002). Les caractéristiques du chant représentent donc un indicateur fiable de la qualité de l'individu car les structures cérébrales nécessaires à leur apprentissage se développent précocement, à une période où l'individu a un risque non-négligeable de subir un stress développemental de nutrition (deKogel & Prijs, 1996; Spencer *et al.*, 2003; Buchanan *et al.*, 2004; Brumm, Zollinger, & Slater, 2009; Holveck & Riebel, 2010). La structure du chant est plus stable s'il est adressé à une femelle en particulier (« directed song ») que s'il n'est pas dirigé (« undirected song » ; Sossinka & Böhner, 1980; Kao, Doupe, & Brainard, 2005). Le chant non dirigé semble être une forme d'entrainement et d'exploration motrice qui pourrait conduire à la performance réussie du chant dirigé (Kao *et al.*,

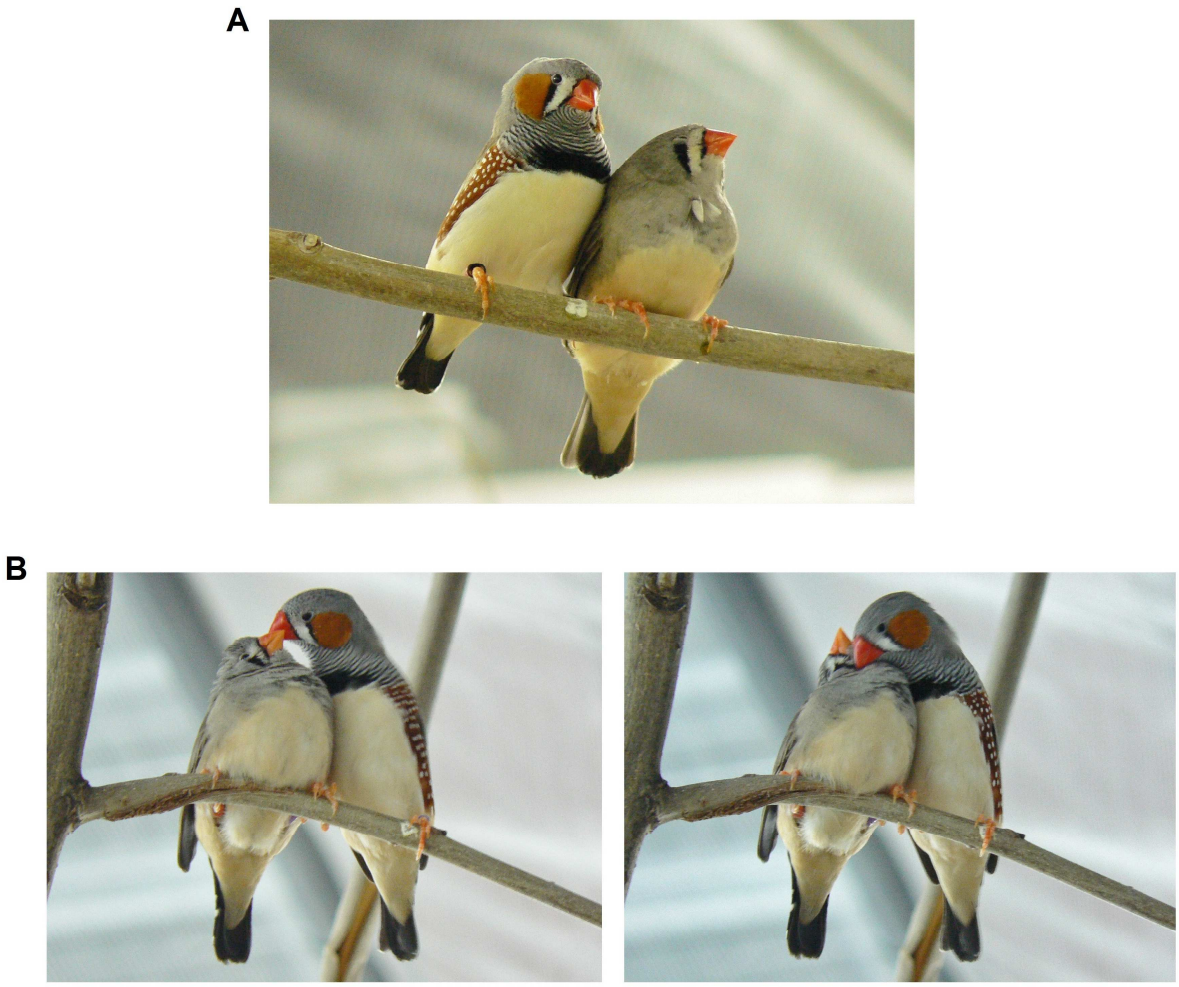

Figure 4. Comportements affiliatifs chez le diamant mandarin : A) « clumping », et B) « allopreening ».

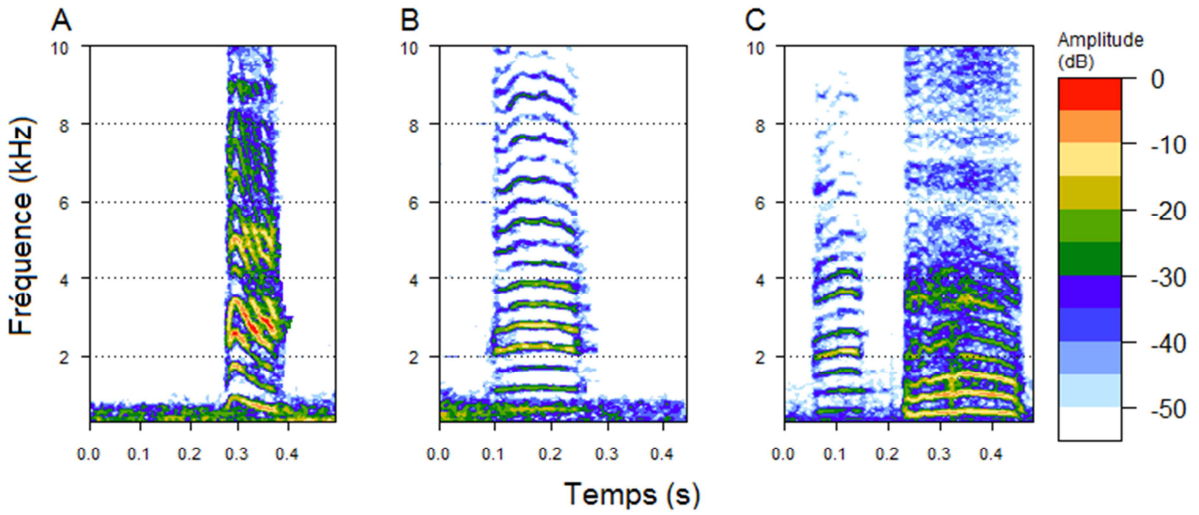

Figure 5. A) Cri de distance d'un mâle, B) cri de distance d'une femelle et D) « tet » suivi d'un « whine ».

2005). Les femelles peuvent distinguer un chant dirigé d'un chant non-dirigé et se dirigent préférentiellement vers le chant dirigé (Woolley & Doupe, 2008) qui va induire des comportements

de sollicitation sexuelle (Clayton & Pröve, 1989). Les chants non dirigés pourraient servir à attirer des femelles soit pour trouver une partenaire soit pour copuler en dehors du couple (Dunn & Zann, 1996a,b). De plus ils pourraient limiter les copulations extra-couple de la part de la femelle en l'incitant à passer plus de temps dans le nid pendant sa période de fertilité (Dunn & Zann, 1996a). Enfin, les diamants mandarins ne défendant pas de territoire et ne montrant pas d'agressivité envers ou de la part des chanteurs, le chant n'a pas de fonction dans la défense d'un territoire (Zann, 1996).

Les poussins utilisent des cris de quémande lorsqu'ils sont nourris par le mâle et la femelle. Ces cris possèdent une signature individuelle qui permet aux parents de les reconnaitre (Levrero *et al.*, 2009). D'autres cris apparaissent ensuite à l'âge adulte. En tout, une douzaine de cris ont été décrit chez le diamant mandarin (Zann, 1996; Elie & Theunissen, 2015) et sont utilisés à la fois par les mâles et par les femelles. Seuls les cris de distance sont différents selon les sexes (Fig. 5A et 5B). De plus ils ont la particularité d'être appris par les mâles et non pas par les femelles (Simpson & Vicario, 1990). Les cris de distance sont généralement utilisés lorsque deux individus se perdent de vue et présentent une signature individuelle qui permet aux partenaires d'un couple de se reconnaitre, et ce même à des distances supérieures à 100m (Vignal *et al.*, 2008; Mouterde *et al.*, 2014a,b). Ces cris permettent également aux juvéniles de reconnaitre leur parents après l'envol du nids (Mulard *et al.*, 2010). Lorsque les individus sont plus proches les uns des autres ils utilisent alors les « Tets » pour rester en contact (Fig. 5C). Une étude préliminaire a montré que ces cris permettent sans doute également la reconnaissance entre partenaires (Elie *et al.*, 2010). Les diamants mandarins ne possèdent pas de cris d'alarme mais leur stress peut être codé dans les cris de distance (Perez *et al.*, 2012), ce stress étant alors communiqué à leur partenaire (Annexe 1).

Lorsqu'un couple choisit l'emplacement de son nid, mâle et femelle utilisent des « Whines » (Fig. 5C), des « Kackles » et des claquements de bec (« nest ceremony » ; Zann 1996). Une communication au nid similaire à récemment été décrite à différents stades de la reproduction et pourrait servir aux parents à synchroniser leurs activités (Elie *et al.*, 2010).

I.6. La mésange charbonnière

I.6.a. Mâle et femelle ont des rôles différents durant la reproduction

Les mésanges charbonnières sont des passereaux communs que l'on trouve dans tous types de zones boisées en Europe, en Afrique du nord et dans une partie de l'Asie (Fig. 6 ; Cramp & Perrins, 1993). Elles nichent dans des cavités naturelles et acceptent également très bien les nichoirs artificiels (Fig. 6B ; Cramp & Perrins, 1993). Les mésanges sont plutôt insectivores en été mais se nourrissent également de graines qui représentent l'essentiel de leur nourriture en hiver (Perrins, 1979).

Les mésanges charbonnières se déplacent en groupes, d'une douzaine d'individus en moyenne, de la fin de l'été jusqu'au début du printemps (Hinde, 1952). Durant cette période, les couples ne sont pas clairement visibles car les oiseaux ne montrent pas de préférence pour la proximité de leur partenaire par rapport aux autres membres du groupe (Hinde, 1952). C'est lorsque le groupe se disperse au début du printemps que les couples apparaissent clairement. Certains

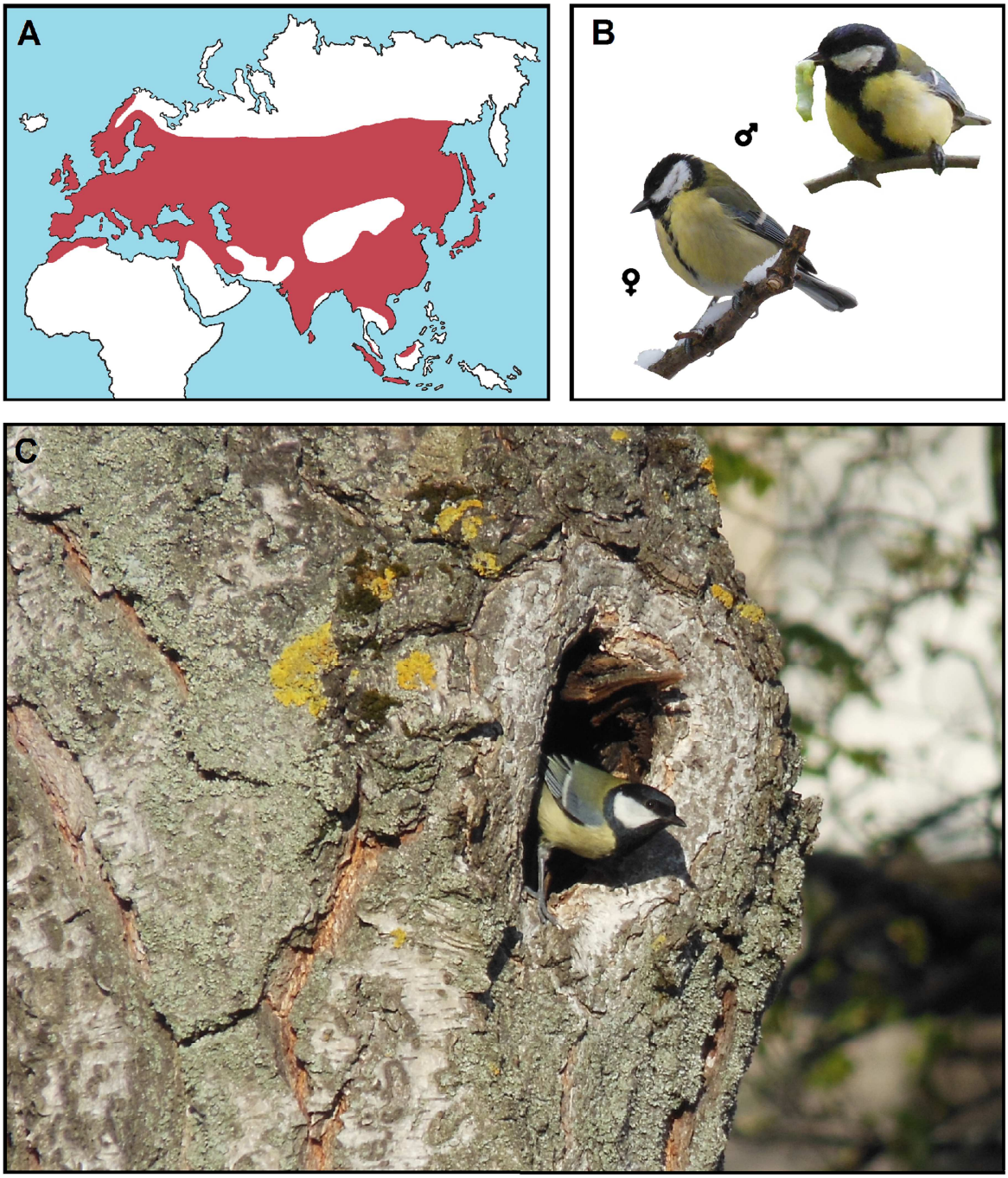

Figure 6. A) Aire de répartition de la mésange charbonnière, B) mâle et femelle adultes se distinguant par la largeur et la longueur de la bande noire se trouvant sur le ventre, et C) individu nichant dans une cavité naturelle.

peuvent être des couples qui se sont déjà reproduits l'année d'avant, les autres se forment probablement à cette période (Hinde, 1952). Les territoires se forment ensuite progressivement jusqu'au début de la construction du nid (Hinde, 1952).

Pendant la saison de reproduction, les mésanges charbonnières peuvent faire une ou deux nichées (Hinde, 1952; Perrins, 1979). Les premières pontes ont lieu en mars/avril. Le début de la reproduction dépend de plusieurs facteurs comme la température (Schmidt, 1984), la disponibilité en nourriture (Källander, 1974), l'habitat, l'altitude et l'âge de la femelle (Cramp & Perrins, 1993). La première nichée est généralement démarrée de façon à ce que les poussins arrivent lorsque la disponibilité en nourriture est la plus importante (Perrins, 1979). La femelle seule s'occupe de la construction du nid et de l'incubation (Hinde, 1952; Cramp & Perrins, 1993). Elle pond de 3 à 18 œufs (Cramp & Perrins, 1993) à raison de un chaque matin, généralement avant qu'elle n'émerge du nid après y avoir passé la nuit (Perrins, 1979). L'incubation dure de 12 à 15 jours (Cramp & Perrins, 1993). Durant cette période, la femelle sort régulièrement du nid pour se nourrir (Perrins, 1979). Elle reste encore très présente dans le nid les jours suivant l'éclosion pour couver les poussins. C'est alors majoritairement le mâle qui nourrit les jeunes (Hinde, 1952). Petit à petit la femelle passe moins de temps dans le nid et plus à nourrir les poussins. Mâle et femelle finissent par faire le même nombre de nourrissages en alternant leurs visites au nid (Johnstone *et al.*, 2014). Après l'envol, les jeunes sont encore dépendants de leurs parents pendant une semaine (Hinde, 1952).

Pendant la saison de reproduction, le mâle nourrit la femelle (Fig. 7). Les nourrissages commencent pendant la ponte, s'intensifient pendant l'incubation et continuent jusqu'à l'envol des jeunes (Hinde, 1952; Royama, 1966). Royama (1966) estime que le mâle nourrit la femelle en moyenne 57 fois par jour pendant la ponte et 160 par jour pendant l'incubation, suggérant que ces nourrissages pourraient être un apport énergétique conséquent pour la femelle, comme cela a été montré chez d'autres espèces ('female nutrition hypothesis' ; Moreno *et al.*, 2011).

I.6.b. La mésange charbonnière a une communication complexe

Le chant consiste en des séquences de motifs composés en général de 2 notes, mais qui peuvent contenir jusqu'à 4 notes (Fig. 2C ; Hinde, 1952). Ce sont principalement les mâles qui chantent et le chant a alors des fonctions dans la défense du territoire (Hinde, 1952; Krebs, Ashcroft, & Webber, 1978) et dans l'attraction de partenaires (Krebs, Avery, & Cowie, 1981). Les femelles chantent plus rarement et les fonctions de leur chant ne sont pas connues (Hinde, 1952). Les adaptations des chants des mâles aux bruits urbains sont très étudiées chez cette espèce. Plusieurs études ont notamment montré que les chants produits en ville étaient plus aigus, ce qui leur permettaient d'être dans des fréquences au-dessus de celles des bruits ambiants et donc d'être potentiellement mieux perçus (Slabbekoorn & Peet, 2003; Slabbekoorn & den Boer-Visser, 2006; Mockford & Marshall, 2009). Les mâles possèdent un répertoire de 1 à 7 types de chants différents qui sont appris (Mcgregor & Krebs, 1982; Lambrechts & Dhondt, 1986; Rivera-Gutierrez, Pinxten, & Eens, 2010). Ces chants portent la signature individuelle du mâle qui peut ainsi être reconnu par ses voisins (Krebs, 1971) ainsi que par sa femelle (Lind, Dabelsteen, & McGregor, 1996).

Le répertoire de cris de la mésange charbonnière est particulièrement varié. Gompertz (1961) a décrit 10 catégories différentes de cris et a observé jusqu'à 40 cris distincts chez un individu.

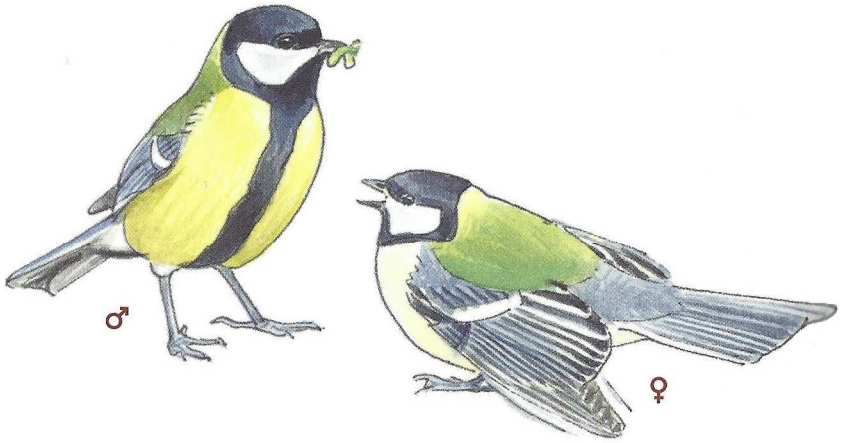

Figure 7. Femelle sollicitant son mâle pour obtenir de la nourriture.
(Lesaffre G. 2007. Le traité Rustica des oiseaux du jardin. Paris.)

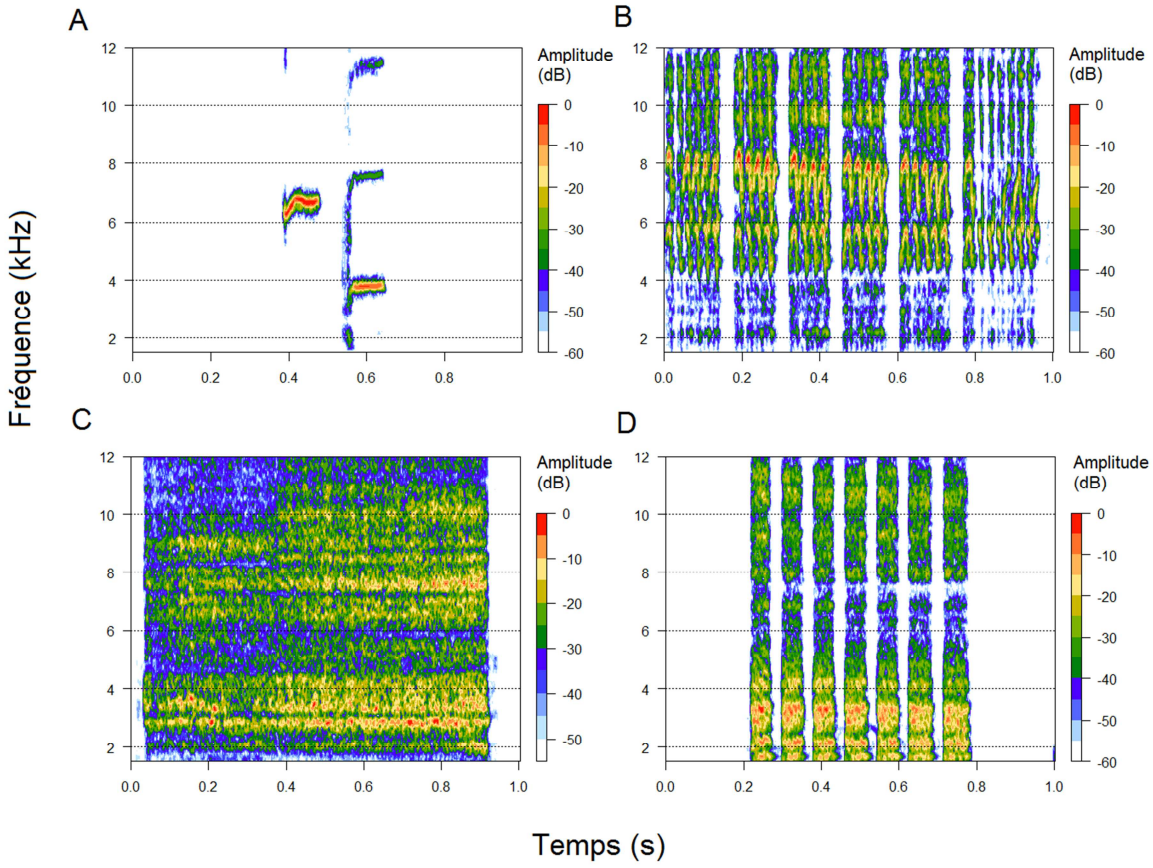

Figure 8. Notes « tsee » et « pee » (A), ainsi que différents cris produits par les femelles depuis l'intérieur du nid : B) « chattering », C) « screaming » et D) « churring ».

Comme chez le diamant mandarin, les poussins n'utilisent que des cris de quémande lorsqu'ils sont encore au nid. Peu à peu ils développent des cris spécifiques aux juvéniles puis acquièrent les cris des adultes (Gompertz, 1961). Parmi ces cris, trois notes (« Tsee », « pee » et « tink ») peuvent être combinées de différentes façons et utilisées dans des contextes variés (Gompertz, 1961; Cramp & Perrins, 1993 ; Fig. 8A). Certaines combinaisons sont utilisées lorsque les oiseaux se déplacent et pourraient correspondre à des cris de contact (Gompertz, 1961). Les femelles, comme les jeunes après l'envol, utilisent des cris de quémande qui sont associés à une posture particulière : les oiseaux s'aplatissent au sol en faisant vibrer leurs ailes et en étalant les plumes de leur queue (Fig. 7 ; Hinde, 1952; Perrins, 1979). Pendant la saison de reproduction, les femelles ont également été observées faire des cris avant d'entrer ou de sortir du nid (Perrins, 1979) et plus récemment il a été montré que les femelles pouvaient répondre à leur mâle depuis l'intérieur du nid en utilisant 8 types de cris différents (Gorissen *et al.*, 2004; Gorissen & Eens, 2005; Halfwerk *et al.*, 2011 ; Fig. 8B, 8C et 8D).

I.7. Approche : étude de la communication des couples au nid

Pour chaque espèce, j'ai placé des micros dans et à proximité des nids de façon à pouvoir enregistrer la communication entre le mâle et la femelle. Ces enregistrements ont pu être accompagnés d'observations visuelles et/ou d'enregistrements vidéo à l'intérieur du nid. Pour chaque espèce mon étude s'est faite en deux temps. Parce que très peu de choses étaient connues sur la communication des couples au nid, j'ai observé sur le terrain dans quels contextes cette communication avait lieu et comment elle différait d'un contexte à un autre. J'ai également fait une étude expérimentale pour chaque espèce (sur le terrain pour la mésange charbonnière et en volière pour le diamant mandarin) afin de déterminer plus précisément les fonctions possibles de cette communication.

I.7.b. Les couples de diamants mandarins communiquent-ils pour se partager le temps d'incubation ?

A part dans le cas de la recherche d'un emplacement pour faire un nid (Zann, 1996), la communication au nid entre le mâle et la femelle diamants mandarins n'a été décrite que très récemment. Elie et *al*. (2010), ont décrit deux types d'échanges vocaux : les « meetings » et les « sentinels ». Les « meetings » ont lieu lorsqu'un oiseau retrouve son partenaire dans le nid, par exemple en apportant des matériaux de construction. L'oiseau qui est revenu pourra alors soit repartir, soit relever son partenaire au nid et prendre son tour d'incubation. Les « sentinels » ont lieu, quant à eux, avec un oiseau à l'intérieur du nid et l'autre à l'extérieur. Ce dernier semble alors vigilant à la présence de potentiels prédateurs (la prédation des nids naturels est responsable de l'échec de plus de 60% des nichées chez cette espèce et les adultes comme les poussins peuvent être touchés ; Zann, 1996; Griffith, Pryke, & Mariette, 2008). La proportion de « meetings » est plus importante pendant la construction du nid et la ponte, puis diminue progressivement en faveur des « sentinels » au cours de la reproduction. Les cris les plus utilisés pendant ces échanges sont les « tets », les « whines » (Fig. 5D) et les cris de distance. Les « tets » sont plus utilisés par l'oiseau qui se trouve en dehors du nid au début de l'échange alors que les « whines » sont plus utilisés par celui qui se trouvait dans le nid.

Dans cette thèse j'ai d'abord enregistré des couples en milieu naturel de façon à comparer les différents types d'échanges vocaux produits pendant l'incubation. J'ai pu observer que les oiseaux

faisaient bien des duos de « sentinel » et j'ai distingué les « meetings » en relèves et en simples visites (Chapitre 1). Les diamants mandarins ont la particularité d'être très coordonnés pendant l'incubation en se partageant de façon parfaitement équitable le temps passé dans le nid (El-Wailly, 1966; Delesalle, 1986; Zann & Rossetto, 1991; Gorman *et al.*, 2005; Gilby *et al.*, 2013). J'ai donc testé si les différents duos observés pouvaient avoir un rôle dans cette coordination en indiquant à quel moment la relève devait se faire. De plus, j'ai testé expérimentalement cette hypothèse dans un deuxième temps en retardant le retour du mâle au nid en volière. J'ai étudié l'impact de ce retard sur la communication du couple pendant la relève mais également l'impact de cette communication sur le temps que chaque oiseau va passer dans le nid par la suite (Chapitre 2).

I.7.c. Les femelles de mésanges charbonnières indiquent-elles leur besoins en nourriture à leur partenaire lorsqu'elles doivent rester au nid pour incuber les œufs ?

Chez la mésange charbonnière**,** c'est la femelle qui passe du temps dans le nid pour couver les œufs et plus tard les jeunes poussins (Hinde, 1952). Dès les années 1986-1987, Mace, remarquait que le pic de chant des mâles durant le chorus de l'aube a lieu pendant la ponte, alors que les couples et les territoires sont déjà bien établis (Mace, 1987). De plus, le mâle diminue fortement la fréquence à laquelle il chante juste après l'émergence de la femelle qui a passé la nuit dans le nid (Mace, 1986). Ces observations indiquent que le chant ne semble pas avoir seulement un rôle dans la défense du territoire et l'attraction de nouvelles partenaires mais doit être adressé en partie à la femelle. Ces observations sont confirmées plus tard par une étude montrant que les femelles sortent de leur nid en réponse à un playback de chant de leur mâle pendant l'incubation (Lind *et al.*, 1996). De plus, les femelles peuvent répondre à leur mâle depuis l'intérieur du nid (Gorissen *et al.*, 2004; Gorissen & Eens, 2005) et les mâles ajuster leur réponse en chantant plus près du nid si les femelles mettent plus de temps à répondre (Halfwerk, Bot, & Slabbekoorn, 2012).

Dans cette thèse, j'ai observé que cette communication avait lieu dans différents contextes, en partie liés au nourrissage de la femelle par le mâle (Chapitre 3). Chez certaines espèces, ces nourrissages peuvent jouer un rôle important sur la qualité de l'incubation en permettant aux femelles de passer plus de temps dans le nid (Lyon & Montgomerie, 1985; Lifjeld & Slagsvold, 1986; Hałupka, 1994; Klatt *et al.*, 2008; Lloyd *et al.*, 2009; Stein *et al.*, 2009; Matysioková *et al.*, 2011; Moore & Rohwer, 2012; Matysiokova & Remes, 2014). J'ai dans un deuxième temps testé si la femelle pouvait utiliser ces échanges vocaux pour communiquer son niveau de faim à son mâle (Chapitre 4). J'ai dans ce but enregistré des couples dans deux conditions pendant l'incubation : une condition dans laquelle la femelle disposait de nourriture à volonté dans le nid et une situation contrôle sans apport en nourriture.

RESULTATS

Chapitre 1

Vocal duets at the nest in the wild zebra finch: mates signal when they are ready to take turns incubating and foraging

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En préparation pour soumission

Synthèse du chapitre 1

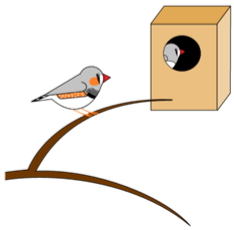
Question : Les partenaires signalent-ils lors des duos s'ils vont rester dans le nid ou sortir?

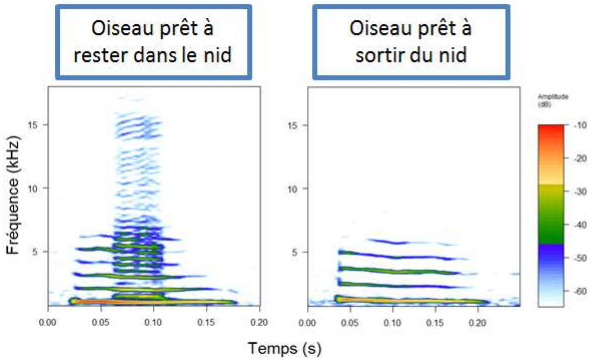
Méthodes : Couples enregistrés au nid en milieu naturel pendant l'incubation

Résultats principaux

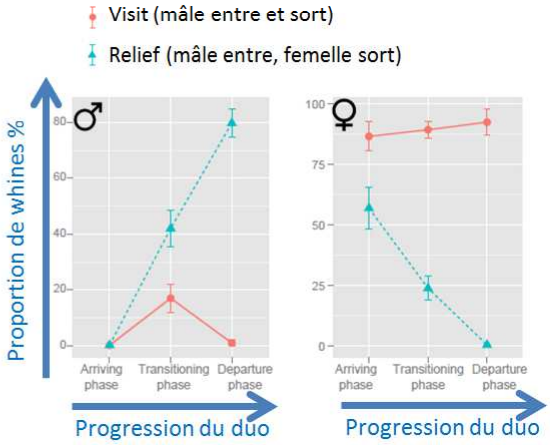
→ Les diamants mandarins font des duos dans trois contextes différents :

- Sentinel : un oiseau est dans le nid tandis que son partenaire est à l'extérieur
- Visit : un oiseau rejoint son partenaire dans le nid (en général avec des matériaux de construction) et ressort
- Relief : un oiseau entre dans le nid et prend la place de son partenaire sur les œufs et ce dernier sort se nourrir





→ Les cris des mâles et des femelles ('whines') sont plus graves et moins bruités quand les oiseaux s'apprêtent à sortir du nid



→ Mâle et femelle font plus de 'whines' lorsqu'ils vont rester dans le nid à la fin du duo

Conclusion

Les oiseaux indiquent dans leur répertoire vocal et dans la structure de leurs cris s'ils sont prêts à rester dans le nid ou non. Les duos semblent donc jouer un rôle dans le partage de l'incubation entre le mâle et la femelle.

ABSTRACT

In species with bi-parental care, parents adjust their workload to the one of their partner. In birds, the coordination between mates might be particularly important in species in which both male and female incubate the eggs. Whether mates communicate to adjust their behaviour during this period is poorly know. In zebra finch, mates performed vocal duets at the nest. In a previous study, we found that duets performed during the nest-relief (when birds take turns incubating and foraging) play a role in the share of incubation time between mates. In the present study, we investigated the role of the duets produced outside the reliefs. During each incubation bout, the foraging mate may visit its incubating partner, by coming in the nest or staying outside, and birds perform a duet during these encounters. We tested whether the structure of the duet predicted the outcome of the exchange (nest-relief or not). To do so, we recorded wild zebra finches at the nest during incubation. We found, that partners indicated their readiness to leave or to stay in the nest by modifying their vocal repertoire as well as the acoustic structure of one call type which is typically used inside the nest. Duets produced during incubation may thus allow deciding when it is time for mates to do a nest-relief.

Keywords: Acoustic communication - Bi-parental care – Calls – Coordination – Incubation - Intra-pair communication – Monogamy - Negotiation – Songbird

INTRODUCTION

In species with bi-parental care, parents adjust their workload to the one of their partner. Models predict that parents may either compensate (Houston & Davies, 1985; Jones, Ruxton, & Monaghan, 2002; J. M. McNamara, Houston, Barta, & Osorno, 2003; John M. McNamara, Gasson, & Houston, 1999) or match (Johnstone & Hinde, 2006) the parental effort of their mate. Several empirical studies have tested these models by experimentally decreasing or increasing the parental effort of an individual to observe modifications in the behaviour of its partner (Lessells, 2012). But fewer studies have investigated the mechanisms allowing this adjustment. For example, matching can be reached by coordinating parental activities. In the zebra finch (*Taeniopygia guttata*) and in the long-tailed tit (*Aegithalos caudatus*), parents coordinate their foraging trips and their visits to the nest to feed the nestlings (Mariette & Griffith, 2012, 2015; Meade, Nam, Lee, & Hatchwell, 2011), and in the great tit, parents alternate their visits to feed the nestlings (Johnstone et al., 2014).

The coordination between mates might be particularly important in species with bi-parental incubation. In these species, partners take turns incubating the eggs and the transition when the foraging partner relieves the incubating partner, a nest-relief, is a crucial step. A failure of relief would leave the nest unattended and could have important impacts on the clutch (Ball & Silver, 1983; Spoon, Millam, & Owings, 2006). Incubation maintains the eggs in an optimal range of temperature for embryonic development (Farmer, 2000). Nest attentiveness has thus positive impacts on hatching success (Carey, 1980; Lyon & Montgomerie, 1985; Reid, Monaghan, & Ruxton, 2002), fledglings condition (Reid et al., 2002), but also parents' and offspring's survival because it shortens the duration of the incubation period and consequently the exposition to nest predators (Magrath, 1988; T E Martin, Scott, & Menge, 2000; Thomas E Martin, 2002). Moreover, re-adjusting eggs' temperature that has changed during absences may represent a major energetic cost for parents. For instance, the metabolic rate of the incubating parent during re-warming phases may increase threefold in the zebra finch *Taeniopygia guttata* (Vleck, 1981).

In species with bi-parental incubation there is substantial variation in male participation. Males can spend less time incubating than females (eg.Kleindorfer, Fessl, & Hoi, 1995; Klimczuk et al., 2015; Smith & Montgomerie, 1992) or both partners can share incubation more or less equally (e.g. Bulla, Valcu, Rutten, & Kempenaers, 2014; Yoon, Ha, Jung, & Park, 2015). There is also great variation in the duration of an incubation bout, from few tens of minutes (e.g. Yoon et al., 2015) to several hours (e.g. Bulla et al., 2014; Ekanayake et al., 2015), and even up to several days in some species (e.g. Widmann et al., 2015; Williams & Croxall, 1991). The duration of the incubation bout may be controlled by the returning bird, especially in species that have to forage far from the nest (e.g. Widmann et al., 2015; Williams & Croxall, 1991). But in species in which mates can stay in contact during incubation, the incubating bird can control the duration of a bout by deciding to leave the nest when its partner is nearby (Ball & Silver, 1983). Finally, mate may decide together when it is time to do the relief and this process may involve communication.

Whether the nest-relief involves any communication between partners is still poorly known. In the ring dove (*Streptopelia risoria*), male and female incubate the eggs and the non-incubating bird can initiate a nest-relief by various behaviours such as allopreening (Ball & Silver, 1983). In several species, mates perform a display, referred as a nest-relief ceremony, when taking turn incubating and foraging (Wachtmeister, 2001). Intra-pair acoustic communication at the nest has been described in several species (Elie et al., 2010; Gorissen, Eens, & Nelson, 2004; Grunst, Grunst, & Rotenberry, 2014; Halfwerk, Bot, & Slabbekoorn, 2012; Halkin, 1997) and could be used by mates to coordinate nest-relief. Because female vocalizations have long been neglected (Odom, Hall, Riebel, Omland, & Langmore, 2014; Riebel, 2003; Riebel, Hall, & Langmore, 2005), little is known on

interactive communication between males and females (Wachtmeister, 2001) with the exception of acoustic duets (Benedict, 2008; Dahlin & Benedict, 2013; Farabaugh, 1982; M. Hall, 2004; M. L. Hall, 2009). Avian duets are joint acoustic displays between two birds that make temporally coordinated vocal or non-vocal sounds. Among them, the highly coordinated song duets of tropical songbirds are the most studied. These duets have been hypothesized to fulfil several functions, such as pair-bond maintenance, mate guarding, territory defence or synchronization of reproductive effort. But the possibility that duets could play a role in parental care, and more precisely in the coordination of bi-parental incubation, has never been explored.

Although song duetting is rare (approximately 4% of bird species), interactive communication between mates involving simpler and less conspicuous vocalizations is likely to be more widespread (Lamprecht, Kaiser, Peters, & Kirchgessner, 1985; Morton & Derrickson, 1996; Todt, Hultsch, & Duvall, 1981; Wright & Dahlin, 2007). Until recently, most research on bioacoustics has focused on high amplitude signals that project over long distances and can simultaneously influence multiple types of receivers (Anderson & Reichard, 2015; Reichard & Anderson, 2015). But in songbirds there is increasing evidence that not only songs but also calls, which are usually discrete low amplitude signals, play a role during breeding (Elie et al., 2010; Gill, Goymann, Ter Maat, & Gahr, 2015; Ter Maat, Trost, Sagunsky, Seltmann, & Gahr, 2014).

Zebra finches maintain life-long pair bonds and both parents contribute to nest building, incubation and chicks' provisioning (Zann, 1996). Mates show a high degree of coordination of their activities during nestlings feeding (Mariette & Griffith, 2012, 2015) and by sharing incubation time equally (Delesalle, 1986; El-Wally, 1966; Gilby, Mainwaring, & Griffith, 2013; Gorman, Arnold, & Nager, 2005; R. Zann & Rossetto, 1991). Zebra finches use lots of calls throughout the year and some of them are particularly used during breeding (Elie et al., 2010; Gill et al., 2015; R. A. Zann, 1996). Moreover, during breeding, parents perform call duets each time they meet at the nest (Boucaud, Mariette, Villain, & Vignal, *in press*.; Elie et al., 2010). In a previous study, we demonstrated that the duets performed when partners take turns incubating and foraging - the nest-relief - function as vocal negotiation processes over incubation share (Boucaud et al. *in press*). Indeed, we found that disrupting the pair's usual nest-relief pattern by delaying the male's return induced shorter duets with higher call rates. Moreover, females spent less time incubating during their subsequent shift, and females´ time off-nest was best predicted by their mate's calling behaviour in the previous duet. These results show that duets of nest-relief play a role in the adjustment of partners' contribution to incubation. Between two reliefs, mates also use other duets that do not end with a nest exchange. So mates might use duets to communicate if it is time or not to make a relief. In that case, duets would encode mates' readiness to leave or to stay in the nest. If this holds true, duets ending with a nest-relief should be acoustically different from other duets. In this study, we tested this hypothesis by recording wild zebra finch pairs during incubation. We first compared female and male behaviours by analyzing the structure of relief duets produced by (1) the returning male and the incubating female and (2) the returning female and the incubating male. Then we compared the duets ending with a relief with duets that did not. We analyzed both the temporal structure of the duets and the acoustic structure of one call type specifically used by both male and female in the nest.

METHODS

Study site

Data were collected from October to early November 2012 at Fowlers Gap Arid Zone Research Station, western New South Wales, Australia (31°05'S, 142°42'E). The study area at Gap Hills is a breeding area of typical zebra finch habitat (Acacia spp. trees and shrubs) situated adjacent to a permanent dam, which was likely used as the main water source for all individuals (S. C. Griffith, Pryke, & Mariette, 2008). It had been provided with 191 identical nest boxes constructed of 8-mm plywood with internal dimensions of 140mm (length) x 93mm (breadth) x 180mm (depth at the back) x 120mm (depth at the front). Birds entered the box through a round hole, 30mm in diameter. All nest-boxes were fixed on individual steel stakes 1–1.8 m off the ground and within 2 m of an acacia bush (S. C. Griffith et al., 2008).

Recording

Eighteen breeding pairs were recorded during incubation stage on two consecutive days (except for two pairs recorded for the second time two days after the first recording). The birds were not banded but no pair or individual could have been recorded at two different nests because all recordings took place in a short time window (25 days) relative to the length of a pair's breeding attempt and because monogamy is strict in this species (S. Griffith, Holleley, Mariette, Pryke, & Svedin, 2010; R. A. Zann, 1996).

Just before recording, a tie microphone (Audio-Technica AT803) was installed inside the nest-box and connected to a digital recorder (Zoom H4n, 44.1 kHz, 16 bit). During the recording session an observer, sitting in a hide 10-15m from the nest, recorded partners' behaviour (presence of partners in the nest area and movements in and out of the nest-box). Recordings started between 07:30 and 14:00. The equipment inside and around their nest-box did not significantly disturb the birds. The observer stopped the recording after observing three relief duets. Thus the mean duration of a recording was 3.7 ± 0.2 hours.

Duet analysis

We define duets as a sequence of male and female calls that either alternate or partly overlap. A duet started with the first call of one mate answered by its partner within 10 seconds, and lasted until one bird left or both birds stopped calling for at least 10 seconds. We distinguished three types of duets:

(i) Relief duets were performed when partners took turns incubating the eggs. The returning bird relieved its incubating partner at the nest, so they alternated foraging trips.

(ii) Nest visit duets were performed when the returning mate just visited its mate at the nest, for instance bringing nest materials, but did not take its turn incubating and left the nest at the end of the duet.

(iii) Sentinel duets were performed with one mate inside the nest and the other staying outside, located between 20 cm and 5 m from the nest (Elie et al., 2010).

For each recording, we used GoldWave software (https://www.goldwave.com/) to extract two reliefs, one with the female returning to the nest and one with the male returning, as well as one nest visit (with the male visiting the incubating female) and one sentinel (with the male outside and the female incubating) when it was possible. Nest visits and sentinels with the male incubating were

rare, so our sample size was too limited for an analysis. We took care to extract only duets occurring after the first relief to be sure that both birds had time to habituate to the equipment inside and around the nest. From a total of 36 recordings (two recording days for 18 pairs), we obtained 35 reliefs with the female returning the nest, 33 reliefs with the male returning the nest, 36 nest visits and 29 sentinels, so a total of 133 duets analyzed.

Reliefs and nest visits can be split into three different phases:

(i) Arriving phase: the duet begins when the returning mate is still outside the nest (usually less than 2 meters from the nest entrance) whereas its incubating partner is inside.

(ii) Transitioning phase: the returning bird meets its partner inside the nest.

(iii) Departure phase: the departure phase starts when the incubating bird (in reliefs), or the returning bird (in nest visit) goes out of the nest. During this phase, mates continue interacting vocally until the duet ends when the leaving bird leaves the nest area (or when both birds stop calling for more than 10 seconds).

Note that in reliefs the transitioning phase could happen outside the nest: the incubating bird met its partner at the nest's entrance. In these cases, the two birds vocalize outside the nest during this phase and the departure phase starts when the returning bird goes inside the nest. The duration of these three phases was measured manually on the acoustic recordings using PRAAT software (http://www.fon.hum.uva.nl/praat/). Birds make characteristic noises when entering or exiting the boxes allowing us to detect precisely these events.

Each extracted duet was manually labeled using Praat software which displays both the oscillogram and the spectrogram of the recording (view range: 0-20kHz; window length: 0.02s; dynamic range: 60dB). For each vocalization, we recorded the identity of the caller and the call type. The identity of the caller was determined by visual observation of the birds' behaviours (described above) and the characteristics of the recordings. At the beginning and the end of the duets, caller identity was easily established because one bird was calling from inside the box and the other one from outside. The bird inside the box was closer to the microphone than the bird outside, and its calls were therefore a lot louder on recordings. When both birds were inside the nest-box, we managed to attribute calls to individuals using the individual signature of the calls.

In zebra finches, males and females use the same vocal repertoire, except song which is produced only by males (R. A. Zann, 1996). Moreover, the repertoire used during duets does not depend on the bird's sex but depends on the bird's position inside or outside the nest (Elie et al., 2010). In this study, we distinguished three types of vocalizations:

(i) Short calls. Short calls are harmonic stacks generally shorter than 100 ms. Zebra finches show a continuum of soft short calls used in close social context (R. A. Zann, 1996); Tets, Cackles, Arks) as well as louder Distance Calls (R. A. Zann, 1996); around 100 ms for males, 200 ms for females). The acoustic conditions of the nest-boxes (creating attenuation and reverberation of the sound) did not allow us to accurately discriminate between these different short calls, so we grouped them into the single call type 'Short Call'.

(ii) Whines. Whine is a soft, long (around 300 ms, but with high variation) and high pitched moan. This 'pleading' sound is a vocalization specifically uttered inside the nest (R. A. Zann, 1996).

(iii) Song. Males sang in 22 out of the 133 duets.

(iv) Bill-clicking. Bill-clicking are beak clattering that have already been described in birds searching for a nest site and performing nest ceremony (R. A. Zann, 1996).

After labeling, each recording was analyzed using custom-written codes implemented in R (R Development Core Team, 2014). From the list of calls, we measured temporal parameters of the duet sequence and of each phase separatly: the total duration, the total number of calls, the average call rate (number of calls per minute defined as the total number of calls divided by the time spent calling). The relative participation of the partners was measured as the proportion of males' call among the total number of calls. To describe the repertoire used, as song was rare and birds used only two call types (whines and short calls), we measured whine proportion, as the percentage of whine calls among all calls in the duet. Because song and bill-clicking were not systematically used in duets we only considered if they were present or not.

Analysis of the acoustic feature of whine calls

Because most short calls were produced either outside the nest or when the two birds were inside the nest-box creating overlaps, the quality of the recordings did not allow analyzing acoustic features. On the contrary, the acoustic features of male and female whine calls could be measured. To automatically select calls without overlap and of good recording quality, we selected calls produced when only one bird was inside the nest-box. We analyzed a total of 625 whines from the females (nest visits: 144; sentinels: 121; reliefs with the female returning: 185; reliefs with the male returning: 175) and 335 whines for the male (reliefs with the female returning: 107; reliefs with the male returning: 228).

Calls were then analyzed using custom-written codes implemented in R. The duration of each call (in s) was measured between edges labeled using Praat software. After bandpass filtering (400Hz-18000Hz encompassing the spectral bandwidth of this type of vocalizations, 'fir' function) the following spectral parameters were computed using the 'specprop' function (FFT using a Hamming window and a window length of 512):

- As the frequency spectrum is a distribution, it can be characterized using classic descriptive statistics (all in Hertz). We used the median, the first (Q25) and third (Q75) quartiles of the spectrum of the call – which represent the frequencies below which lie respectively 50%, 25% and 75% of the energy of the call. The IQR (Inter Quartile Range) was defined as Q75-Q25. We also used the mean and standard deviation (sd) of the spectrum.

- The call's noisiness can be quantified using the Shannon spectral entropy. The Shannon entropy of a noisy signal tends towards 1 whereas it tends towards 0 for a pure tone.

Recording of Birds' Activities

For each recording day, we measured the duration of one male and one female incubation bout (time spent inside the nest by one bird between two reliefs). During these two incubation bouts we counted the number of nest visits and sentinel duets.

To determine whether birds carried nest material when returning to the nest during relief or visits, we used video recordings simultaneous to the acoustic recordings inside the nest (DVR-500-L recorder connected to a micro video camera 120JN-OBJ from Active Media Concept). Direct observations were not sufficient because birds can bring small nest materials not always visible to the observer at a distance from the nest. Four nests were successfully video-recorded during the two days of acoustic recording and three nests during only one day. Only events during which the beak of the returning bird was clearly visible on the video were taken into consideration. We thus observed 82 nest visits with the male returning, 19 nest visits with the female returning, 16 reliefs with the

male returning and 18 reliefs with the female returning, so a total of 135 events. We calculated the proportion of nest visits and reliefs during which the returning bird carried nesting material.

Ethical Note

This work was conducted under the authorities of the Animal Ethics Committees at the University of New South Wales and Macquarie University, and a Scientific Research Permit from the New South Wales Parks and Wildlife Service.

Statistical Analysis

All statistical tests were performed using R software (R Development Core Team, 2014). Comparisons of proportions were performed using tests of equal or given proportion ('prop.test' and 'pairwise.prop.test' functions). Following all linear mixed models (LMM, 'lmer' function of the lme4 library), equivariance and distribution of the residuals were graphically checked using 'plotresid' function (RVAideMemoire library). For each model, marginal and conditional coefficients of determination are presented (r2m and r2c, 'r.squaredGLMM' function of the MuMIn library, Nakagawa & Schielzeth, 2013). P-values on models were obtained using Wald Chi-square tests ('Anova' function, car library). Post-hoc tests were performed using the 'lsmeans' function (lsmean library) using Tukey contrasts.

To avoid multiple testing, PCA were used to compute composite scores whenever possible, i.e. when more than three parameters were available on one aspect (e.g. parameters describing the temporal structure of the duet: number of calls, duration, duration of the arriving phase, duration of the transitioning phase). A total of four PCA were performed. Before being included in the PCA, some parameters were transformed to reach a symmetrical distribution (Table 1). Principal components with eigenvalue above 1 (Kaiser criterion) were selected. All variable loadings are given in table 1.

Do female and male behaviours differ during incubation?

The effect of the sex on the mean time spent in the nest by each bird between two nest reliefs was tested using a LMM with the sex of the bird (two levels) as fixed factor and the pair identity (18 levels) as random factor. Because the time spent in the nest was an average over the two recording days, the day of recording was not included as a random factor. The LMM was written as follows: Time spent in the nest ~ Sex of the bird + (1|Pair identity).

Does the Acoustic Structure of Relief Duets Depend on the Sex of the Returning Bird?

To test the effect of the sex of the returning bird on the temporal structure of relief duets, a first PCA was computed on the number of calls, the duration, the duration of the arriving phase, and the duration of the transitioning phase (Table 1). Two other PCA were performed on the acoustic parameters of the whine calls to compare 1) calls of returning and leaving females, 2) calls of returning and leaving males during relief duets. After each PCA, PC1 and PC2 were analyzed using LMMs. LMMs included the sex of the returning bird (two levels) as fixed factor and the pair identity

Table 1. Variable loadings of the PCA on a) the structure of relief duets, b) the acoustic structure of male whine calls during relief duets, c) the acoustic structure of female whine calls during relief duets and, d) the acoustic structure of female whine calls in the arriving phase of sentinel, visit and relief duets with the male returning.

a)	**Variable**	**Transformation**	**PC1**	**PC2**
	Explained variance (%)		**58.13%**	**27.57%**

Number of calls	log10	-0.88	0.06
Duration of the duet	log10	-0.94	-0.07
Duration of the arriving phase	log10	-0.52	-0.79
Duration of the transitioning phase	log10	-0.63	0.68

b)

Variable	**Transformation**	**PC1**	**PC2**
Explained variance (%)		**50.45%**	**29.57%**
Duration	square-root	-0.10	-0.62
Mean	none	-0.97	0.06
Sd	none	-0.53	0.73
Mode	log10	-0.15	-0.77
Median	log10	-0.77	-0.51
First quartile (Q25)	none	-0.43	-0.83
Third quartile (Q75)	none	-0.95	0.19
Inter quartile range (IQR)	log10	-0.88	0.39
Shannon spectral entropy	none	-0.91	-0.05

c)

Variable	**Transformation**	**PC1**	**PC2**
Explained variance (%)		**58.31%**	**22.06%**
Duration	square-root	-0.12	-0.67
Mean	none	-0.98	0.10
Sd	none	-0.67	0.58
Mode	log10	-0.32	0.71
Median	log10	-0.87	-0.29
First quartile (Q25)	none	-0.63	-0.67
Third quartile (Q75)	none	-0.95	0.21
Inter quartile range (IQR)	log10	-0.90	0.32
Shannon spectral entropy	none	-0.92	0.02

d)

Variable	**Transformation**	**PC1**	**PC2**
Explained variance (%)		**50.19%**	**25.05%**
Duration	square-root	-0.18	0.63
Mean	none	-0.97	-0.09
Sd	none	-0.61	-0.57
Mode	log10	-0.39	0.68
Median	log10	-0.74	0.47
First quartile (Q25)	none	-0.53	0.74
Third quartile (Q75)	none	-0.93	-0.26
Inter quartile range (IQR)	log10	-0.80	-0.46
Shannon spectral entropy	none	-0.82	-0.08

(18 levels) and the day (two levels: 1st day and 2nd day) as random factors. LMMs were written as follows: Variable ~ Sex of the returning bird + (1|Pair identity) + (1|Day). The same LMM was used to test the effect of the sex of the returning bird on the whine proportion.

To test the effect of the sex of the returning bird on the relative participation of partners (measured as the proportion of male's calls) during the relief duets, a similar model was performed including the phase (three levels: arriving, transitioning, departure) as an additional fixed factor and the interaction. This LMM was written as follows: Proportion of male calls ~ Sex of the returning bird * Phase + (1|Pair identity) + (1|Day).

Does the Acoustic Structure of the Duet Predict Male Entrance In the Nest?

To test whether the structure of the duet predicts male entrance, we compared the arriving phase between sentinel, visit and relief duets with the male returning. A PCA was performed on the acoustic structure of the whine calls of incubating females (Table 1). PC1 and PC2 of this PCA, as well as the parameters describing the temporal structure of the arriving phase (duration, number of calls, call rate, whine proportion) were compared using LMMs with the context (three levels: sentinel, visit and relief) as fixed factor and the pair identity (18 levels) and the day (two levels: 1st day and 2nd day) as random factors. LMMs were written as follows: Variable ~ context + (1|Pair identity) + (1|Day).

Does the Acoustic Structure of the Duet Predict That the Male will take its turn incubating?

Finally, the parameters of visit and relief duets (duration, number of calls, call rate, whine proportion of the male, whine proportion of the female, proportion of male calls) were compared using LMMs with the context (two levels: visit and relief), the phase (three levels: arriving, transitioning, departure) and their interaction as fixed factors and the pair identity (18 levels) and the day (two levels: 1st day and 2nd day) as random factors. LMMs were written as follows: Variable ~ Context * Phase + (1|Pair identity) + (1|Day).

RESULTS

Do female and male behaviours differ during incubation?

Between two reliefs, a bird spent around 45 minutes on the eggs and this time did not differ between males and females (Table 2). Considering two incubation bouts per bird, visit duets occurred more when the female was incubating than when the male was incubating. Sixteen out of 18 males came to visit when the female was incubating whereas 2 out of 18 females came when the male was incubating (Test of Equal or Given Proportions: females-male=0.11-0.89=-0.78, 95CI=[-1.00; -0.52], Chi^2=18.78, df=1, p<0.001). Fifteen pairs performed sentinel duets when the female was incubating whereas 11 pairs performed sentinel duets when the male was incubating, but this difference was not significant (females-male=0.61-0.83=-0.22, 95CI=[-0.56; 0.12], Chi^2=1.25, df=1, p=0.264).

In almost all nest visits, the returning bird brought nest materials and there was no difference between males and females (females-male=1.00-0.99=0.01, 95CI=[-0.02; 0.05], Chi^2=0, df=1, p=1). Birds also brought nest materials in the majority of the reliefs and again there was no difference

Table 2. Statistical analysis comparing incubation bouts' duration between male and female

	Estimate ± SE	R2m	R2c	Chi²	Df	P-value
Incubation bouts' duration (min)		0.02	0.46	1.31	1	0.253
(Intercept)	48.91 ± 6.21					
Male	- 7.45 ± 6.51					

Table 3. Statistical analysis of the effect of the sex of the returning bird on relief duets.

	Estimate ± SE	R2m	R2c	T-ratio	Chi²	Df	P-value
PC1 temporal structure		0.02	0.27		1.92	1	0.165
(Intercept)	0.19 ± 0.40						
Male	- 0.46 ± 0.33						
PC2 temporal structure		0.01	0.08		0.43	1	0.511
(Intercept)	- 0.11 ± 0.27						
Male	0.17 ± 0.25						
Whine proportion (%)		<0.01	0.19		0.07	1	0.797
(Intercept)	0.25 ± 0.03						
Male	0.01 ± 0.04						
Proportion of male calls		0.21	0.22				
Sex of the returning bird					1.01	1	0.313
Phase					1.18	1	0.554
Sex of the returning bird * Phase					48.02	1	**<0.001**
Sex of the returning bird (arriving phase)				-4.75		174	**<0.001**
Sex of the returning bird (transitioning phase)				1.40		173	0.727
Sex of the returning bird (departure phase)				5.03		174	**<0.001**
(Intercept)	38.95 ± 4.21						
Male	29.70 ± 6.11						
Transitioning phase	13.61 ± 5.81						
Departure phase	24.12 ± 5.94						
Transitioning phase * Male	- 37.41 ± 8.52						
Departure phase * Male	- 59.51 ± 8.58						

Significant P-values are shown in bold. PC: principal component of the principal component analysis.

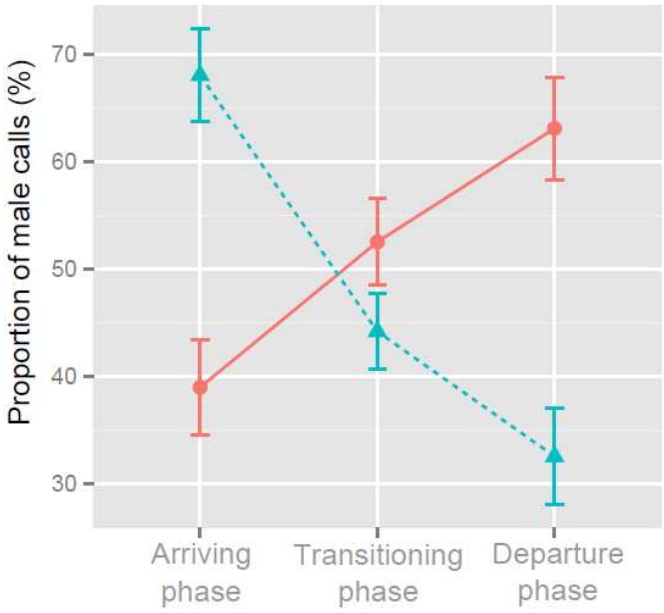


Figure 1. Proportion of male calls during the three phases of a relief duet when the returning partner is the female (F, red) or the male (M, blue).

between males and females (females-male=0.61-0.81=0.20, 95CI=[-0.56; 0.81], Chi^2=0.83, df=1, p=0.363).

Does the Acoustic Structure of Relief Duets Depend on the Sex of the Returning Bird?

The sex of the returning bird during the relief had no impact on the duet's structure (duration, number of calls, and phases' duration; Table 3), the whine proportion (Table 3), the presence of bill-clicking (Test of Equal or Given Proportions: female-male=0.80-0.76=0.04, 95CI=[-0.18; 0.27], Chi^2=0.01, df=1, p=0.897), or the presence of song (female-male=0.17-0.03=0.14, 95CI=[-0.02; 0.31], Chi^2=2.29, df=1, p=0.130). The relative participation of partners to the duet (measured as the proportion of male calls) did not depend on the sex of the birds but on their positions inside or outside the nest: during the arriving phase, the returning bird outside the nest produced more calls than its incubating partner, and during the departure phase, the departing bird produced more calls than its partner who just took its turn incubating (Fig. 1; Table 3).

The structure of whine calls differed between returning and leaving birds. When males returned to the nest, their whine calls had lower values of PC1 and PC2 corresponding to longer calls with energy in higher frequency bands (higher mean, mode, median and Q25), differences in frequency bandwidth (higher IQR but lower sd) and more spectral noise (higher entropy) (Table 4; Fig. 2A). This difference between returning and leaving calls was similar in females. When the females returned to the nest, their whine calls had lower values of PC1 corresponding to calls with energy in higher frequency bands (higher mean, median, Q25 and Q75), larger frequency bandwidth (higher IQR and sd) and more spectral noise (higher entropy)(Table 4; Fig. 2B).

Does the Acoustic Structure of the Duet Predict Male Entrance In the Nest?

When comparing the arriving phase of relief, visit and sentinel duets performed between the returning male and the incubating female, the call rate (call/s) and the whine proportion were not significantly different (Table 5). Contrastingly, the number of calls was significantly lower in visits than in relief duets, and the duration of the duet was significantly lower in visits than in reliefs and sentinel duets (Table 5). In sentinel duets, the incubating female never used bill-clicking during the arriving phase whereas she did in visit and relief duets (Test of Equal or Given Proportions: relief-visit=0.30, visit=0.28, sentinel=0.00, Chi^2=10.63, df=2, p=0.005, p(relief-sentinel)=0.012, p(relief-visit)=1.000, p(visit-sentinel)=0.012). Moreover the males sang more often in sentinel duets (relief=0.15, visit=0.00, sentinel=0.48, Chi^2=24.52, df=2, p<0.001, p(relief-sentinel)=0.022, p(relief-visit)=0.050, p(visit-sentinel)<0.01).

Whine calls of the incubating female had higher PC1 values in relief than in sentinel duets and tended to have higher values in relief than in visit duet. Higher values of PC1 corresponded to more energy in lower frequency bands (lower mean, median and Q75), smaller frequency bandwidth (lower IQR and sd) and less spectral noise (lower entropy; Table 4; Fig. 3). Whines calls had also lower PC2 values in relief than in sentinel, so were shorter with more energy in lower frequency bands (lower mode and Q25; Table 4; Fig. 3).

Table 4. Statistical analysis of the acoustic structure of whine calls of a) returning and leaving males, b) returning and leaving females, c) females depending on the context (relief, sentinel or visit).

a)

	Estimate ± SE	R2m	R2c	Chi²	Df	P-value
PC1		0.02	0.33	9.11	1	**0.002**
(Intercept)	0.42 ± 0.58					
Returning	-0.74 ± 0.25					
PC2		0.05	0.37	19.48	1	**<0.001**
(Intercept)	0.99 ± 0.40					
Returning	-0.78 ± 0.18					

b)

	Estimate ± SE	R2m	R2c	Chi²	Df	P-value
PC1		0.06	0.48	21.17	1	**<0.001**
(Intercept)	-0.36 ± 0.43					
Leaving	1.16 ± 0.25					
PC2		<0.01	0.44	0.16	1	0.685
(Intercept)	-0.49 ± 0.34					
Leaving	-0.06 ± 0.16					

c)

	Estimate ± SE	R2m	R2c	T-ratio	Chi²	Df	P-value
PC1		0.05	0.35		16.83	2	**<0.001**
Relief vs visit				-2.12		435	0.086
Sentinel vs visit				1.67		435	0.219
Sentinel vs relief				3.46		436	**0.001**
(Intercept)	0.13 ± 0.53						
Relief	0.49 ± 0.27						
Sentinel	-0.63 ± 0.30						
PC2		0.05	0.38		20.38	2	**<0.001**
Relief vs visit				1.80		398	0.171
Sentinel vs visit				-1.92		86	0.14
Sentinel vs relief				-3.53		307	**0.001**
(Intercept)	0.51 ± 0.27						
Relief	-0.68 ± 0.17						
Sentinel	-0.02 ± 0.19						

Significant P-values are shown in bold. PC: principal component of the principal component analysis.

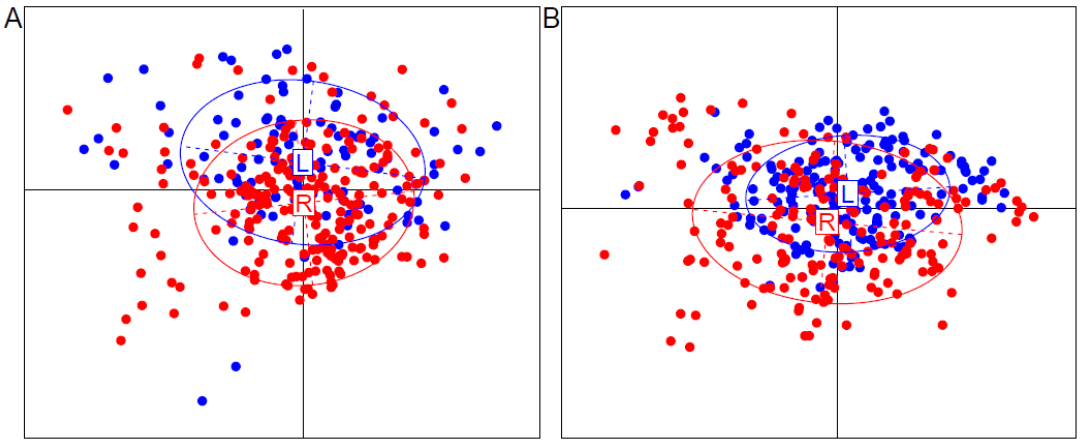


Figure 2. Acoustic structure of whine calls of returning and leaving birds A) male whine calls and B) female whine calls (R: returning; L: leaving). In A) lower values of PC1 indicate energy in higher frequency bands (higher mean, median and Q25), larger frequency bandwidth (higher IQR) and more spectral noise (higher entropy). Lower values of PC2 indicate longer calls with energy in higher frequency bands (higher mode and Q25) and lower frequency bandwidth (lower sd). In B) lower values of PC1 indicate energy in higher frequency bands (higher mean, median, Q25 and Q75), larger frequency bandwidth (higher IQR and sd) and more spectral noise (higher entropy). Lower values of PC2 indicate longer calls with energy in higher frequency bands (higher mode and Q25). Ellipses show 67% of the data points in each group.

Table 5. Statistical analysis comparing the structure of the arriving phase between relief, visit and sentinel duets.

	Estimate	±	R2m	R2c	T-ratio	Chi²	Df	P-value
Duration (s, log10)			0.09	0.16		9.45	2	**0.008**
Relief vs visit					-2.31		77	0.059
Sentinel vs visit					-2.59		71	**0.031**
Sentinel vs relief					-0.46		74	0.889
(Intercept)	0.85 ± 0.08							
Relief	0.25 ± 0.11							
Sentinel	0.30 ± 0.11							
Number of calls			0.08	0.24		10.13	1	**0.006**
Relief vs visit					-3.16		76	**0.006**
Sentinel vs visit					-1.52		71	0.288
Sentinel vs relief					1.33		74	0.382
(Intercept)	0.92 ± 0.07							
Relief	0.29 ± 0.09							
Sentinel	0.15 ± 0.09							
Call rate (call/s)			0.03	0.14		3.49	2	0.174
(Intercept)	0.4 ± 0.05							
Relief	0.02 ± 0.05							
Sentinel	0.09 ± 0.05							
Whine proportion (%; log10)			0.03	0.28		3.29	2	0.19
(Intercept)	0.06 ± 0.01							
Relief	0.004 ± 0.02							
Sentinel	0.02 ± 0.02							

Significant P-values are shown in bold.

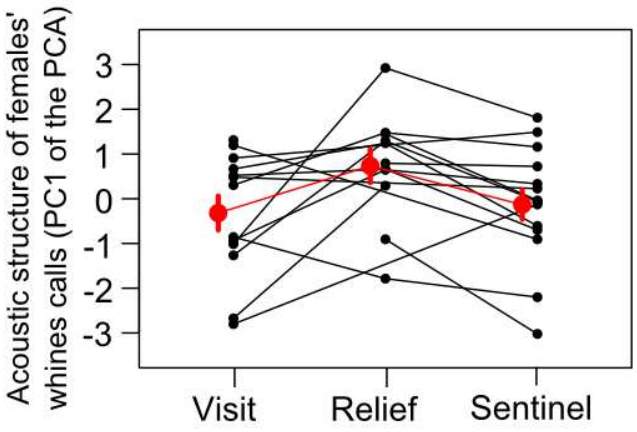


Figure 3. Effect of the context on the acoustic structure of the whine calls of the incubating female. Higher values of PC1 indicate more energy in lower frequency bands (lower mean, median and Q75), smaller frequency bandwidth (lower IQR and sd) and less spectral noise (lower entropy).

Does the Acoustic Structure of the Duet Predict That the Male will take its turn incubating?

When comparing the whole duet performed between the returning male and the incubating female, relief duets were longer with more calls than visit duets (Table 6). During the transitioning phase, the call rate was higher in relief than in visit duets (Table 6). The proportion of whine calls used by returning males was higher during the transitioning and departure phases in relief than in visit duets (Table 6, Fig. 4). The proportion of whine calls used by incubating females was lower in all phases of relief duets compared to visit duets (Table 6, Fig. 4). Finally, the proportion of male calls in the departure phase was higher in visit than in relief duets (Table 6), so here again we found that the bird outside the nest contributed more to the duet.

DISCUSSION

Both males and females had whine calls that differed in acoustic structure depending on whether the bird was returning or leaving the nest. On average, before a bird left the nest its whine calls had energy in lower frequency bands and less spectral noise. Thus, birds seem to indicate their readiness to return or leave the nest. This result is confirmed by the comparison of the whine calls of the incubating female between the three contexts of duet (sentinel, visit or relief). Whine calls had energy in lower frequency bands and less spectral noise in relief duets thus in the context in which the female subsequently left the nest. In the zebra finch, a physiological stress induces modifications of the acoustic structure of contact calls (Perez et al., 2012) and mates can perceive this difference in their partner's calls (Perez et al., 2015). In males stressed by social isolation or by an oral administration of corticosterone, contact calls had energy in higher frequency bands (Perez et al., 2012). In our results, the bird using whine calls with energy in higher frequency bands was the bird ready to stay in the nest. If stress is encoded in whine calls using the same parameters as in contact calls, birds ready to stay would be more stressed than birds ready to leave the nest. This is inconsistent with the idea that the duration of the incubation bout is determined by an energy reserves threshold, because in that case birds ready to leave the nest should be more stressed. Another hypothesis is that the information about stress is encoded differently in contact calls and whine calls.

The proportion of whine calls among total calls also seems to predict the end of the duet, as it was more important in visit than in relief duets for the incubating female and in relief than in visit for the returning male. We found that both males and females used relatively less whine calls when they left the nest. To sum up, both the phonology (repertoire use, i.e. proportion of whine calls) and the prosody (spectral structure of whine calls) predict which bird will stay in the nest at the end of a duet.

The temporal structure of the duet also changed between visit and relief duets. Relief duets were longer with more calls and a higher call rate. Such modifications of the temporal structure of the duets have been found to play a role in incubation share between male and female (Boucaud et al., *in press*). Indeed, we found in a previous study that a delay in male return to the nest induced shorter relief duets with higher call rates. Moreover, the more accelerated the duet, the longer the time off nest of the female after the relief. A higher call rate could thus code the urgency to do the relief: a low call rate indicating that the relief can be done later and a high call rate that the relief is rather late. As previously described by other studies, we found that male and female share incubation time equally (Delesalle, 1986; El-Wailly, 1966; Gilby et al., 2013; Gorman et al., 2005; R.

Table 6. Statistical analysis comparing the structure of relief and visit duets.

	Estimate ± SE	R2m	R2c	Chi^2	T-ratio	Df	P-value

Duration (s; log10)		0.12	0.20				
Context				27.17		1	**<0.001**
Phase				0.26		2	0.879
Context * phase				1.84		2	0.392
(Intercept)	0.85 ± 0.07						
Relief	0.25 ± 0.09						
Transitioning phase	- 0.002 ± 0.09						
Departure phase	- 0.09 ± 0.09						
Relief * transitioning phase	- 0.05 ± 0.13						
Relief * departure phase	0.12 ± 0.13						
Number of calls (log10)		0.30	0.34				
Context				72.35		1	**<0.001**
Phase				11.62		2	**0.002**
Context * phase				3.84		2	0.146
(Intercept)	0.92 ± 0.06						
Relief	0.28 ± 0.08						
Transitioning phase	0.06 ± 0.08						
Departure phase	- 0.06 ± 0.08						
Relief * transitioning phase	0.22 ± 0.11						
Relief * departure phase	0.11 ± 0.11						
Call rate (calls/s; log10)		0.12	0.17				
Context				6.96		1	**0.008**
Phase				13.20		2	**0.001**
Context * phase				9.60		2	**0.008**
Context (arriving phase)					-0.34	176	0.999
Context (transitioning phase)					-4.05	175	**0.001**
Context (departure phase)					-0.14	176	1.000
(Intercept)	0.40 ± 0.03						
Relief	0.02 ± 0.05						
Transitioning phase	0.03 ± 0.05						
Departure phase	0.02 ± 0.05						
Relief * transitioning phase	0.17 ± 0.07						
Relief * departure phase	- 0.01 ± 0.07						
Whine proportion of the male		0.61	0.63				
Context				106.00		1	**<0.001**
Phase				95.96		2	**<0.001**
Context * phase				102.46		2	**<0.001**
Context (arriving phase)					0.03	165	1.000
Context (transitioning phase)					-4.65	164	**<0.001**
Context (departure phase)					-13.57	169	**<0.001**
(Intercept)	0.35 ± 3.87						
Relief	- 0.18 ± 5.46						
Transitioning phase	16.71 ± 5.23						
Departure phase	0.92 ± 5.30						
Relief * transitioning phase	25.00 ± 7.61						
Relief * departure phase	78.72 7.90						
Whine proportion of the female		0.63	0.70				
Context				262.71		1	**<0.001**

Phase			30.81		2	**<0.001**
Context * phase			37.57		2	**<0.001**
Context (arriving phase)				4.00	149	**0.001**
Context (transitioning phase)				10.73	142	**<0.001**
Context (departure phase)				13.02	146	**<0.001**
(Intercept)	85.13 ± 5.67					
Relief	- 29.27 ± 7.26					
Transitioning phase	4.90 ± 6.68					
Departure phase	5.94 ± 7.39					
Relief * transitioning phase	- 37.22 ± 9.47					
Relief * departure phase	- 60.67 ± 9.96					
Proportion of male calls		0.32 0.36				
Context			27.12		1	**<0.001**
Phase			41.39		2	**<0.001**
Context * phase			26.44		2	**<0.001**
Context (arriving phase)				1.58	178	0.612
Context (transitioning phase)				0.26	177	0.999
Context (departure phase)				7.13	178	**<0.001**
(Intercept)	78.05 ± 4.36					
Relief	- 10.08 ± 6.37					
Transitioning phase	- 32.29 ± 6.01					
Departure phase	- 1.19 ± 6.06					
Relief * transitioning phase	8.45 ± 8.89					
Relief * departure phase	- 34.20 ±8.88					

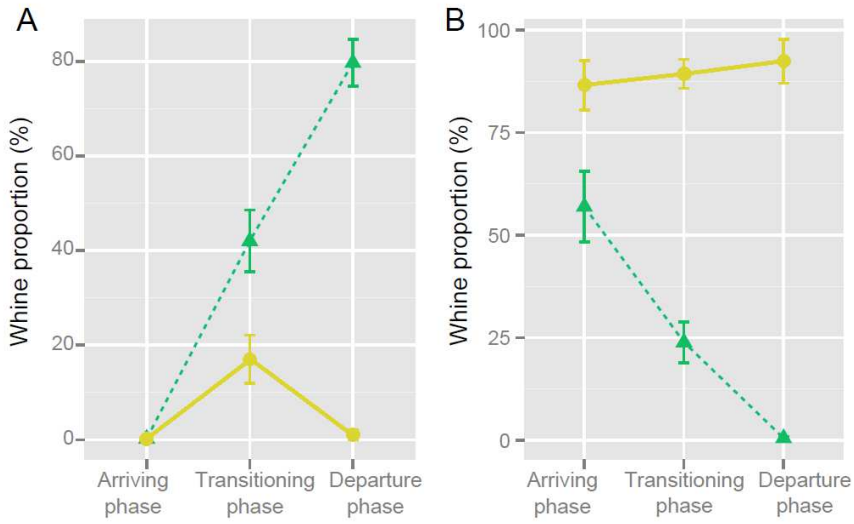


Figure 4. Effect of the context (green: relief duet; yellow: visit duet) on the proportion of whine calls used by A) the returning male and B) the incubating female throughout the duet. Points are means and bars are standard errors.

Zann & Rossetto, 1991). How birds reach this equality was unknown so far but duets performed at the nest may participate in this coordination. As found in our previous study in captivity (Boucaud et al., *in press*.), we showed here that the sex of the returning bird did not impact the structure of relief duets. This confirms that both sexes are similarly involved in these duets and that they probably encode the same information in their vocalizations.

By comparing visit and relief duets, we compared two contexts that are identical in their beginning (e.g. the male returned and entered the nest) but differ in their ending (either the male or the female left). In both contexts, we found that males may bring nest materials to the nest, so this behaviour does not determine the issue of the duet. This strengthens the hypothesis that the duet performed by the pair predicts whether partners will take turns incubating or not.

More males than females performed sentinel duets when their partner was incubating but this difference was not significant. We also observed that returning males used more song during sentinel than visit or relief duets. During the laying period, a positive relationship was found between the amount of singing given by the male after the female entered the nest and the time she subsequently spent inside the nest (Dunn & Zann, 1996b). If male song encourages the female to remain in the nest during laying, it may have a similar function during incubation by informing that the male is not ready to enter the nest. Interestingly, in our recordings the vocal behaviour of the female was also different between sentinel and other duets as females almost never used bill-clicking in sentinel duets. Bill-clicking has been described as part of the nest ceremony occurring when a pair is searching for a nest site (Zann, 1996). In other species, bill-clicking or clattering is considered as part of the courtship display (Eda-Fujiwara et al., 2004; Soma & Mori, 2015; Yoon et al., 2015). Here we describe for the first time their use during incubation in the zebra finch. Because, bill-clicking was rarely used in sentinel duets in our study, but mainly when birds met in the nest (visits, reliefs), it could be part of a greeting ceremony (Wachtmeister, 2001). Greeting or meeting ceremonies in birds are observed when the partners meet after a period of separation and in many species occur throughout the duration of the pair bond (Wachtmeister, 2001).

Here we show that sentinel duets may participate in the coordination of incubation between partners but they may have other functions. Sentinel duets got their name from the observation that the bird outside the nest appeared to be highly vigilant to the presence of potential predators (Elie et al., 2010). In the zebra finch, nest predation in natural nests accounts for the failure of over 60% of breeding attempts, and both the adult inside the nest and the offspring may be predated (S. C. Griffith et al., 2008; R. A. Zann, 1996). Moreover, an adult taken by surprise in a nest is very vulnerable because the only exit is a funneled entrance hole. A study recently showed that when experimentally approached by a human observer during incubation, birds flushed from their nests at significantly greater distances when their reproductive partner was present (Mainwaring & Griffith, 2013). Sentinel duets may indicate to the incubating bird the presence or absence of a danger.

When one bird met its partner in the nest and then left (visit duet), it almost always brought nest materials. Males visited the nest more often than females, thus brought more nest materials. This is consistent with previous observations that males bring the nest material and initially position it, and females put it in its final position (R. A. Zann, 1996). Most of nest building is done before egg laying, so the function of these supplementary nest materials brought during incubation is unknown. They may either be used for complementary nest building or signal mates' investment and represent "nuptial gifts" (Hunter & Davis, 1998).

In this study we showed that duets performed by zebra finch mates during incubation differed in temporal structure and more importantly in vocal repertoire and spectral structure of calls depending on the context (sentinel, visit or relief). The vocal behavior of each bird indicated its readiness to stay or leave the nest. Duets may thus be used by mates to decide when it is time to make a nest-relief. These results are consistent with our previous study showing that relief duets play a role in the share of incubation time in this species (Boucaud et al., *in press*).

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Chapitre 2

Vocal negotiation over parental care? Acoustic communication at the nest predicts partners´ incubation share

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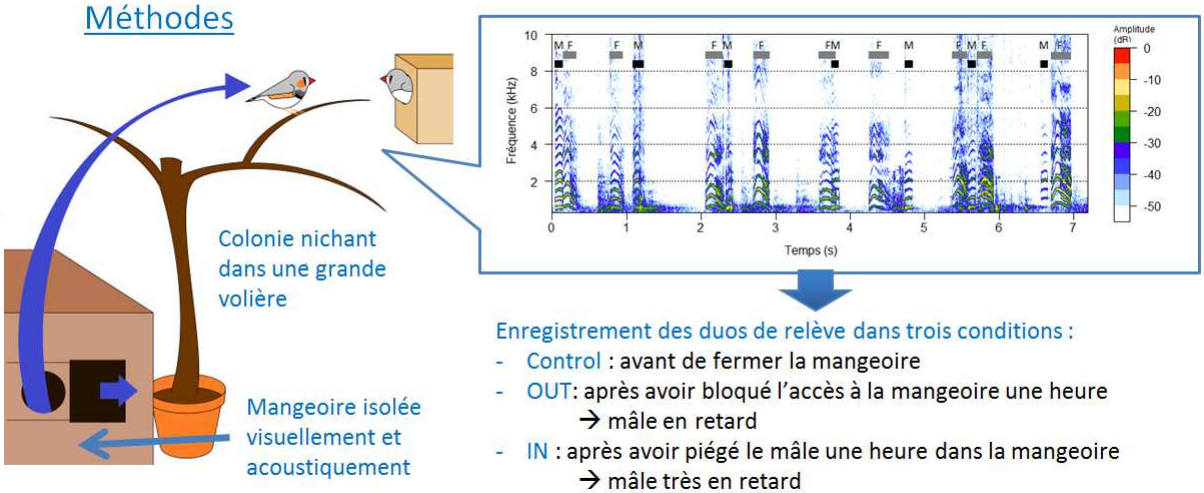
Synthèse du chapitre 2

Question : les duos vocaux des couples de diamants mandarins jouent ils un rôle dans le partage du temps d'incubation entre le mâle et la femelle ?

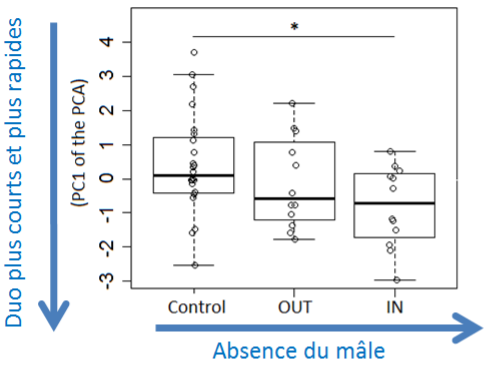
Méthodes



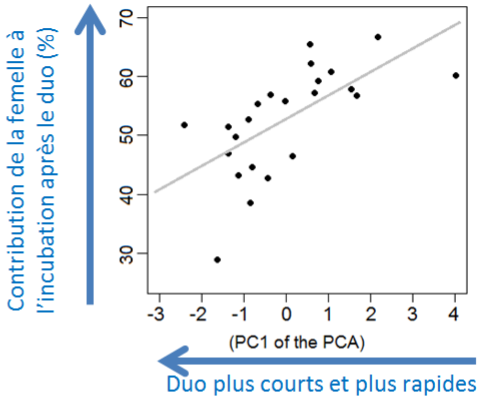
Enregistrement des duos de relève dans trois conditions :

- Control : avant de fermer la mangeoire
- OUT: après avoir bloqué l'accès à la mangeoire une heure
  → mâle en retard
- IN : après avoir piégé le mâle une heure dans la mangeoire
  → mâle très en retard

Résultats principaux



→ Les duos de relève sont plus courts et plus rapides quand le mâle est en retard



→ Plus le duo est court et rapide, plus le mâle va contribuer à l'incubation par la suite

Conclusion

Ces résultats suggèrent que les duos réalisés lors des relèves d'incubation permettent aux oiseaux de se partager le temps d'incubation et représentent une forme de négociation vocale sur l'organisation des soins parentaux.

ABSTRACT

In species with biparental care, individuals adjust their workload to that of their partner to either compensate or match its investment. Communication within a pair might be crucial for achieving this adjustment. Zebra finches, *Taeniopygia guttata*, form life-long monogamous pair bonds, in which partners are highly coordinated and both incubate the eggs. When relieving each other during incubation, partners perform a structured call duet at the nest. If this duet functions to coordinate incubation workload, disrupting the pair's usual nest-relief pattern by delaying the male's return to the nest should affect the structure of the duet. Using domesticated birds breeding in a large aviary, we found that delaying the male's return induced shorter duets with higher call rates. In addition, we tracked the location of individuals with a transponder at the nest and the feeder, and showed that these accelerated duets were associated with an increased haste of the partners to take turns incubating and foraging. Females also spent less time incubating during their subsequent shift, and females´ time off-nest was best predicted by their mate's calling behaviour in the previous duet. Taken together, these results suggest that duets may function as 'vocal negotiation' over parental care.

Keywords: coordination – duet – monogamy – negotiation – pair bond – zebra finch

INTRODUCTION

Biparental care in birds has been a long-term focus of studies testing theoretical models on cooperation versus conflict equilibrium between unrelated individuals (Trivers, 1972). In particular, sexual conflict theory predicts that each parent increases its lifetime reproductive success by reducing its investment in current offspring care at the expense of the other sex that compensates for this reduction (Trivers, 1972). Subsequent research has examined how two parents reach their division of labour not only through evolutionary responses, but also through behavioural adjustment (i.e. sequential modification of one's parental effort in response to the prior effort of its mate) (McNamara, Gasson & Houston, 1999). Specifically, when an individual decreases its investment, its partner may either increase its own workload to at least partially compensate for that loss (full or partial compensation models; McNamara et al., 1999) or decrease its own workload to match the reduced effort of its partner (matching model; Johnstone & Hinde, 2006). Overall, there is more empirical support towards partial compensation (meta-analysis; Harrison et al., 2009); however, some cases clearly do not follow that pattern (Hinde, 2006; Hinde & Kilner, 2007; Meade et al., 2011).

The adjustment of an individual's workload to that of its partner could also derive from the coordination of their activities resulting in a cooperative teamwork (Black, 1996). Many long-term monogamous species increase their breeding success with increased pair-bond duration, which may be attributed to the improved coordination of partners over time (mate familiarity effect: Forslund & Part, 1995; Black, 1996, 2001). Mates may progressively fine-tune how they function as a pair, making the division of labour during breeding more efficient. In some species, parents coordinate their foraging activities or synchronize their nest visits to feed the chicks (Lee, Kim & Hatchwell, 2010; Mariette & Griffith, 2012, 2015; Van Rooij & Griffith, 2013). In species where both parents incubate, hatching success may be increased when partners synchronize foraging trips (Coulson, 1966; Morris, 1987; Davis, 1988) or coordinate incubation bouts (measured by periods during which only one parent at a time incubates; Spoon, Millam & Owings, 2006). The coordination of partners during breeding could also reflect their coordination in other activities and be linked to pair-bond strength. Mates can alert each other to danger (Krams, Krama & Igaune, 2006; Mainwaring & Griffith, 2013), fight off predators by giving alarm calls or aggressive flights (Regelmann & Curio, 1986), join forces to compete over food resources or nest sites (Black & Owen, 1989; Black, 2001), and alternate vigilance and foraging (McGowan & Woolfenden, 1989; Sedinger & Raveling, 1990).

Regardless of whether conflict or coordination between breeding partners is occurring, the mechanisms allowing pairs to reach the adjustment of parental workload is an important but largely unstudied question. Previous research on the division of labour during biparental care has experimentally altered the workload of one partner by either handicapping it (wing clip, weight) (Schwagmeyer, Mock & Parker, 2002) or by modifying offspring begging calls (Hinde, 2006) and then measured how each partner adjusts its own workload as a consequence. However, we do not know how partners estimate each other's effort, and whether they somehow communicate with each other to reach a given level of parental investment.

If communication between partners is involved in parental care adjustment, acoustic communication may play a key role in this process. Birds produce a wide variety of vocalizations, especially during the breeding period. Male vocal behaviour is very well studied in birds, although much less is known about female vocalizations (Riebel, Hall & Langmore, 2005) and interactive communication between male and female, with the exception of acoustic duets (Farabaugh, 1982;

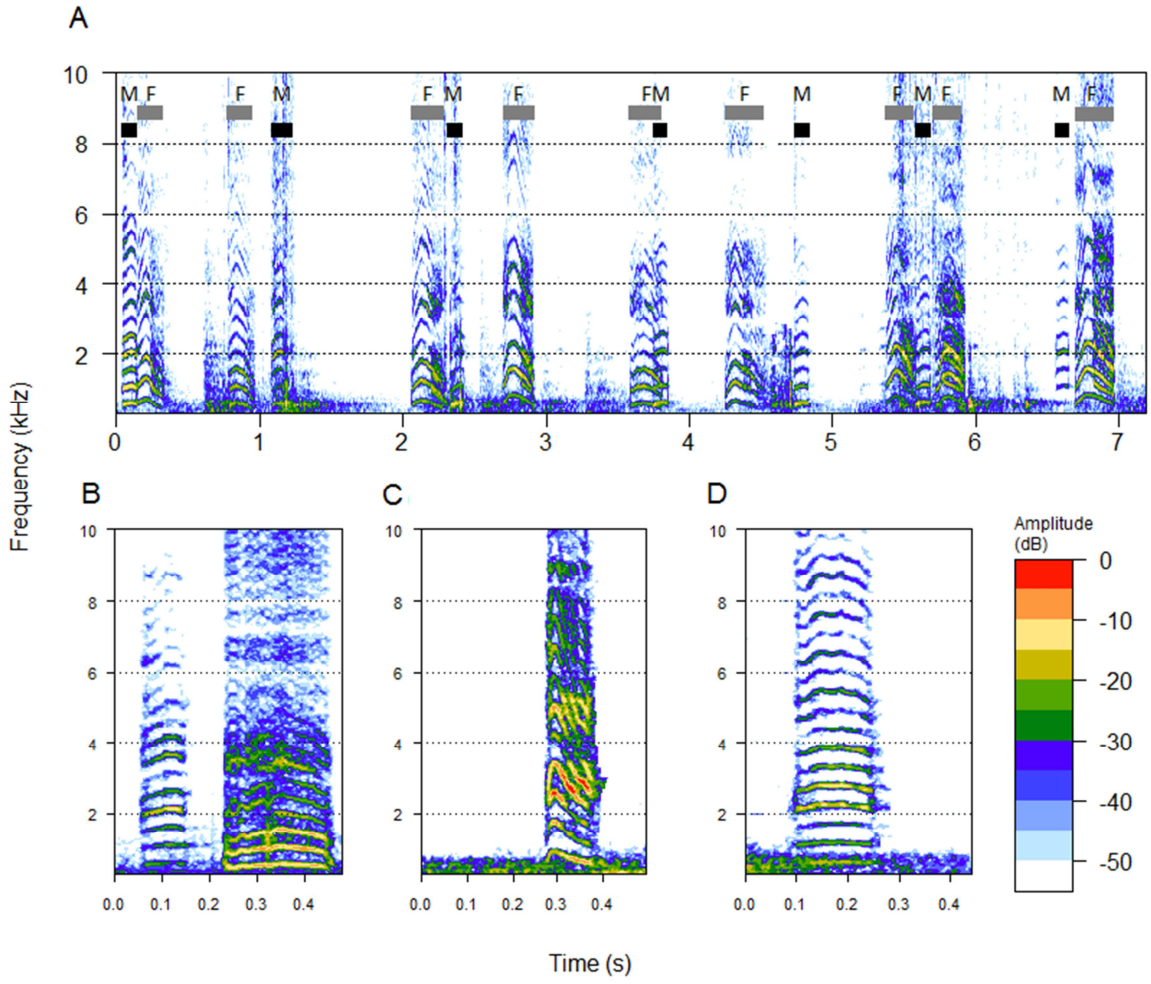


Figure 1. A, duet performed by zebra finch mates when they take turns incubating (M, male vocalization; F, female vocalization). Mates perform only one duet per incubation nest relief (see Supporting information, File S1). B–D, examples of call types used during these sequences, (B) one Tet (Short Call) followed by a Whine (see Supporting information, File S2), (C) one male Distance Call (Short Call) (see Supporting information, File S3), and (D) one female Distance Call (Short Call) (see Supporting information, File S4). Spectrograms were prepared using SEEWAVE (Sueur et al., 2008) in R (R Development Core Team, 2014).

Hall, 2004, 2009; Benedict, 2008; Dahlin & Benedict, 2013). Avian duets are joint acoustic displays between two birds that make temporally coordinated vocal or nonvocal sounds. Among them, the highly coordinated song duets of tropical songbirds are the most studied. These duets have been hypothesized to fulfil several functions, such as pair-bond maintenance, mate guarding, territory defence or synchronization of reproductive effort. However, the possibility that duets could play a role in parental care has never been explored.

Although song duetting is rare (approximately 4% of bird species), interactive communication between mates involving simpler and less conspicuous vocalizations is likely to be more widespread (Todt, Hultsch & Duvall, 1981; Lamprecht et al., 1985; Morton & Derrickson, 1996; Wright & Dahlin, 2007). In particular, the sounds produced inside the nest by females and used in interactive communication between mates may be more widespread than previously assumed (Ritchison, 1983; Beletsky & Orians, 1985; Yasukawa, 1989; McDonald & Greenberg, 1991; Halkin, 1997; Halfwerk et al., 2011; Halfwerk, Bot & Slabbekoorn, 2012). We recently described a simple and quiet form of call duets at the nest in wild zebra finches *Taeniopygia guttata* (Elie et al., 2010). Each time partners meet at the nest, they perform simple coordinated mutual vocal displays that can be described as call duets (Fig. 1A; see also Supporting information, File S1) (Elie et al., 2010). These duets involve calls that are softer than many others in this species (Zann, 1996) and may therefore represent a private communication between mates at the nest that could be involved in parental care adjustment.

Zebra finches maintain life-long pair bonds and mates show high coordination of activities during reproduction (Mariette & Griffith, 2015). Both parents contribute to nest building, incubation, and the provisioning of chicks (Zann, 1996). The male and female start incubation on the same day (Gilby, Mainwaring & Griffith, 2013) and multiple studies have found partners share incubation time equally, both in the wild and in captivity (El-Wailly, 1966; Delesalle, 1986; Zann & Rossetto, 1991; Gorman, Arnold & Nager, 2005; Gilby et al., 2013). Typically, the pair takes turns incubating and foraging. The call duets (thereafter referred to as 'duets' rather than 'call duets' for simplicity) occur during the transition where the foraging partner relieves the incubating partner: a nest-relief (Elie et al., 2010). Which partner controls the length of an incubation bout and whether incubation sharing involves any communication between partners remains to be determined. In addition, zebra finch mates are highly coordinated during the nestling period; in the wild, partners visit the nest and foraging patches together as a pair rather than independently of each other (Mariette & Griffith, 2012, 2015). Pairs increase visit coordination when brood size is experimentally increased, and better-coordinated pairs produce heavier nestlings (Mariette & Griffith, 2015). Similarity in the personality traits of partners might contribute to this effect if more similar partners have heavier nestlings as suggested in domestic zebra finches (Schuett, Godin & Dall, 2011; but see also Schielzeth et al., 2011). Coordination of breeding activities between partners could be reached by communication, with duets being part of the process.

In the present study, we aimed to investigate whether duets at the nest could play a role in the adjustment of incubation shifts between zebra finch partners. Using domesticated birds breeding in a large aviary, we disrupted the usual nest-relief pattern of a pair by delaying male return to the nest, and investigated whether duetting between mates was affected, and how birds responded to these modifications. We delayed the male's return to relieve its partner by trapping the male in an acoustically and visually isolated feeder. To control for the contact opportunities between partners in a second experimental condition, we again closed the feeder but without trapping the male inside. This again delayed male return to the nest because he postponed his visit to the feeder until after it was re-opened but did not affect a pair's ability to communicate.

MATERIAL AND METHODS

Housing Conditions

The present study was conducted in spring 2011 at the University of Saint-Etienne, France. We banded 54 males and 54 females with a unique combination of two plastic colour bands and a transponder tag. Birds were allowed to breed freely in a large indoor aviary (6.5 x 5.5 x 3.5 m; temperature: 20–30 °C, daylight: 07.30–20.30 h) and were provided with 50 nest-boxes (dimensions 13 x 12 x 17 cm). To simulate the separation of the partners that occurs in the wild when one partner incubates and the other one is foraging, all food and water in the aviary were provided in a separate room (thereafter 'feeder', dimensions: 3.2 x 1 x 1.30 m). This feeder was visually separated from the breeding area and acoustically insulated using acoustic foam (the intensity of a white noise broadcasted in the breeding area at 2 m from the feeder was 19 dB SPL lower inside than outside the feeder at the same distance). Moreover, birds entered the feeder using two small entrances (diameter 14 cm) oriented in the direction opposite to the breeding area. Birds were fed with finch seed mix, cuttlefish bones and water ad libitum and supplemented with salad and boiled eggs twice a week. The feeder was also equipped with perches and baths. Dried grass was provided as nest material *ad libitum* and cotton wool three times a week.

Recording and Experimental Approach

Out of the 108 birds, 32 pairs bred during the experiment and, among them, 12 pairs were successfully recorded in all experimental conditions (see below). Breeding pairs were recorded in the morning (between 09.00 and 14.00 h), during incubation. The day before recording, a tie microphone (C417; AKG) was installed inside the nest-box and connected to a digital recorder (PMD-671; Marantz). During the recording session an observer, sitting either in a hide inside the aviary or behind a one-way mirror outside the aviary, recorded the behaviour of partners during nest relief (presence of partners in the nest area and movements in and out of the nest-box). We used a within pair design to limit the number of possible confounding factors between our two experimental conditions. Each pair was recorded on 2 days (interval of 0– 6 days). On each recording day, the pair was recorded under the control (i.e. non-manipulated) condition, as well as under one of the two different experimental conditions (i.e. male inside the feeder or outside; see below). The order of experimental days was randomized across pairs. We successfully recorded 12 pairs in all four conditions, and so our sample size was 48 recordings. Our sample size is limited because incubation only last 10 days and most nests started at the same time so we could not record all the nests. In addition, monitoring individuals outside the nest to trap the male inside the feeder was challenging given the size of the aviary and the large number of birds.

On each recording day, the nest was observed and recorded under undisturbed conditions until the female returned to the nest and the male went out (except for two nests showing the reverse). This nest-relief represents the 'control' (i.e. non-manipulated) condition. Then the feeder was closed for 1 h to obtain one of two experimental situations: (1) the male was trapped inside the feeder ('IN') when the female was in the nest or (2) both the male and the female remained outside the feeder ('OUT'). In the second situation, mates continued to carry out nest reliefs and were not separated for the duration of the feeder closure, although they were both unable to forage for 1 h. In both IN and OUT conditions, we analyzed the first nest-relief after re-opening the feeder (referred to as 'experimental duets'), which corresponded to the male returning to the nest to relieve the female in 23 out of 24 nest-relieves. In summary, duets were obtained for three conditions:

1. Control: last duet before closing the feeder (one duet per pair per day, N = 24 duets; female returning in 22 duets, male in two).
2. IN: first duet after re-opening the feeder when the male had been trapped inside it (one duet per pair, N = 12 duets; male returning in all 12 duets).
3. OUT: first duet after re-opening the feeder when the male had remained outside (one duet per pair, N = 12 duets; male returning in 11 duets, female in one). In most cases, the sex of the returning bird differed between control and experimental conditions.

However, in a different dataset (N = 18 pairs), the sex of the returning bird had no effect on the parameters of the duet analyzed here (see Supporting information, Data S1). To test the effect of our manipulations on duet characteristics, all 48 duets were therefore included and classified on the basis of the experimental condition rather than on the basis of the sex of the bird returning to the nest. However, for simplicity, in analyses based on experimental duets only (see below), the one duet with the female returning was excluded because these analyses were specifically linking each partner calling pattern with its subsequent incubation behaviour. Including the duet with a female returning did not change any of the results.

Duet Analysis

When zebra finch mates take turns incubating, they perform a duet (i.e. a sequence of male and female calls that either alternate or partly overlap). There is typically only one duet per nest-relief, according to certain criteria: a duet started with the first call of one mate answered by its partner within 10 s, and lasted until one bird left or both birds stopped calling for at least 10 s. We measured the duration of three phases of the duet:

1. Arriving phase: the duet begins when the returning mate is still outside the nest (usually < 2 m from the nest entrance) whereas its incubating partner is inside.
2. Transitioning phase: the returning bird meets its partner either inside the nest or just outside. In most cases, the returning bird enters the box before the incubating partner gets out; however, in six out of 48 cases, the incubating bird met its partner at the nest's entrance. In these cases, the two birds vocalize outside the nest during this phase.
3. Departure phase: if the transitioning phase took place inside the nest, the departure phase starts when the incubating bird goes out of the nest; if the transitioning phase took place outside the nest, the departure phase starts when the returning bird goes inside the nest. During this phase, mates continue interacting vocally until the duet ends when the previously incubating bird leaves the nest area (or when both birds stop calling for more than 10 s).

The duration of these three phases was measured manually on the acoustic recordings (44.1 kHz, 16 bit) using PRAAT (http://www.fon.hum.uva.nl/praat/), which displays both the oscillogram and the spectrogram of the recording (view range: 0–20 kHz; window length: 0.02 s; dynamic range: 60 dB). Birds make characteristic noises when entering or exiting the boxes, allowing us to precisely detect these events.

To analyze the temporal structure of the duet, we used a semi-automatic detection of the calls. Using SEEWAVE (Sueur, Aubin & Simonis, 2008) in R (R Development Core Team, 2014), we segmented the recording according to an amplitude threshold to obtain a list of start and end times of each detected call. In a second step, all recordings were checked manually using PRAAT to add any call missed by the automatic detection or separate overlapping calls wrongly counted as one call.

In zebra finches, males and females use the same vocal repertoire, except song, which is produced only by males (Zann, 1996). Moreover, the repertoire used during duets does not depend

on the bird's sex but instead on the bird's position inside or outside the nest (Elie et al., 2010). In the present study, we distinguished three types of vocalizations:

1. Short Calls: these are harmonic stacks generally shorter than 100 ms. Zebra finches show a continuum of soft short calls used in close social context (Zann, 1996) (tets, cackles, arks) (Fig. 1B; see also Supporting information, File S2) as well as louder distance calls (Zann, 1996) (Fig. 1C, D; see also Supporting information, Files S3, S4; approximately 100 ms for males and 200 ms for females). The acoustic conditions of the nest-boxes (creating attenuation and reverberation of the sound) in the aviary (with partly overlapping vocalizations from other birds) did not allow us to accurately discriminate between these different calls, and so we grouped them into the single call type 'Short Call'.
2. Whines: these are soft, long (around 300 ms, with high variation) and high-pitched moans. This 'pleading' sound is a vocalization specifically uttered inside the nest (Zann, 1996) (Fig. 1B; see also Supporting information, File S2).
3. Song: males sang in only five out of the 48 duets analyzed. Each song syllable was counted as a
4. call.

The identity of the caller was determined by visual observation of the birds' behaviours (described above) and the characteristics of the recordings. During arriving and departure phases, caller identity was easily established because one bird was calling from inside the box and the other one from outside. The bird inside the box was closer to the microphone than the bird outside, and its calls were therefore a lot louder on recordings. During the transitioning phase, we managed to attribute calls to individuals using the individual signature of the calls.

From the list of calls, we measured call duration, as well as temporal parameters of the duet sequence: the total duration, the average call rate (number of calls per minute defined as the total number of calls divided by the time spent calling), and, for each sex: the total number of calls, the time spent calling (i.e. the duration between the first and the last calls), and the average call rate. To describe the repertoire used, because Song was rare (see above) and birds used only two call types, we measured Whine proportion, as the percentage of Whines among all calls in the duet.

Recording of Birds' Activities

Each bird was identified by a PIT-tag (Passive Integrated Transponder tag; Trovan ID100A) glued on one band. Antennas (Dorset Identification BV, The Netherlands) were installed in the nest-boxes around the nest built by the birds with dried grass and cotton, and at the entrance of the feeder. In this way, time spent in the nest as well as exits and entrances at the feeder were recorded for each bird (unless technical issue, see below).

The time spent away from the nest by one partner was equivalent to the time spent in the nest by its partner because, during our observations, parents never ended their incubation bout before their partner returned to the nest. To estimate incubation effort, we measured 'incubation bout' duration as the time spent inside the nest by one bird before (or after) the analyzed duets. In addition, we quantified the 'contribution to incubation' between partners by measuring the relative participation of each partner to incubation using the percentage of time the female incubated over the total incubation time. This female contribution to incubation was measured on an equal number of incubation bouts for the male and the female, corresponding to ~2 h of PIT-tag recordings before the analyzed duets (and after in the case of experimental duets).

By closing the feeder, birds were unable to eat during 1 h. To estimate females' motivation to feed at the time of the experimental duet (after re-opening the feeder), we measured the time

between their exit from the nest box (i.e. the last detection in the nest) and their entrance into the feeder (i.e. first detection at the feeder door). To estimate the readiness of males to come back to the nest after the treatment, we measured the difference between their last detection at the feeder door and their first detection in the nest. We then compared these times with those in the last relief before closing the feeder when the male was returning to the nest and the female was leaving (the experimental duet in which the female rather than the male was returning to the nest was removed from this analysis). Here, we took care to compare reliefs with the same sex returning because it is possible that the time to return to the nest and to join the feeder is individual specific. Lastly, because of technical problems (saturation of the PIT-tag data loggers as a result of intense bird activity), pit-tag data at the feeder entrance were available for 39 out of 48 relieves for the females and 36 out of 48 relieves for the males.

Ethical Note

Experiments were performed under the authorization number 42-218-0901-38 SV 09 (ENES Lab, Direction Departementale des Services Vétérinaires de la Loire) and were in agreement with the French and European legislations regarding experiments on animals.

Statistical Analysis

Overview

The purpose of our experiment was, in a first step, to delay male return to the nest (IN and OUT), at the same time as controlling for mate separation effects (OUT), and to investigate the effect on mate communication at the nest. In a second step, we tested whether mate communication or incubation share, which were both affected by our experiment, best predicted subsequent incubation behaviour. For the first step, we checked that our manipulation had the intended effect on birds' incubation behaviour, and tested whether it directly affected duet characteristics. Even though both experimental conditions delayed male return, there was a lot of overlap between conditions, and so we also tested whether duet characteristics responded linearly to the duration of the preceding incubation bout (the condition and the duration of the incubation bout could not be included in the same model as they were collinear). In an attempt to reduce the number of parameters tested, and still performing a thorough analysis given the absence of a priori knowledge, we grouped duet characteristics under (1) overall temporal structure (duration of the duet, duration of the different phases and call rate) and (2) detailed vocal repertoire (type and duration of calls given by each partner). Because the latter did not differ between experimental conditions, we did not include it further when investigating the linear effect of incubation bout duration on the prediction of post-manipulation incubation behaviour. Lastly, to improve our interpretation of the effect of our manipulation on birds' incubation behaviour, we also investigated the effect of the manipulation on birds' visits to the feeder.

All statistical tests were performed using R (R Development Core Team, 2014). Normality of the data was tested using Shapiro–Wilk test. Some parameters were transformed to reach normality (male time to return to the nest was log-transformed and female time to enter the feeder was square-root-transformed). In addition, all models were checked for normality and homogeneity by visual inspection of plots of residuals against fitted values. The analysis of variance function of the car package was used to obtain P-values on models. $P < 0.05$ was considered statistically significant. All post-hoc tests were performed using multiple comparisons tests (glht function of the multcomp package, with Tukey contrasts). All linear mixed models (LMM) used restricted maximum likelihood

estimation, except for the model comparison approach, which was instead based on maximum likelihood. Estimates and SEs were calculated using the lsmeans function.

Principal component analysis (PCA)

All three PCA were performed using the function dudi.pca of the ade4 package, which performs a normed PCA (i.e. a PCA on correlation matrix). Principal component with Eigen value above one (Kaiser criterion) were selected. Two PCA were performed to test the effect of delaying males' return to the nest on first (1) the overall temporal structure of the duet and then (2) its detailed call composition for each partner. Lastly, (3) a third PCA, identical to the first but using only experimental duets, was used to test the effect of experimental duets' characteristics on subsequent incubation behaviour. Control duets, which occurred just before closing the feeder, were omitted from this third PCA because the feeder manipulation was specifically designed to interfere with usual incubating schedule.

1. The first PCA summarized four temporal parameters of the duets: call rate, duet total duration, duration of arriving phase, and duration of transitioning phase. These four parameters were chosen to avoid linear dependencies (e.g. as duet total duration = duration of arriving phase + duration of transitioning phase + duration of departure phase, we chose only duet total duration, duration of arriving phase, duration of transitioning phase). The first principal component PC1 explained 50.16% of the variance and was described by the equation: -0.81 call rate + 0.87 duet duration + 0.64 arriving phase duration + 0.44 transitioning phase duration. PC2 explained 28.70% of the variance and was described by the equation: -0.11 call rate -0.03 duet duration -0.67 arriving phase duration + 0.83 transitioning phase duration.
2. The second PCA describing the vocal repertoire of each partner in the duet included the duration of Whines and Short Calls for each sex, and the proportion of Whine. Because of missing data when
3. one of the two partners did not produce one of the two call types (and therefore no duration for that call type was available), the sample size was 41 (instead of 48) duets. Considering the proportion of Whines in all 48 duets or the duration of each call type singly ($44 < N < 48$) gave the same results (i.e. all nonsignificant; data not shown). PC1 explained 39.98% of the variance and was described by the equation: 0.77 Whine proportion + 0.62 male Short Calls duration + 0.68 female Short Calls duration + 0.52 male Whines duration + 0.53 female Whines duration. PC2 explained 24.24% of the variance and was described by the equation: -0.21 Whine proportion -0.25 male Short Calls duration + 0.42 female Short Calls duration -0.66 male Whines duration + 0.70 female Whines duration.
4. The third PCA on experimental duets only, had similar loadings to the first PCA on all duets. Indeed, PC1 explained 50.78% of the variance and was described by the equation: -0.82 call rate + 0.86 duet duration + 0.31 arriving phase duration + 0.72 transitioning phase duration. PC2 explained 25.51% of the variance and was described by the equation: 0.12 call rate + 0.08 duet duration -0.91 arriving phase duration + 0.42 transitioning phase.

Statistical models

For the first step, to test the effect of our experimental treatments on (1) incubation behaviour, (2) birds' movements from and to the feeder, and (3) duets' overall and detailed characteristics (first and second PCA), we used LMM (lmer function of the lmerTest package). All had the experimental condition (three levels: control, IN, OUT) as a fixed factor and the recording day (two levels: first day and second day) nested within the pair identity (12 levels) as random factors. In

addition, we tested for a linear effect of incubation duration on duet's overall temporal structure in another model using the same random factors but with the duration of the incubation bout as a covariate instead of the three-level experimental condition.

In a second step, to test the effect of experimental duets' characteristics on subsequent incubation behaviour, we first investigated whether duet overall temporal structure (third PCA) predicted incubation behaviour, and we then tested which duet characteristic or previous incubation behaviour best explained subsequent incubation, using a model comparison approach. First, we performed two LMMs, using, respectively, PC1 or PC2 from the third PCA as a covariate and the pair identity (12 levels) as a random factor (recording day was not a random factor in this model because the dataset limited to experimental duets had only one recording per day for each pair). Second, to test which parameter best explained subsequent incubation behaviour, we built three LMM model sets to test three response variables: (1) the duration of male's incubation bout (immediately following the experimental duet), (2) the duration of female's incubation bout (when she returned from her off-nest bout after the experimental duet), and (3) female contribution to incubation over the 2 h following the experimental duet. Each model set used eight fixed factors: six acoustic parameters of the experimental duet (for each sex: number of calls, call rate, time spent calling) and two incubation parameters (duration of females' last incubation bout before the experimental duet, or females' contribution to incubation in the 2 h prior to the experimental duet). The pair identity (12 levels) was used as a random factor. Collinearity was checked for all fixed factors using the variance inflating factor (vif function of the car package). Time spent calling was excluded from the analysis because it had a vif > 10, indicating high collinearity with the other predictors (other parameters all had vif < 5.3). The correlation between call rates from the male and the female was high ($r = 0.81$, Spearman correlation), although this factor was not correlated with other parameters; other correlations between parameters never exceeded $r = 0.5$. A model selection was then performed on the six remaining parameters using the dredge function from the MuMIn package, which compares all possible models built using subsets of the initial factors of the complete model, including null model. Models were compared using Akaike information criteria corrected for small sample size (AICc). When more than one model had some support (i.e. DAICc < 2; Burnham & Anderson, 2002), the importance of explanatory terms was evaluated by calculating the predictor weight for each term (i.e. the sum of the Akaike weights for each model that contained that variable).

RESULTS

Effect of Closing the Feeder on the Duration of Incubation Bouts and on Females' Contribution to Incubation

By closing the feeder for 1 h with the male either inside or outside, we obtained three different conditions with differences in the duration of incubation bout (i.e. time spent in the nest between two nest reliefs) and the proportion of incubation time carried out by females (Table 1). The duration of incubation bout just prior to the relief was significantly increased by trapping the male in the feeder (IN condition) and, to a lesser extent, by preventi3ng access to the feeder for both partners (OUT condition) (Fig. 2A, Table 1). In the control condition, mates shared incubation time equally (Fig. 2B, Table 1). In the OUT condition, birds were still able to perform nest relief normally, with a nest relief every 20–40 min, although they were unable to forage because the feeder was closed. In this condition, the duration of the incubation bouts just before the experimental duet (and therefore females' contribution to incubation) increased (Fig. 2B, Table 1) because the male, which was off the nest when the feeder re-opened, went to eat before returning to the nest (100% of the nine males with PIT-tag data were detected in the feeder before returning to the nest; the female returning to the nest in this condition was not detected). Finally, by trapping the male in the feeder (IN condition), we forced the female to carry out most of the incubation time (Fig. 2B, Table 1). In sum, for both types of experimental duets, the female had not eaten for over 1 h and the male was late coming back from the feeder.

Effect of Delaying Male's Return on Birds' Movement Timing

When males had been trapped inside the feeder, males were significantly faster to return to the nest after exiting the feeder (i.e. seven out of nine males with PIT-tag data) (Fig. 3A, Table 1) and females tended to be faster to go to the feeder after the nest relief, although this was not statistically significant (seven out of 10 females) (Fig. 3B, Table 1).

Acoustic Structure of the Duets

During nest-reliefs, calls were always emitted, normally by both birds thereby producing a duet (96% of the nest-reliefs observed) or, more rarely, only by the bird inside the nest (4% of the reliefs).

Duet temporal structure (PC1 of the first PCA) was significantly affected by the experimental condition: when the male had been trapped inside the feeder (IN condition), PC1 was significantly lower (shorter duets, shorter arriving and transitioning phases, higher call rate) compared to control (PC1 reduced in 75% of the pairs) (Fig. 4A, Table 2). The second principal component was not affected by the conditions (Table 2). Similarly, across treatment groups, PC1 significantly decreased when the duration of the incubation bout increased (LMM, $Chi^2 = 8.83$, d.f. = 1, P = 0.003, N = 48) (Fig 4B), whereas the second principal component was not affected (LMM, $Chi^2 = 0.12$, d.f. = 1, P = 0.720, N = 48). Therefore, the longer the incubation bout, the shorter and more accelerated the duet.

Table 1. Statistical analysis of the effect of delaying male's return on nest-relief and birds' movement timing

	Df	Chi² or Z value (post hoc)	P value	estimate ± SE control condition	estimate ± SE OUT condition	estimate ± SE IN condition
Female contribution to incubation (%)						
	2	50.34	**<0.001**	51 ± 3	64 ± 4	74 ± 4
				N=24	N=12	N=12
OUT - control		3.98	**<0.001**			
IN - control		6.86	**<0.001**			
IN - OUT		2.50	**0.033**			
Incubation bout duration (min)						
	2	56.84	**<0.001**	38 ± 4	55 ± 6	85 ± 6
				N=24	N=12	N=12
OUT - control		2.79	**0.015**			
IN - control		7.53	**<0.001**			
IN - OUT		4.11	**<0.001**			
Female time to go to the feeder (min; log transformed)						
	2	0.35	0.842	0.77 ± 0.11	0.74 ± 0.17	0.66 ± 0.15
				N=19	N=9	N=11
Male time to go to the nest (min; square-root transformed)						
	2	9.85	**0.007**	28 ± 3	27 ± 4	14 ± 4
				N=17	N=9	N=10
OUT - control		-0.22	0.974			
IN - control		-3.00	**0.007**			
IN - OUT		-2.41	**0.042**			

Significant P-values are shown in bold. N values below the full sample size of 48 (12 pairs with four duets each: two duets in control condition, one duet in IN condition, and one duet in OUT condition) are a result of missing values.

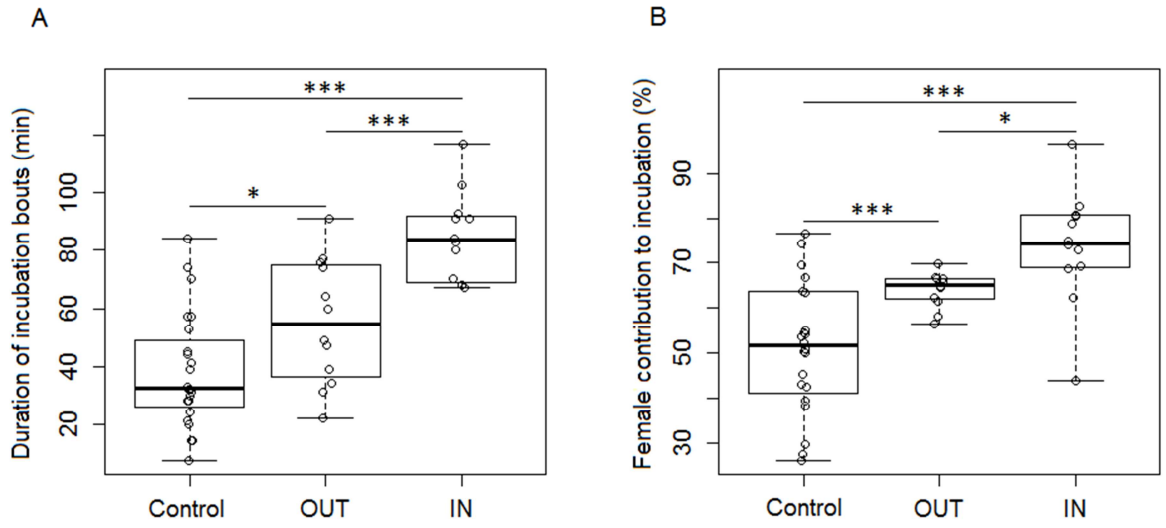


Figure 2. Duration of incubation bouts (time spent in the nest between two nest-reliefs) (A) and female contribution to incubation (B) in the three experimental conditions. Boxes are the first to third quartiles, lines are medians, whiskers are extreme values, and each point represents one duet (control: N = 24; OUT: N = 12; IN: N = 12; linear mixed model: ***P < 0.001; *P < 0.05).

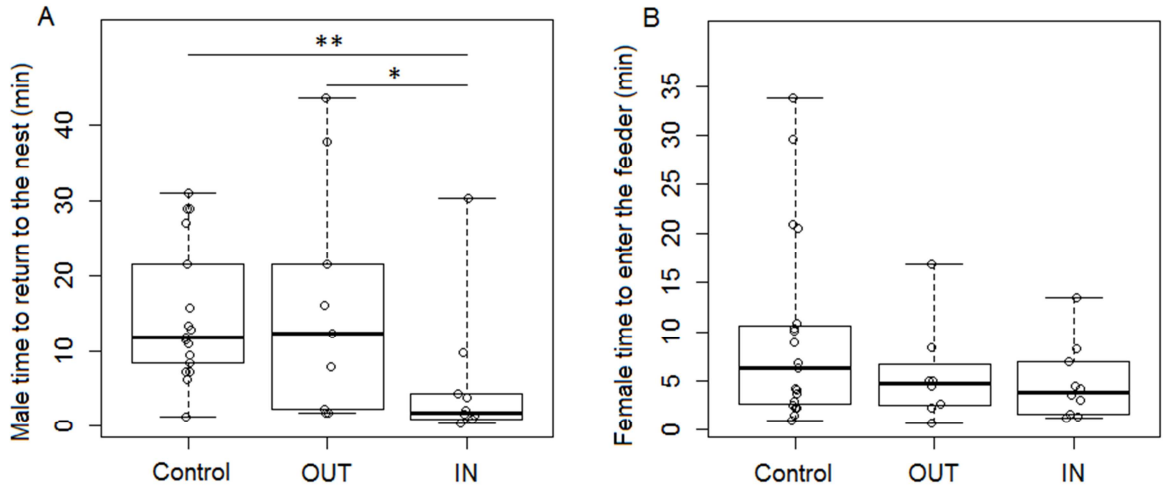


Figure 3. Time to return to the nest for the male (A) and time to enter the feeder for the female (B) in the three experimental conditions. Boxes are the first to third quartiles, lines are medians, whiskers are extreme values, and each point represents a duet (control: N = 24; OUT: N = 12; IN: N = 12; linear mixed model: **P < 0.01; *P < 0.05).

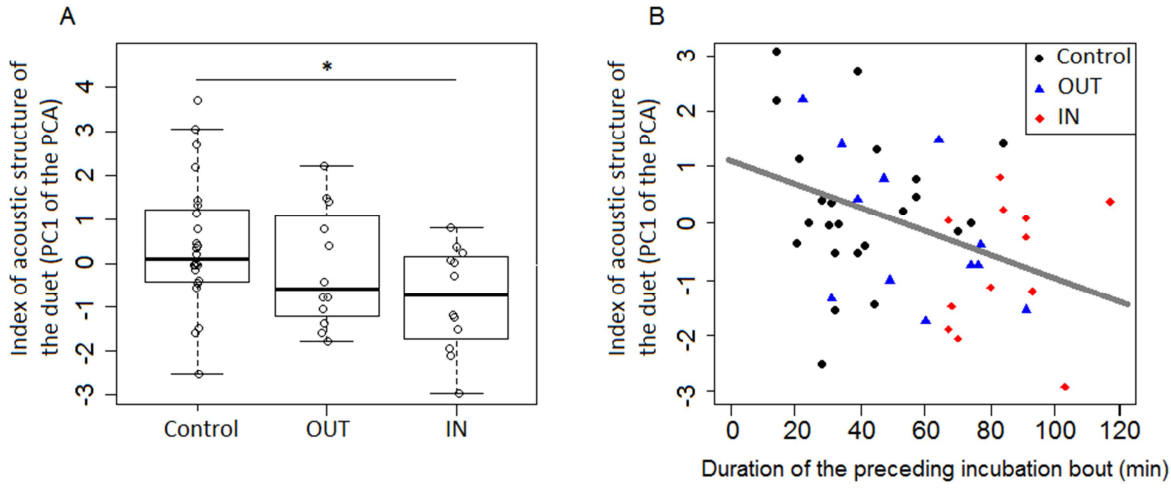


Figure 4. Effects of delaying male return to the nest on duet structure. Principal component (PC)1 is the first principal component of a principal component analysis, and low values of PC1 indicate shorter duets with higher call rate, and, to a lesser extent, shorter arriving and transitioning phases. In (A), boxes are the first to third quartiles, lines are medians, whiskers are extreme values, and each point represents a duet. In (B), the regression line of PC1 is shown as a function of the duration of the preceding incubation bout.

Table 2. Statistical analysis of the effect of delaying male's return on duet characteristics

	Df	Chi² or Z value (post hoc)	P value	estimate ± SE control condition	estimate ± SE OUT condition	estimate ± SE IN condition
Index of acoustic structure of the duet (PC1 of the PCA)						
	2	6.63	**0.036**	0.44 ± 0.28 N=24	-0.10 ± 0.39 N=12	-0.78 ± 0.39 N=12
OUT - control		-1.12	0.498			
IN - control		-2.56	**0.028**			
IN - OUT		-1.24	0.428			
Index of acoustic structure of the duet (PC2 of the PCA)						
	2	0.71	0.702	-0.13 ± 0.22	0.10 ± 0.32	0.16 ± 0.32

* Significant P values are shown in bold. PC, principal component; PCA, principal component analysis.

Vocal Repertoire for Each Partner

The first and second principal components (PC1 and PC2 of the second PCA) were not affected by the conditions (LMM, PC1: LMM, $Chi^2 = 0.76$, d.f. = 2, P = 0.684, N = 41; PC2: $Chi^2 = 1.62$, d.f. = 2, P = 0.444, N = 41). Therefore, delaying the male's return had no effect on the proportion of Whine versus Short Calls and the duration of the calls of either partner.

Predictors of the Contribution of Partners to Incubation After Experimental Duets

Pairs did not appear to compensate for the additional female incubation time caused by male's delay in OUT and IN conditions because the females' contributions to incubation before and after the experimental duets were not negatively correlated (LMM, $Chi^2 = 1.37$, d.f. = 1, P = 0.242, N = 23) (Fig. 5A). Instead, the incubation behaviour of pairs varied with the characteristics of their duet under experimental conditions. Indeed, females' contribution to incubation decreased when the previous duet had a lower PC1 for the third PCA (i.e. was shorter and more accelerated; LMM, $Chi^2 = 14.97$, d.f. = 1, $P < 0.001$, N = 23) (Fig. 5B). Female's contribution to incubation was not affected by PC2 (LMM, $Chi^2 = 0.37$, d.f. = 1, P = 0.540, N = 23).

The parameter that best explained subsequent incubation behaviour was the number of male calls: the more the male called, the shorter his following incubation bout (Fig. 5C, Table 3). Including the female contribution to incubation before the experimental duet did not improve the model because the model with both predictors was equivalent to that with male call number only (delta AIC < 2). Moreover, the sum of the Akaike weights for each model that contained male call number is higher (0.605) than those of female incubation bout before the experimental duet (0.468) (Fig. 5D). Similarly, females' call rate was the best predictor of female's first incubation bout when she returned to the nest after being off-nest following the experimental duet (Table 3). The higher the female's call rate, the shorter her incubation bout. Finally, females' vocal behaviour (call rate, sum of the Akaike weights: 0.819), as well as males' vocal behaviour (number of call and call rate, respectively, 1.764 and 0.573), best explained female contribution to incubation in the 2 h following the experimental duet (Table 3). In agreement with the analysis of the third PCA above, female contribution to incubation decreased with duets with fewer male's calls and higher call rate from both the male and the female.

DISCUSSION

We found that the duration of the incubation bout was mostly determined by the returning partner because, under our experimental conditions, parents never ended their incubation bout before their partner returned to the nest. Delaying males' return to the nest increased females' contribution to incubation, and also modified the vocal exchange of partners, which was shorter with a higher call rate, corresponding to an accelerated duet. Furthermore, the incubation behaviour of pairs after that accelerated experimental duet varied with the characteristics of that duet rather than with their previous incubation effort. Indeed, the shorter and more accelerated the experimental duet, the less females subsequently contributed to incubation. It was difficult to separate male and female contributions to duets, especially features such as call rate, as a result of the high level of contribution of both partners. Nevertheless, the vocal behaviour of each mate was the best predictor

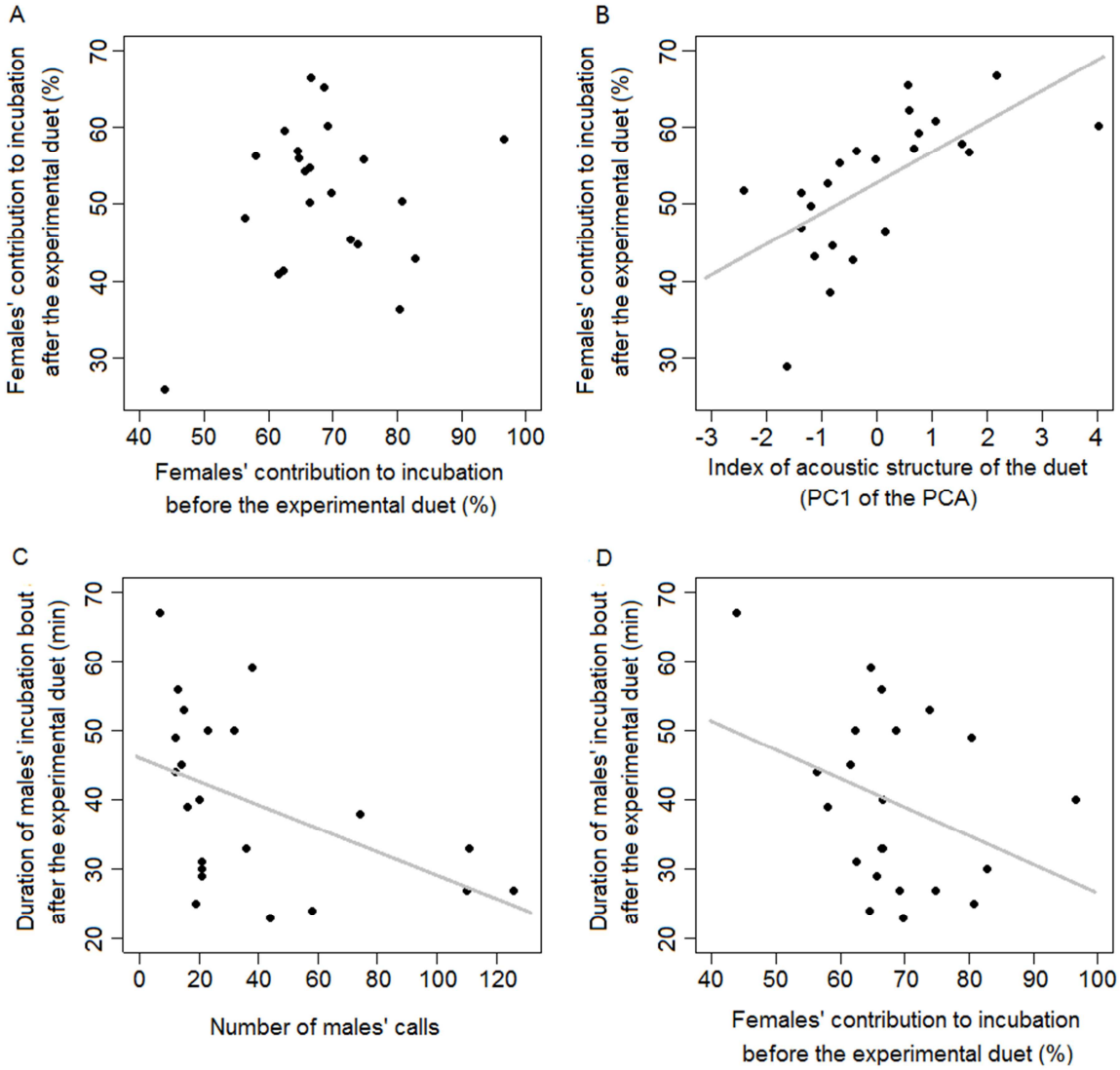


Figure 5. Predictors of the contribution of partners to incubation after experimental duet (11 pairs in OUT and 12 pairs in IN conditions: N = 23). A, female contribution to incubation before the experimental duet does not predict female contribution to incubation after the experimental duet. B, principal component (PC1) is the first principal component of a principal component analysis, and low values of PC1 (shorter duets with higher call rate, and, to a lesser extent, shorter arriving and transitioning phases) predict a decrease in female contribution to incubation after the experimental duet. C, fewer male calls predict an increase in male contribution to incubation after the experimental duet. Male call number is a better predictor of male contribution to incubation after the experimental duet than female contribution to incubation before the experimental duet (D).

Table 3. Model comparison to test which of the correlated acoustic and behavioural parameters best explained incubation behaviour after the experimental duet: a) duration of male's incubation bout, b) duration of female´s incubation bout and, c) females´ contribution to incubation.

a)

Model number	(Intercept)	Female call number	Male call number	Female call rate	Male call rate	Female incubation bout before the experimental duet	Female contribution to incubation before the experimental duet	df	LogLikelihood	AICc	ΔAICc	Weight
1	**46.12**		**-0.1692**					**4**	**-83.682**	**177.7**	**0.00**	**0.166**
2	**70.26**		**-0.1576**				**-0.3584**	**5**	**-82.303**	**178.4**	**0.64**	**0.120**
3	46.66	-0.2166						4	-84.770	179.9	2.18	0.056
6	39.64							3	-86.489	180.3	2.59	0.045

b)

Model number	(Intercept)	Female call number	Male call number	Female call rate	Male call rate	Female incubation bout before the experimental duet	Female contribution to incubation before the experimental duet	df	LogLikelihood	AICc	ΔAICc	Weight
1	**50.76**			**-0.1717**				**4**	**-96.175**	**202.7**	**0.00**	**0.218**
2	83.56			-0.1870			-0.4626	5	-95.476	204.7	2.00	0.080
3	63.88			-0.1485		-0.2186		5	-95.531	204.8	2.11	0.076
4	38.77							3	-98.858	205.0	2.35	0.067
5	61.18					-0.3324		4	-97.550	205.5	2.75	0.055
6	48.26				-0.1293			4	-97.607	205.6	2.86	0.052

c)

Model number	(Intercept)	Female call number	Male call number	Female call rate	Male call rate	Female incubation bout before the experimental duet	Female contribution to incubation before the experimental duet	df	LogLikelihood	AICc	ΔAICc	Weight
1	**54.60**		**0.1037**		**-0.0863**			**5**	**-71.911**	**157.6**	**0.00**	**0.200**
2	**58.72**			**-0.0927**				**4**	**-73.920**	**158.2**	**0.62**	**0.147**
3	**55.24**		**0.0657**	**-0.0790**				**5**	**-72.843**	**159.4**	**1.87**	**0.079**
4	58.92		0.1123		-0.0844	-0.0710		6	-71.266	160.1	2.56	0.056
5	58.91				-0.0908			4	-74.940	160.2	2.66	0.053
32	52.24							3	-79.157	165.6	8.08	0.004

* Only models with weight > 0.05, as well as null models, are presented. Significant models are shown in bold.

of its subsequent incubation bout duration. Our results therefore support the hypothesis that the vocal exchange of partners at the nest plays a role in incubation shift coordination, in addition to the experimental conditions that had altered this duet.

The male appeared to communicate his willingness to spend time incubating by calling less during the duet and the female indicated that she will decrease her subsequent incubation duration by increasing her call rate. Importantly, because the duration of an incubation bout depends on when the returning partner is coming back, this suggests that birds responded to their partner's demand by coming back and relieving them early or late depending on the information they received during the relief. Each partner may thus use the vocal behaviour of its mate during the duet to predict its future behaviour and adjust its own behaviour pre-emptively. This is consistent with a vocal negotiation process where each partner signals its capacity to invest in parental care and its mate adjusts its own contribution in response to this information (McNamara et al., 1999; Johnstone & Roulin, 2003; Johnstone & Hinde, 2006; Bell et al., 2010). Our results therefore suggest that duets are used to adjust incubation duties between partners and may function as vocal negotiation processes. In the present study, we manipulated male's time to return to the nest during the female's incubation shift, and we thus studied the impact of a male delay on the process of negotiation over parental care. This process might be different in the case of a female delay, and it would be very interesting to design the reverse experiment by delaying the female during the male's incubation shift.

The decrease in duration of the arriving phase and the transitioning phase indicated that birds did the physical relief faster after delaying male return to the nest: the returning male did not spend as much time vocalizing outside the nest but instead went quickly inside. The transitioning phase inside the nest was also shorter and females left quickly. It appears that both birds were accelerating the relief. This is confirmed by the transponders data, showing a significantly shorter time for the male to return from the feeder to the nest compared to the control condition. The haste of both partners could be explained by the motivation of the female to eat after her extended nest attendance and of the male to return to the nest after an extended absence (Dearborn, 2001; Cresswell et al., 2003; but see also Bulla et al., 2014). Nevertheless, the experimental extension of the female's nest attendance remains within the range of bout duration for the species and it is possible that greater disruptions (e.g. a longer absence of the male) would cause some females to terminate their incubation bout before their mate returns (Chaurand & Weimerskirch, 1994).

Our results showed clear patterns even though the sample size was only moderate, with 12 pairs per experimental group, possibly because the within-pair design partly compensated for that limitation. The effects that we report were followed by the large majority of individuals in our sample, although it is possible that more subtle differences were missed. Moreover, future studies could directly manipulate pair communication by using playback to artificially increase calling rate, although achieving convincing conditions to birds may be technically challenging. In addition, it remains to be investigated whether more information is encoded in the acoustic characteristics of the calls themselves because the recording conditions in the present study did not allow for the analysis of calls' spectral structure. In particular, if the stress level of individuals increases as they are forced to incubate for long periods, it is possible that the spectrum of their calls shift to higher frequency bands (Perez et al., 2012) and that their partner is sensitive to such modulations (Perez, 2015). Nevertheless, vocal repertoire (calls duration and proportion of Whines) was maintained in accelerated experimental duets. This suggests that the pace of the duet could be sufficient to cause functional changes in the behaviour of individuals, and thus be a relevant signal for pair coordination.

The present study found that duets in domesticated zebra finches are similar to those in wild birds (Elie et al., 2010). Insights that we learn regarding the structure and function of duets in a captive environment may thus be applicable to wild birds. Because females of many bird species vocalize at the nest (Ritchison, 1983; Beletsky & Orians, 1985; Yasukawa, 1989; McDonald & Greenberg, 1991; Halkin, 1997; Halfwerk et al., 2011, 2012), call duets between partners at the nest are likely to be widespread. The coordination of parental care via call duets at the nest is therefore likely to also occur in some other species. Although many functions have been attributed to duets in a range of species (Farabaugh, 1982; Hall, 2004, 2009; Benedict, 2008; Dahlin & Benedict, 2013), their role in parental care negotiation had not been considered previously.

Overall, we have demonstrated that partners modify their vocal exchange when their incubation routine is experimentally altered by delaying male return, and they also adjust their time off-nest according to their calling behaviour in the previous duet. Taken together, these results suggest that acoustic communication may play a role in the negotiation of parental care between breeding partners.

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Supplementary material: Analysis of the effect of the sex of the returning partner on the structure of the duet.

METHODS

To control for the potential effect of the sex of the returning partner on the structure of the duet performed during incubation relief, additional data collected on a different group of 18 zebra finch pairs were used. Duets were recorded in 2013, in exactly the same aviary conditions and with the same set up as the ones used in 2011 for the main data set. On each nest, duets were recorded during two consecutive incubation shifts, in order to obtain one duet when the male returned to relieve the incubating female and one duet when the female returned to relieve the incubating male. We analysed partners' participation in the duet (proportion of male calls), vocal repertoire, and duet temporal structure. In addition, we considered which sex initiated the duet.

A- parameters

- Duet temporal structure was assessed using a PCA on four parameters: number of calls, call rate, duration of the arriving phase and duration of the transitioning phase (see variable loading table ESM 1).
- Vocal repertoire was assessed using a PCA on five parameters: the proportion of Whine, the duration of Short Calls in both sexes and the duration of Whines in both sexes (see variable loading table ESM 1). Only one PC had an Eigen value above one and was kept for the analysis.
- Male-female participation was assessed with the proportion of male calls.
- Duet initiation: the sex of the first caller was identified using behavioural observations.

Table ESM 1: Variable loadings of the PCA on: (a) duet temporal structure (complete data set of 34 duets, from 18 pairs) and (b) vocal repertoire (23 duets from 15 pairs, after removal of missing values before running the PCA).

a-	**PC1**	**PC2**
Explained variance (% cumulative)	61.8	89.6
Duet duration (s)	0.963802949	-0.026500614
Call rate (calls/s)	-0.823336513	0.230238034
Arriving phase duration (s)	0.071884863	-0.981681594
Transitioning phase duration (s)	0.928363813	0.307716429

b-	**PC1**
Explained variance (% cumulative)	37.8
Whine Proportion (%)	-0.750438223
Male Short Calls duration (s)	-0.730434936
Female Short Calls duration (s)	-0.543667033
Male Whines duration (s)	-0.681056833
Female Whines duration (s)	-0.178839946

B- Statistics.

All statistical tests were performed using R software. For duet temporal structure, vocal repertoire and partners' participation we used linear models (lmer function of lme4 package), using the sex of the returning bird as a fixed factor and pair identity as a random factor. P-values were calculated with the Anova function (car package). All models were validated using plotresid (RVAideMemoire package) for equivariance and normality of residuals.

Duet initiation was analyzed with a generalized mixed effect model for binomial distribution (glmer function of lme4 package), using the sex of the returning bird as a fixed factor and pair identity as a random factor.

RESULTS

Linear models run on PCs resulting from the PCA on duet temporal structure showed no effect of the sex of the returning partner neither on PC1 (61.8% of explained variance), nor on PC2 (27.8% of explained variance) (fig ESM 1a, table ESM 2).

Linear models run on the only PC having an Eigen value above one (37.8% of variance explained) from the PCA on vocal repertoire found no effect of the sex of the returning partner (Fig ESM 1b, Table ESM 2).

The proportion of male calls was significantly higher when the male was returning (Fig. ESM 1c, Table ESM 2).

Last, a significant effect of the sex of the returning partner was found on the sex of the first caller; when the male was returning, the probability that he was the first caller increased 15 times ([2-80] confidence interval) (Binomial GLM: Chi^2=10.0, Df=1, P=0.002, Fig. ESM 1d).

CONCLUSION

Duet temporal structure and vocal repertoire, as computed in the main experiment using indexes computed from PCA, did not differ depending on the sex of the returning partner. The results of our main experiment, showing differences between control and experimental duets, were therefore not confounded by an effect of the sex of the returning bird. It was thus appropriate to use the very last and very first duets around the feeder closure to investigate the effects of this manipulation. However, we found that the returning partner was the one initiating the duet. In addition, the returning partner also called more during the duet. This is consistent with the observation that the returning partner usually emits several calls before the incubating partner utters a first call in response. We thus excluded these two features from our analysis of the main experiment.

Figure ESM 1: Effect of the sex of the returning partner on (a) duet temporal structure, (b) vocal repertoire, (c) partner' participation and (d) duet initiation. In (d): number of duets initiated by the male or the female, depending on the sex of the returning partner. In (a), (b), (c): Boxes are 1st-3rd quartiles, lines are medians and whiskers are extreme values. Linear mixed effect models, see Table ESM 2 **P<0.01, ***P<0.001.

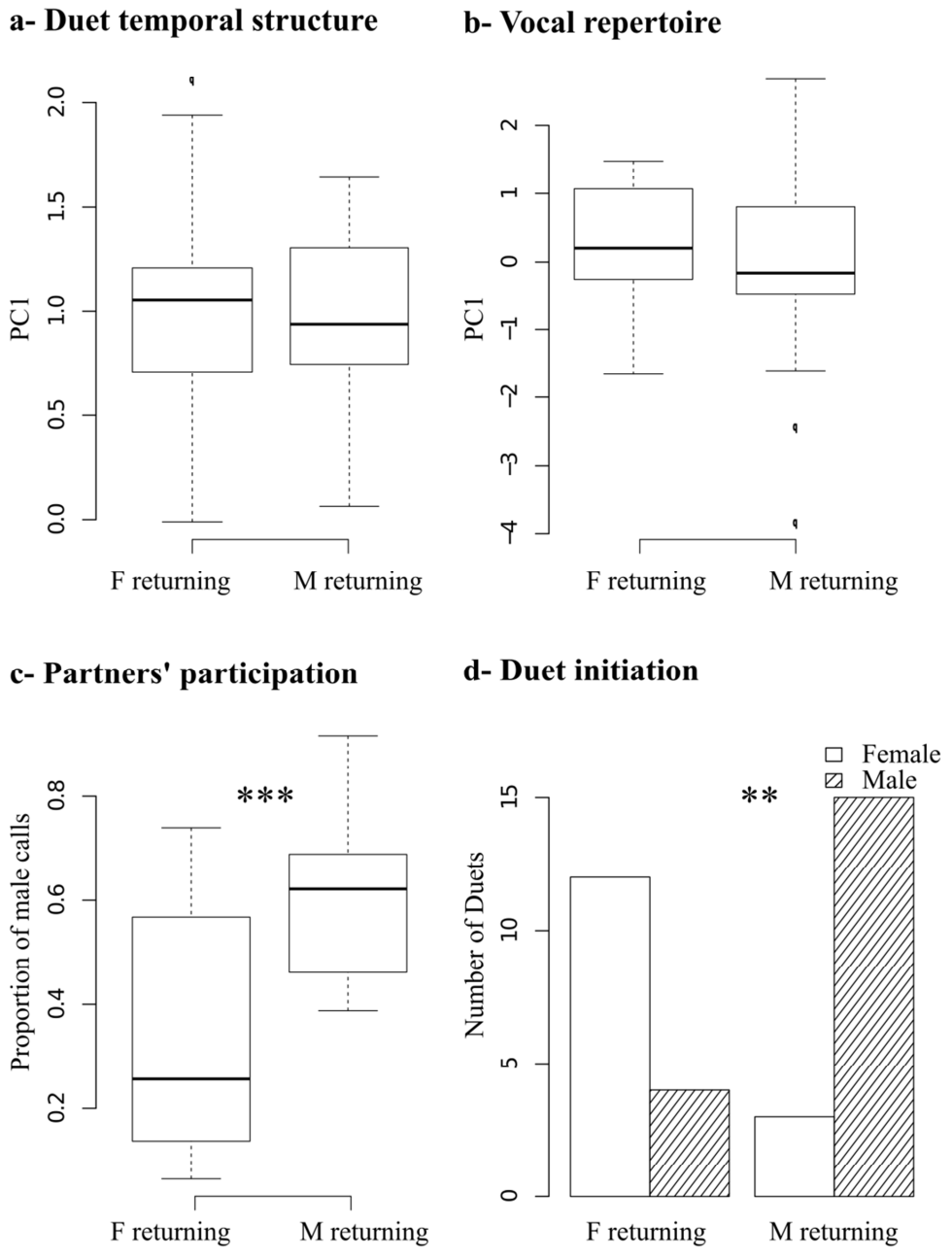

Table ESM 2: Effect of the sex of the returning partner on duet structure. Results from models ('lmer' function, 'lme4' R package) testing the sex of the returning partner as fixed factor, with pair identity as random factor.

	Chisq	DF	P-value	N duets	Estimate ± SE (F returning)	Estimate ± SE (M returning)
Duet temporal structure						
PC1(log+3)	0.362	1	0.547	34	1.0336 ± 0.124	0.9312 ± 0.117
PC2(box-cox[1], λ=2)[2]	0.007	1	0.934	34	12.4894 ± 1.111	12.6153 ± 1.047
Vocal repertoire						
PC1	0.586	1	0.444	23	0.2715 ± 0.487	-0.1212 ± 0.370
Male-female participation						
Proportion of male calls (%)	16.925	1	<0.001	34	0.3284 ± 0.048	0.6008 ± 0.045

1: The box cox transformation computes one parameter transformation using the following formula: parameter (λ)=parameter (λ) – 1 /λ, if $\lambda \neq 0$ and ln(parameter (λ)) if λ=0. The boxcox function of the Mass package automatically finds the appropriate λ value to reach a distribution as close as possible to the Gaussian distribution.
2: This model was not validated by the 'plotresid' graphical tool, even after data transformation, additional non-parametric Friedman test was performed (on a complete block design, n=16 pairs): χ_1=0.25, p= 0.62. The estimates are then to be interpreted precociously.

Chapitre 3

Interactive vocal communication at the nest between mates during breeding in Great Tits (*Parus major*)

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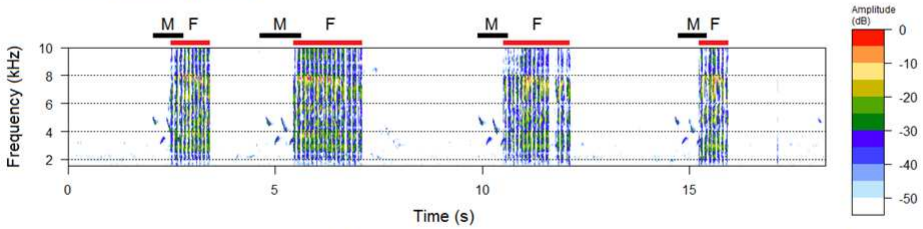
Soumis à IBIS

Synthèse du chapitre 3

Question : dans quels contextes les mésanges charbonnières font elles des duos vocaux et ces échanges diffèrent-ils selon les contextes ?

Méthodes : Couples enregistrés au nid à trois périodes de la reproduction : ponte, incubation, poussins de moins de 4 jours

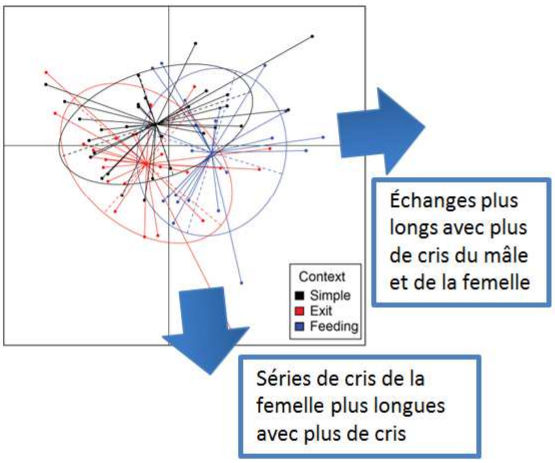
Résultats principaux

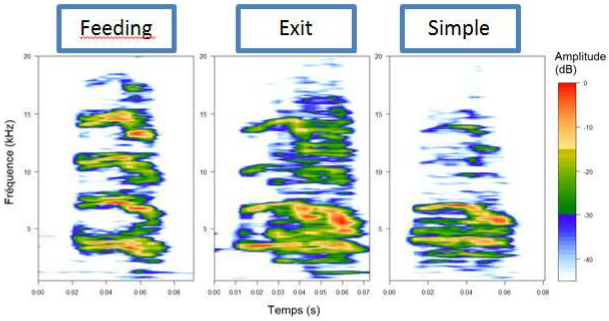


→ Les mésanges charbonnières font des échanges vocaux coordonnés dans lesquels la femelle adopte le rythme du mâle.

→ Ces échanges vocaux peuvent se terminer de trois façons:

- Exit : la femelle sort du nid et peut-être nourrie par le mâle
- Feeding : le mâle entre dans le nid nourrir la femelle
- Simple : l'échange s'arrête sans qu'un oiseau n'entre ou ne sorte du nid





→ La structure des cris de la femelle est différente selon l'issue de l'échange. Les cris sont notamment plus aigus dans les échanges Feeding que Simple avec Exit en intermédiaire

Conclusion

La structure des cris et des échanges vocaux prédit la façon dont ces derniers vont se terminer. Ces résultats suggèrent que les femelles pourraient indiquer aux mâles leur niveau de faim de façon à être nourries par ceux-ci.

ABSTRACT

Although most bird species show monogamous pair bonds and bi-parental care, little is known on how mated birds coordinate their activities. Whether or not partners communicate with each other to adjust their behaviors remains an open question. During incubation and the first days after hatching, one parent – generally the female – stays in the nest, and might depend on acoustic communication to exchange information with its mate outside. The Great Tit (*Parus major*) represents an interesting study system to investigate intra-pair communication at the nest, as males address songs to their female while she is in the nest cavity, and females answer their male from the cavity with calls. But what might be the functions of this communication remains unknown. In this study, we recorded the vocalizations and observed the behavior of Great Tit pairs around the nest at different breeding stages (laying, incubation and young nestlings). We observed vocal exchanges, *i.e.* vocalization bouts alternated on the same tempo, between the female inside the nest and her male outside in three contexts with different outcomes: (i) the female left the nest, (ii) the male entered the box with food, and the female then used specific call types, (iii) mates stopped calling but did not move out/in the nest. The structure of vocal exchanges was globally stable between contexts, but females used calls with up-shifted spectrum during exchanges at the end of which they left the nest or the male entered the nest. Birds vocalized more and at higher tempo during exchanges that ended up in feeding inside the nest. Birds also vocalized more during exchanges taking place during laying – a period of active mate guarding – than incubation. We conclude that vocal exchanges could signal females' need for food and males' mate guarding behavior, and discuss other possible functions of this communication.

Keywords: acoustic communication - bi-parental care – coordination - hole-nesting - intra-pair communication – monogamy - pair bond - songbird

INTRODUCTION

The monogamous pair bond in birds represents a real partnership (Black 1996). Bi-parental care can be considered as a form of cooperation in which parents have to coordinate their activities to maximize their reproductive success (Black 1996, Mariette and Griffith 2012). In addition to the partners' team-work during parental care, the pair bond brings several advantages to the mates. Mates are able to alert each other in case of danger (Krams et al. 2006), repel together predators or join forces in aggressive encounters with competitors (Black 1996). Surprisingly, little is known on how pair members achieve their division of labour during parental care (McNamara et al. 1999) or synchronize their behaviours during cooperation. During these processes, each mate could benefit from both sending and receiving information to/from its partner. Whether or not mates communicate with each other to coordinate their activities remains an open question, but if they do, acoustic communication may play a key role, as it does in many other social interactions in birds.

The vocal behavior of male birds, and especially male song, has been deeply studied during mate attraction and territory defense, but also after pair formation in the contexts of pair maintenance, mate guarding and territory tenure (Catchpole and Slater 2008). On the contrary, female songs and calls (Riebel 2003, Riebel et al. 2005, Odom et al. 2014) as well as interactive communication between male and female during and outside reproduction (Wachtmeister 2001) have received little attention so far, with the exception of acoustic duets (Farabaugh 1982, Hall 2004, 2009, Benedict 2008, Dahlin and Benedict 2013). Avian duets are joint acoustic displays between two birds that make temporally coordinated vocal or non-vocal sounds. Among them, the highly coordinated song duets of tropical songbirds have attracted most of the interest. These duets have been hypothesized to fulfil several functions like pair bond maintenance, mate guarding, joint territory defense or synchronization of breeding. While song duetting is rare (c.a. 4 % of bird species, Hall 2009), mates may use interactive communication involving simpler and less conspicuous vocalizations in many species. Mates, just as other social affiliates, exchange contact calls while foraging, flight calls before take-off, and separation calls after visual separation (Marler 2004). Mates can also use vocal interactions that are specific to the pair bond. Females may communicate their sexual receptivity to the male by calling (Nagle et al. 2002) or singing (Langmore et al. 1996). In Budgerigars (*Melopsittacus undulatus*), males signal that they are mated by imitating the contact call of their female (Hile et al. 2000). In Dusky Antbirds (*Cercomacra tyrannina*), both pair members can sing, but when one mate sings, the non-singing bird overlaps its mate's song with low-amplitude calls produced in close proximity to ensure the mate will not advertise for a new mate (Morton and Derrickson 1996). Finally, mates may use vocal cues to recognize each other in a crowd: in several species of penguins, birds identify their mate using the acoustic cues of the display call given when one bird returns from its foraging trip and meets its mate in the colony (Aubin and Jouventin 2002). In the gregarious Zebra Finches (*Taeniopygia guttata*), monogamous mates can recognize each other using long distance calls (Vignal et al. 2004, 2008) but also short distance contact calls (Elie et al. 2010). In conclusion, pair communication has been described in some contexts, but whether mates use vocal interactions to coordinate their activities is poorly known.

Pair interactions around the nest might represent a particularly propitious context to test whether mates use acoustic communication to coordinate their behaviors. During eggs incubation as well as young nestlings brooding, the parent staying in the nest has no or limited visual information about

the surroundings, because the nest is either completely enclosed except for the small entrance opening (cavities, burrows, sphere or bottle-shaped nests) or limiting drastically the bird's movements and positions (cups or platforms). Thus, acoustic signals are likely to play a key role for the communication between mates when one of them is at the nest.

Acoustic cues may be used by the bird at the nest to assess the situation outside, such as the proximity of its mate or the presence of predators. When their mate sings outside the nest, incubating female Great Tits (*Parus major*) may leave the cavity, often to be fed by the male (Hinde 1952, Royama 1966, Lind et al. 1996). Incubating female House Wrens (Troglodytes aedon) wait to leave the nest until their mate sings nearby, and this song may serve as an "all-clear" signal indicating to the female that it is safe to leave the cavity (Burns 1983, Johnson and Kermott 1991, Ziolkowski et al. 1997). In Zebra Finches, one of the mates can act as a sentinel outside the nest, exchanging calls with its incubating partner (Elie et al. 2010) and allowing its faster escape from an approaching predator (Mainwaring and Griffith 2013).

A bird may also use acoustic communication from the nest to give information to its distant mate. In Northern Cardinals (*Cardinalis cardinalis*), the female expresses her own needs for food or the need of her nestlings by singing from the nest and allows her male to adjust his feeding visits to the nest, limiting predation risks as males are highly conspicuous with their bright red plumage (Halkin 1997). In Red-winged Blackbird (*Agelaius phoeniceus*), females vocalize upon departing and arriving at their nest, and this might inform their polygynous mate of their activities and reproductive state (Beletsky and Orians 1985) as well as increase mate's nest-defense against predators (Yasukawa 1989). Females of several species use nest-departure calls which might promote anti-predator vigilance by mates, and are also supposed to signal females' identity and thus reduce mate harassment, a common behavior in species in which the males' threshold for an aggressive response toward any conspecific is low (McDonald and Greenberg 1991). For instance, females of the Common Village Weaverbird (*Textor cucullatus*) use a nest call to avoid being disturbed by the male during nest building (Collias 1963).

Finally, pair partners might use vocalizations when they meet at the nest. Each time they meet at the nest during incubation or nestling period, Zebra Finch mates perform simple coordinated mutual vocal displays that can be described as call duets (Elie et al. 2010). Nest meeting ceremonies using vocalizations have been described in several species, particularly in water birds (Johnsgard 1965) and seabirds (Aubin and Jouventin 2002), and are supposed to participate in pair bond maintenance but their functions remain largely unknown (Wachtmeister 2001).

The Great Tit (*Parus major*) provides an excellent study system to investigate intra-pair communication at the nest and test whether it relates to partners' coordination during breeding. Great Tits are cavity-nester and the female builds the nest, incubates the eggs and broods the hatchlings alone (Cramp and Perrins 1993). Males are known to address songs to their mate located in the cavity, as they stop the dawn chorus just after the emergence of their female from the nest (Mace 1986) and sing more at dawn with approaching laying period (Mace 1987). This song seems to secure paternity by favoring copulation after female emergence from the cavity (Mace 1987). Females can respond to their mate song by leaving the nest not only at dawn during the egg laying period (Hinde 1952, Mace 1987) but also throughout the day during incubation (Lind et al. 1996). In addition, during nest building, laying and incubation stages, females may vocally respond to their mate song from the nest by using nine different call types (Gorissen and Eens 2005) and this interactive vocal communication occurs both during and outside the dawn chorus (Gorissen et al.

2004, Halfwerk et al. 2011). Because most of these female calls are produced with a low sound pressure level, they are probably used in short-range communication directed at the mate only (Gorissen et al. 2004, Gorissen and Eens 2005). Males adjust their singing behavior depending on the female response. Indeed, males sing closer to the nest when their female is exposed to a playback of noise inside the cavity (Halfwerk et al. 2012), confirming that male song in this context is addressed to the mate and suggesting that female response is used as a feedback by the male. Great Tit mates thus show interactive vocal communication at the nest, but it remains unknown what pieces of information are exchanged and whether this communication participates in coordinating partners' activities.

In this study, we aimed at investigating the contexts and possible functions of vocal exchanges between Great Tit mates at the nest. We recorded vocal interactions at the nest between pair members over a breeding attempt, i.e. from egg laying to the young nestlings stage, both during and after the dawn chorus. Simultaneously, we made direct observations of the pair behavior to describe the contexts in which vocalizations are emitted. To decipher the possible functions of this communication, we compared the acoustic structure of vocal exchanges between contexts and between breeding stages.

METHODS

Study site and species

Great Tits are common territorial passerine birds that breed in natural tree holes but also readily accept artificial nest boxes in all types of wooded area throughout Europe and parts of Asia and North Africa (Cramp and Perrins 1993).

Our study was conducted in wooden areas close to the University of St-Etienne campus, in France (45°25'N, 4°25'E) provided with 24 nest boxes in 2013 and 54 nest boxes in 2014 (wood nest boxes, 150x150x250 mm, with an entrance hole of 32 mm diameter). To record occupation by a breeding pair, nest-boxes were checked once a week starting at the end of March, and when occupied, three times a week to monitor breeding.

Recording of parental acoustic and behavioral activities around the nest

Data were collected in April-May 2013 on seven Great Tit pairs and in April-May 2014 on 12 pairs. In 2013, each pair was recorded on one morning during three different reproductive stages: laying, incubation and young nestlings (i.e. four days old or less, at this stage females spend most of their time in the nest to brood the nestlings before they thermoregulate). In 2014, each pair was recorded on one morning during incubation. On the evening before the recording day, a recorder (SongMeters SM2+, 16-bit, 44100-Hz sampling rate; Wildlife Acoustics Inc., Concord, MA, USA) was positioned near the nest box, connected to a microphone (SMX-II, Wildlife Acoustics Inc., Concord, MA, USA; omnidirectional, flat frequency response 20 Hz-20 kHz, sensitivity -36±4 dB) inside the nest box just below the ceiling (gain of +24 dB) and a microphone outside, fixed on the tree trunk at the height of the nest box (gain of +42 dB). Pairs were recorded between 05:30 and 09:00 in the laying and young nestlings stages. The duration of recording was increased in the incubation stage (between 05:30 and 14:30) because behavioral events were less frequent at this stage. During

incubation and young nestlings stages, direct visual observations were performed simultaneously to acoustic recordings: an observer sat under a camouflage net at 10-15 m from the nest box and used a headphone to record simultaneously vocalizations and partners' behavior (e.g. birds' entrance and exit from the nest). Direct observations were not necessary during the laying stage, as mates' behavior during this period has been previously described in great detail (Mace 1987): only the female can enter and leave the nest during this stage, and these movements as well as periods of female presence or absence inside the nest were easily identified on acoustic recordings (Halfwerk et al. 2012). Moreover, direct observations were not necessary in 2014 as previous data from 2013 showed that each possible event was easily recognizable using the acoustic recording only. Thus, spectrograms of recordings were visualized using SongScope software (Wildlife Acoustics Inc., Concord, MA, USA) to detect vocal exchanges as well as birds' exits and entrances and their presence inside the nest.

Definition of vocal exchanges

We defined a vocal exchange as the vocal interaction of a female inside her nest with her male. We considered that the vocal exchange started with the male song strophe or call preceding the first vocalization of the female, and ended with the last female vocalization (in some rare cases, male's last vocalization bout overlap the one of the female and ended later; in those cases, the vocal exchange ended with this last male's vocalization bout). A vocal exchange stopped when the female stopped calling for at least 30 seconds, so two successive vocal exchanges were separated by at least 30 seconds.

Sometimes, we observed females vocalizing outside the nest but these vocalizations were difficult to record with our setup, so only female vocalizations produced inside the nest box were considered here.

Vocal exchanges occurred in three different contexts. In all cases, the male initiated the vocal exchange by either singing or calling outside the nest box and the female responded from inside, but the three contexts differed by their outcome:

- Simple exchange: mates stopped calling but did not move out/in the nest (Fig.1a; Supplementary material Sound 1);
- Exit exchange: the female left the nest (Fig.1b; Supplementary materiel Sound 2);
- Feeding exchange: the male entered to feed the female or the nestlings. While the male was inside, the female used specific call types (Fig.1c; Supplementary material Sound 3). Note that the male sometimes enters directly in the nest box, skipping the preceding vocal exchange (incubation: one out of 10 cases in 2013, and 6 out of 20 cases in 2014; young nestlings stage: eight out of 12 cases in 2013).

We extracted two vocal exchanges from each context in each breeding stage. Only one context was sufficiently frequent to be analyzed in laying and young nestlings stages, and three contexts in incubation stage, so in 2013 the maximum sample size per pair is thus of 10 vocal exchanges. One out of the seven recorded females called only twice inside the nest box and thus, was not included in the acoustic analyses of vocal exchanges, but was included in other quantifications. Two other pairs had missing vocal exchanges (four and three respectively), so a total of 53 vocal exchanges were analyzed in 2013. In 2014, whenever possible, we extracted two vocal exchanges per context and per pair (maximum sample size per pair = 6 vocal exchanges). Missing

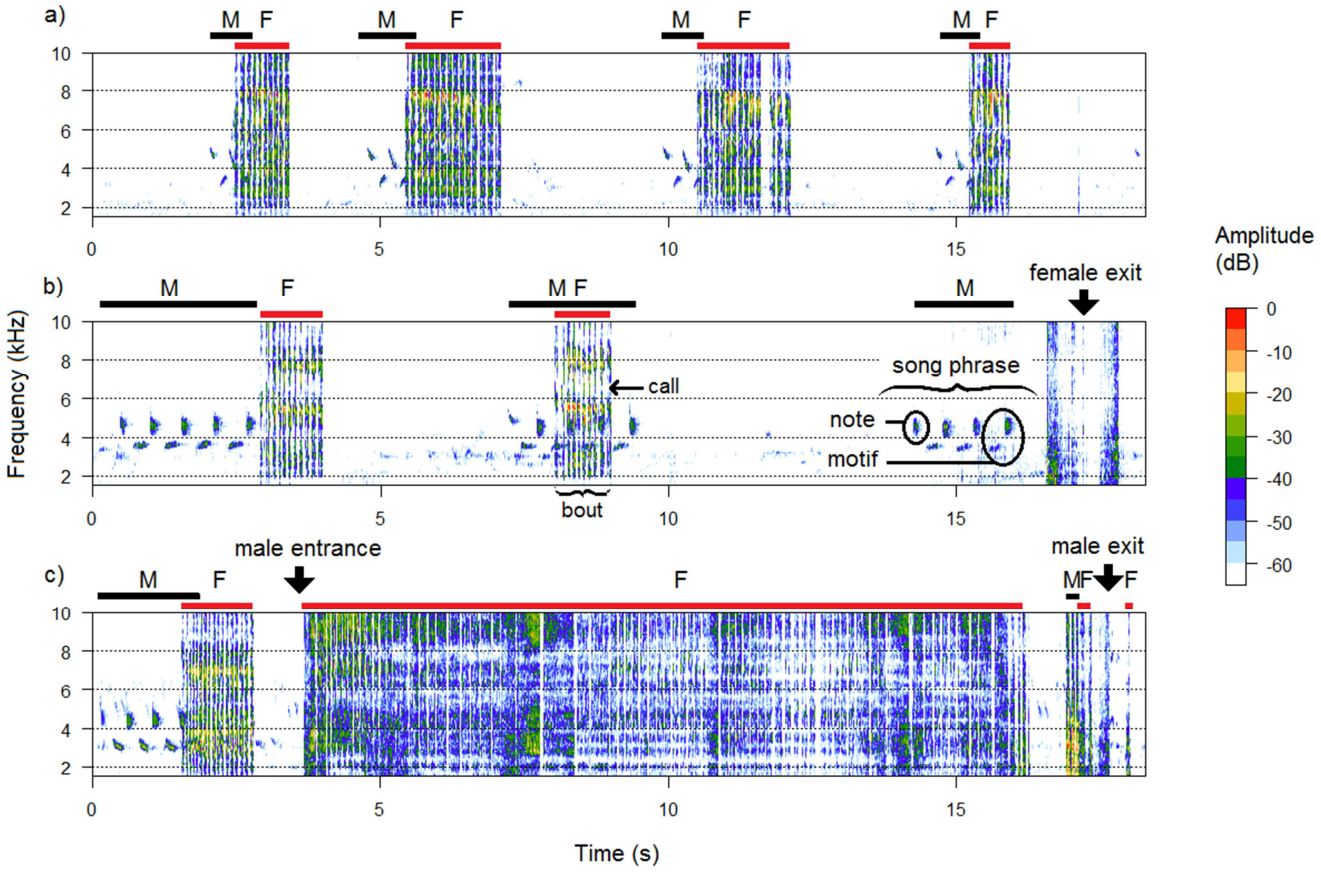


Figure 1. Spectrograms showing extracts of the three contexts of vocal exchanges recorded at the nest between Great Tit mates: a) Simple exchange, b) Exit exchange, c) Feeding exchange (see text for the description of these contexts); M and F: male and female vocalization bout respectively. Spectrograms were prepared using the Seewave package (Sueur et al. 2008) in R software (R Development Core Team 2014).

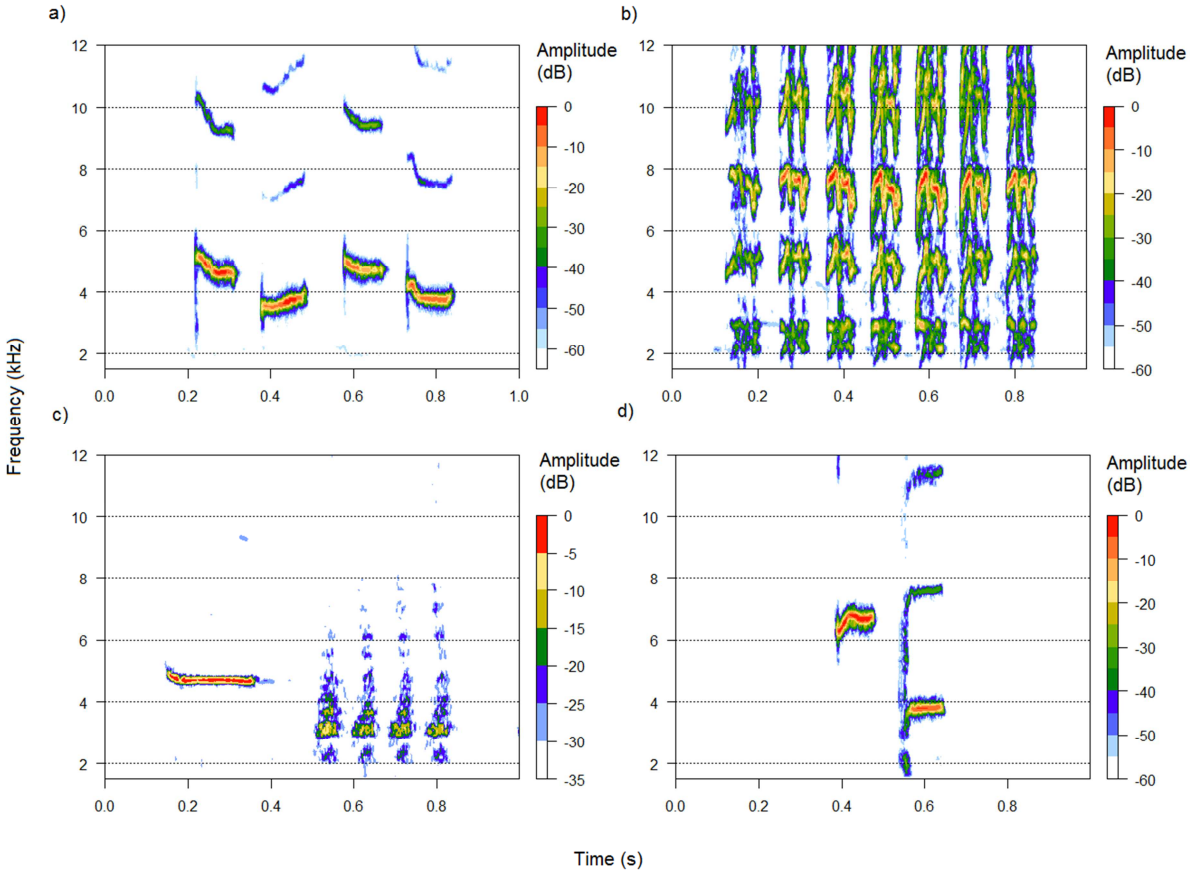


Figure 2. Spectrograms of vocalizations used during exchanges: a) female song, b) female bout of chattering calls, c) male alarm call, d) male contact call. Spectrograms were prepared using the Seewave package (Sueur at al. 2008) in R software (R Development Core Team 2014).

vocal exchanges in some pairs led to a total of 51 vocal exchanges analyzed in 2014. On these vocal exchanges, we performed acoustic analyses describing (i) the vocal repertoire and acoustic structure of female calls and (ii) the temporal structure of the exchange.

Vocal repertoire and analysis of the acoustic structure of female calls

Both males and females produced different call and song types during vocal exchanges (Hinde 1952, Gompertz 1961). Females predominantly produced bouts of calls (series of identical calls emitted with silences between calls that were shorter or equal to the call duration; Fig.1). These bouts mainly contained chattering, a high-pitched call with rapid frequency modulations (described by Gorissen et Eens 2005; Fig.2b; Supplementary material Sound 5). Females occasionally used songs (only during incubation, two females out of six in 2013 and three females out of 12 in 2014, Fig.2a; Supplementary material Sound 4). Great Tit songs are notes (continuous traces on the spectrogram separated by silences) assembled to form motifs (generally two different notes per motif), which are repeated to form a phrase. The song type produced by females sometimes matched the song type of their mate, i.e. showed the same spectro-temporal shape and properties. Females also occasionally used contact calls (four females out of 12 in 2014). Contact calls are song-like notes that can be associated to form a motif. Contrary to songs, however, these motifs are not repeated to form a phrase (Fig.2d; Supplementary material Sound 11).
All males produced songs and contact calls in all contexts of vocal exchanges. Males occasionally used alarm calls (four males in a total of six vocal exchanges in 2013 and four males in a total of five vocal exchanges in 2014; Fig.2c; Supplementary material Sound 10). Alarm calls were produced during Simple and Feeding exchanges but never during Exit exchanges.

We performed an analysis of the acoustic structure of female chattering calls extracted from recordings of 2014 (N=1529 calls from 12 individuals, mean□sd=127.4□126.7) using custom-written codes based on the Seewave package (Sueur et al. 2008) implemented in R (R Development Core Team 2014). The duration of each call (in s) was measured between edges manually labeled using Praat software (http://www.fon.hum.uva.nl/praat/). After bandpass filtering (500Hz-20000Hz encompassing the spectral bandwidth of Great Tit vocalizations, 'fir' function), the amplitude of the call was measured as the root-mean-square of the amplitude envelope of the call ('rms' function) and the following spectral parameters were computed using the 'specprop' function (FFT using a Hamming window and a window length of 512):

- As the frequency spectrum is a distribution, it can be characterized using classic descriptive statistics (all in Hertz). We used the median, the first (Q25) and third (Q75) quartiles of the spectrum of the call – which represent the frequencies below which lie respectively 50%, 25% and 75% of the energy of the call. The IQR (Inter Quartile Range) was defined as Q75- Q25. We also used the mean and standard deviation (sd) of the spectrum.
- The frequency of highest amplitude – the dominant frequency – can be measured over the duration of the call (obtained via the 'dfreq' function, window length of 512 and overlap of 50%) and characterized using its mean (average dominant frequency) and its standard deviation (in Hertz).
- The call's noisiness can be quantified using the Shannon spectral entropy. The Shannon entropy of a noisy signal tends towards 1 whereas it tends towards 0 for a pure tone.

Temporal structure of vocal exchanges

The temporal structure of vocal exchanges was manually labeled using Praat software before being analyzed using custom-written codes implemented in R. For each vocalization (call or song motif), we recorded the identity of the caller and the call or song type. Then, we measured temporal parameters for each vocal exchange: the duration of the exchange and, for each sex, the numbers of (i) song motifs, (ii) calls, and (iii) vocalization bouts (call bouts or song phrases). For females, we also measured the number and duration of vocalization bouts, as well as the number of female vocalization bouts per exchange that overlapped a male vocalization bout. For males, we measured the number and duration of vocalizations bouts.

To compare the tempo of male and female vocalizations, we measured Inter Bout Intervals (IBI), i.e. the duration between the start of two successive vocalization bouts of the same individual. To quantify the reaction time of the female to her male's vocalizations, we measured in each vocal exchange the mean latency, i.e. the duration between the beginning of a male vocalization bout and the beginning of the following female vocalization bout.

Ethical note

Experiments were performed under the authorization no. CU13N7-ENES-VIGNAL1 (issued by the Ethics committee of Université de Saint-Etienne) and were in agreement with the French and European legislations regarding experiments on animals.

Statistical analyses

All statistical tests were performed using R software (R Development Core Team 2014).
Following all linear mixed models (LMM, lmer function of the lme4 package), equivariance and distribution of the residuals were graphically checked using plotresid function (RVAideMemoire package). The stability of the models and absence of influential cases were checked by removing levels of the random effects one at a time (infl.model and cook.distance functions of the influence.ME package, Nieuwenhuis et al. 2012). For each model, marginal and conditional coefficients of determination are presented (r2m and r2c, r.squaredGLMM function of the MuMIn package, (Nakagawa and Schielzeth 2013) as well as effect sizes using estimates, standard errors and 95% confidence intervals of fixed factors. P-values on models were obtained using Wald Chi-square tests (Anova function, car library). Post-hoc tests were performed using multiple comparisons tests (glht function of the multcomp package, with Tukey contrasts).
If equivariance and distribution of the residuals were violated or if model was unstable, non-parametric statistics were performed. Non-parametric tests (for paired data: Wilcoxsign_test; for independent data: Wilcox_test, coin library) are presented with their effect size as r=Z/√N, where Z is the statistics and N the total sample size.

To compare between breeding stages the number of vocal exchanges of each context (Simple, Exit, Feeding) as well as the number of female exits without any vocalization and the time spent by the female in the nest during recording, we used non-parametric Friedman tests on 2013 data recorded over the period 5:30 – 9:00. Post-hoc tests were performed using pairwise multiple

comparisons for ranked data (Nemenyi 1963); posthoc.friedman.nemenyi.test function of the PMCMR package).

We tested the temporal coordination between mates' vocal productions using incubation data collected in 2013 and 2014. We did four different analyses:

(1) First, we tested the regressions between the mean numbers of male and female vocalization bouts per exchange, and between the mean male and female Inter Bout Intervals (IBI) per exchange. We used LMM taking into account repeated measures within pairs (random factor) and testing also the potential interaction with the context (3 levels: Simple, Exit, Feeding). As recommended for within subjects design with a covariate (Schielzeth & Forstmeier 2009), the covariate was also included as a random slope as follows: Male parameter ~ Female parameter * Context + (1+ Female parameter | Pair identity). In each LMM, one pair was identified as overly influential on the regression outcomes (see method above) so it was removed from the data set, leading to N=74 exchanges from 17 pairs.

(2) Then, to test whether individuals adjusted the tempo of their vocalizations to that of their partner in alternated bouts (series MFMF…, where M is a male vocalization and F is a female vocalization), we compared each male Inter Bout Interval (IBI) to the corresponding female IBI (in M1F1M2F2M3F3, the interval M1M2 is compared to the interval F1F2, the interval M2M3 is compared to the interval F2F3…). In this analysis, we thus considered only alternated vocalization between mates (Male-Female or Female-Male transitions) so excluding successive bouts of the same individual (for instance in MFMFFF, only MFMF is studied). A positive regression between male and female IBI should indicate than mates adjust the tempo of their vocalizations to each other. This was assessed the following LMM: log10(Male IBI) ~ log10(Female IBI) + (1 + log10(Female IBI)| Pair identity). The sample size of this analysis was N=124 dyads of male and female IBI from 14 pairs.

(3) If male and female have the same vocalization tempo, then we expect that male average IBI will not differ from female average IBI. Moreover, if male and female vocalizations show antiphasic alternation (same duration between female response to the preceding male bout and male response to the preceding female bout), we expect that male IBI as well as female IBI will not differ from twice the latency (delay between the beginning of a male vocalization bout and the beginning of the female response). We tested these hypotheses on interval values (average per exchange) using a LMM with the type of interval (three levels: male IBI, female IBI, or 2*Latency) as fixed factor, the pair identity and the exchange identity as random factors as follows: log10(interval) ~ type + (1 | Pair identity) + (1 | exchange). The sample size was N=240 from 80 exchanges of 18 pairs.

(4) To test whether the number of female vocalization bouts overlapping male bouts was random or occurred more or less often than expected by chance given the vocalization rate of the partners, we used the duty cycle method outlined by Ficken et al. (Ficken et al. 1974) and commonly used to estimate chance levels of overlap (e.g. (Maynard et al. 2012, Hall et al. 2015). We computed the proportion of female vocalization bouts per exchange that overlapped a male vocalization bout (observed proportion of overlaps). We compared this with the proportion of female bouts expected to overlap a male bout by chance, given the amount of time the male was vocalizing (expected proportion of overlaps = male duty cycle = sum of male bouts' durations / exchange's total duration). The comparison between observed and expected proportions of overlaps was done using a Wilcoxon signed rank test on all exchanges showing at least one male-female transition (N=52 exchanges from 17 pairs). We also performed this comparison using only the exchanges showing at least one overlap

Table 1. Variable loadings of the PCA on A) the acoustic structure of females' chattering calls, B) the temporal structure of the three contexts of vocal exchange in incubation, and C) the temporal structure of Simple exchange in egg laying and incubation stages.

Variable	PC1	PC2
Duration	-0.34	0.00
Mean	-0.90	-0.34
Sd	-0.75	0.51
Median	-0.80	-0.48
First quartile (Q25)	-0.33	-0.85
Third quartile (Q75)	-0.94	0.06
Inter quartile range (IQR)	-0.78	0.55
Shannon spectral entropy	-0.86	0.26
Average dominant frequency	-0.54	-0.72
Sd of the dominant frequency	-0.61	0.06
Amplitude	0.47	-0.50

B)

Variable	PC1	PC2
Duration of the vocal exchange	0.86	0.40
Number of female vocalizations	0.95	-0.04
Mean duration of a female bout	0.36	-0.85
Mean number of calls in one female bout	0.31	-0.90
Number of male vocalizations	0.85	0.36
Female's latency response to male vocalizations	-0.38	0.08

C)

Variable	PC1	PC2
Duration of the vocal exchange	-0.85	0.37
Number of female vocalizations	-0.97	0.05
Mean duration of a female bout	-0.43	-0.79
Mean number of calls in one female bout	-0.59	-0.67
Number of male vocalizations	-0.85	0.31
Female's latency response to male vocalizations	0.30	-0.37

(N=29 exchanges from 15 pairs). We confirmed the results of both comparisons using only mean values per pair, controlling for pseudo-replication.

The acoustic structure of females' chattering calls was studied using the 2014 incubation recordings (N=1529 calls from 12 females). To test whether the acoustic structure of calls differed when compared between the three contexts of vocal exchange, we first built composite scores of acoustic structure using a principal component analysis (dudi.pca of the ade4 package). Before being included in the PCA, symmetrical distribution of parameters was verified. The resulting PCA had two principal components with eigenvalue above 1 (Kaiser criterion). The first and second principal components, PC1 and PC2, explained respectively 48.8 % and 22.5 % of the variance, and showed the variable loadings displayed in table 1a. PC1 and PC2 were analyzed using a LMM with the context (three levels: Simple, Exit, Feeding) as fixed factor and the female identity as random factor (12 levels) as follows: PC ~ Context + (1|Female identity). For both LMM on PC1 and PC2, the data set was N= 1529 calls from 12 females.

Because all three contexts of vocal exchange (Simple, Exit, Feeding) were not observed in every breeding stages, it was not possible to test in the same model (1) whether acoustic parameters differed between the three contexts within the same breeding stage, (2) whether acoustic parameters differed between breeding stages for the same context. We thus answered these questions separately.

To test whether the temporal structure of the three contexts of vocal exchange differed when compared within the same breeding stage (which was possible only in incubation, using both 2013 and 2014 data), we first built composite scores of acoustic structure using a principal component analysis (dudi.pca of the ade4 package). To run the PCA, we kept only parameters showing symmetrical distribution (all were log-transformed to reach this criterion). The numbers of male and female bouts and the proportion of male vocalization overlapped by the female were excluded based on this criterion. Inter Bout Intervals (IBI) were also excluded as male (respectively female) IBI had no value in all exchanges showing only one male (respectively female) bout. The resulting PCA had two principal components with eigenvalue above 1 (Kaiser criterion). The first and second principal components, PC1 and PC2, explained respectively 45.7 % and 30.6 % of the variance, and showed the variable loadings displayed in table 1b. PC1 and PC2 were analyzed using a LMM with the context (three levels: Simple, Exit, Feeding) as fixed factor and the pair identity as random factor (18 levels) as follows: PC ~ Context + (1|Pair identity). The LMM on PC1 showed no overly influential pair (see methods above), so data set was N= 77 exchanges from 18 pairs. In the LMM on PC2, two pairs were sequentially identified as overly influential on the outcome of the model, so they were removed from the data set to reach model's stability, leading to N=66 exchanges from 16 pairs. Then, the total number of vocalization bouts and the IBI were compared between contexts using a kruskal-wallis test followed by pairwise multiple comparisons for ranked data (posthoc.friedman.nemenyi.test function of the PMCMR package) on average value per pair and per context to correct for pseudo-replication.

To test whether the temporal structure of the same context of vocal exchange differed between breeding stages (which was possible only on Simple vocal exchanges on 2013 data), we compared the temporal structure of exchanges between laying and incubation stages. In order to do so, we built composite scores of acoustic structure using a principal component analysis (see above).

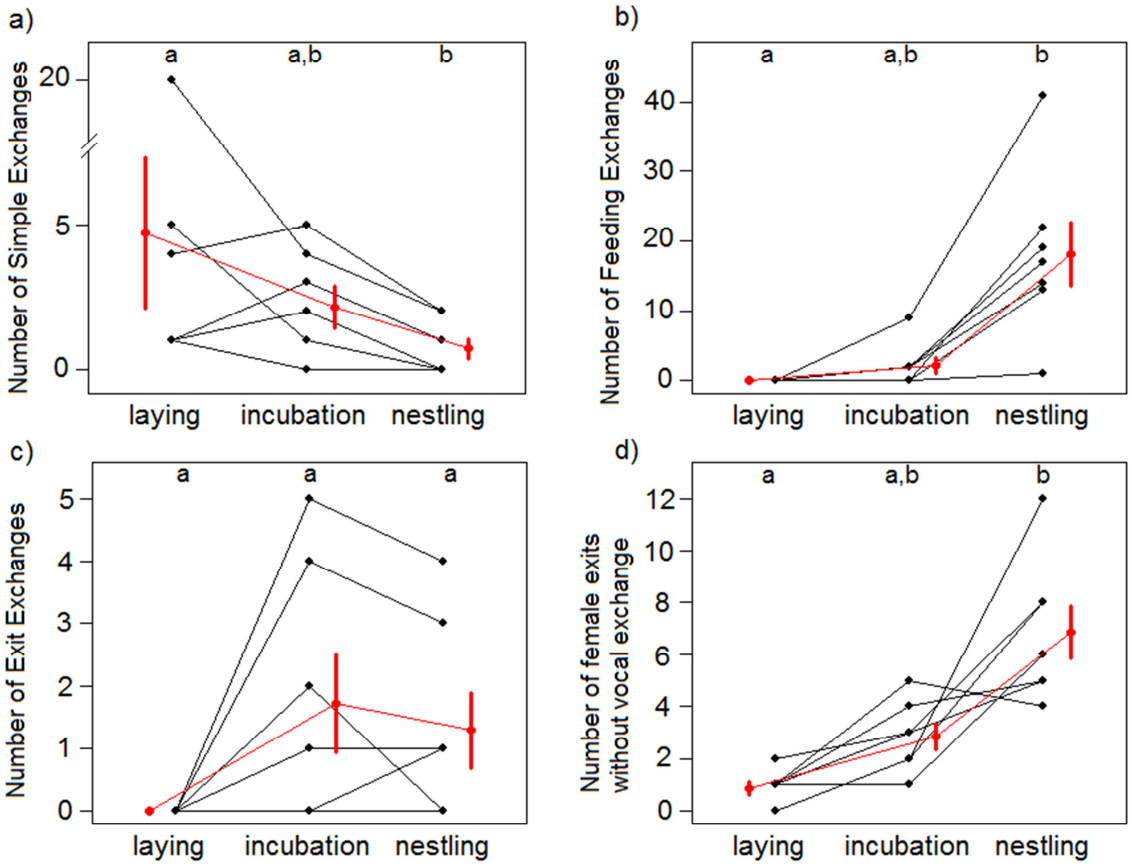


Figure 3. Number of vocal exchanges and female exits recorded between 05:30 and 09:00 along the three phases of the breeding cycle. Lines connect values for each given nest, red points are means with standard error on all individuals. Letters refer to significant differences among breeding stages, N=7.

The first and second principal components, PC1 and PC2, explained respectively 50 % and 24.1 % of the variance, and showed the variable loadings displayed in table 1c.

Because the sample size was small (N= 22 exchanges from 6 pairs), PC1 and PC2 were compared between laying and incubation stages using non-parametric tests on average value per pair (Wilcoxsign_test for paired data). The number of vocalization bouts and the IBI were also compared between stages using non-parametric tests (Wilcoxsign_test for paired data) on average value per pair and per stage to correct for pseudo-replication.

RESULTS

Frequency of the three contexts of vocal exchanges between mates across breeding stages

We observed vocal exchanges, i.e. alternated vocalization bouts between the female inside the nest and her male outside (Fig. 1) in three contexts with different outcomes (see methods): (i) the female left the nest (Exit), (ii) the male entered the box with food (Feeding), (iii) mates stopped calling but did not move out/in the nest (Simple).

All three contexts were observed during incubation and young nestlings stage, but during laying, only Simple exchanges were performed by the birds (Fig. 3). The absence of the two other contexts during laying was not simply explained by a shorter time spent by the female in the nest during this period, as this time was shorter during young nestling stage but not during laying ($\chi^2 2=8$, P=0.02, N=7, posthoc Z=2.37, P=0.018, r=0.63). From laying to young nestlings stage, the number of Simple exchanges decreased ($\chi^2 2=7.44$, P=0.024, N=7; posthoc Z=2.31, P=0.021, r=0.62, Fig.3a) while the number of feeding visits by the male increased ($\chi^2 2=13.04$, P=0.001, N=7; posthoc Z=-3.37, P=0.018, r=0.63, Fig.3b). The number of Exit exchanges also tended to increase across breeding stages ($\chi^2 2=6.12$, P=0.047, N=7; Fig.3c) but post-hoc tests did not show any pairwise significant difference. The number of female exits without vocalization increased ($\chi^2 2=11.62$, P=0.003, N=7; Z=-2.37, P=0.018, r=0.63, Fig.3d).

Temporal coordination between mates during vocal exchanges

Vocal exchanges consisted of vocalization bouts alternated between the female inside the nest and her male outside (Fig. 1). In the three contexts observed during incubation (Simple, Exit, Feeding), the numbers of male and female vocalization bouts were positively related (Table 2a, Fig. 4a), indicating vocal interaction between mates. Male and female Inter Bout Interval (IBI) were also positively related (Table 2b), indicating temporal coordination. This was also true when considering only alternated vocalization bouts (male-female or female-male transitions, see Methods) (Table 2c, Fig. 4b). Moreover, in each exchange, the average female IBI did not differ from the average male IBI (mean (95%CI) in s: female 5.89 (5.01-6.92), male 5.25 (4.47-6.17), Table 2d), confirming that male and female vocalizations had the same tempo. Furthermore, the delay between the beginning of a male vocalization bout and the following female bout (latency: 1.2 (1.04-1.37) s) was shorter than half the male or the female IBI (Table 2d). This result shows that vocal exchanges were not fully antiphasic male-female interactions, but that females responded to male vocalizations more rapidly than the reverse.

Table 2. Results of LMM on the temporal coordination between mates during vocal exchanges.

a)

	Estimate	Std. Error	Chi²	Df	P-value
Number of male vocalization bouts (R2m=0.72, R2c=0.85)					
(Intercept)	0.4544	0.3527			
Number of females vocalization bouts	0.7455	0.1525			
Feeding exchanges	-0.4808	0.5372			
Simple exchanges	-0.1096	0.5109			
Number of females vocalization bouts : Feeding Exchanges	0.2126	0.1465			
Number of females vocalization bouts : Simple Exchanges	0.1393	0.1855			
Number of females vocalization bouts			67.68	1.0	**<2e-16**
Context			0.23	2.0	0.891
Number of females vocalization bouts : Context			2.11	2.0	0.349

b)

	Estimate	Std. Error	Chi²	Df	P-value
Mean male IBI (R2m=0.34, R2c=0.60)					
(Intercept)	3.5268	1.56767			
Female IBI	0.4022	0.2309			
Feeding exchanges	0.49073	2.07317			
Simple exchanges	-0.5414	1.99889			
Female IBI : Feeding exchanges	-0.2216	0.35041			
Female IBI : Simple exchanges	0.08499	0.27723			
Female IBI			10.79	1.0	**0.001**
Context			0.88	2.0	0.643
Female IBI : Context			1.05	2.0	0.593

c)

	Estimate	Std. Error	Chi²	Df	P-value
Male IBI in alternated vocalization bouts (R2m=0.35, R2c=0.47)			54.04	1.0	**<0.001**
(Intercept)	0.23652	0.05085			
Female IBI (log)	0.58772	0.07995			

d)

	Estimate	Std. Error	CI 95%	z-value	Chi²	Df	P-value
IBI type (R2m=0.34, R2c=0.54)					131.39	2.0	**<0.001**
2*Female latency response	0.3812	0.0301	[0.320; 0.442]				
Female IBI	0.7689	0.0348	[0.699; 0.839]				
Male IBI	0.7181	0.0347	[0.648; 0.788]				
Female IBI - 2*Female latency response				10.33			**<0.001**
Male IBI - 2*Female latency response				8.96			**<0.001**
Male IBI - Female IBI				-1.273			0.410

Significant results are displayed in bold.

On average, 26.80% of females' vocalizations bouts overlapped the preceding male bout. This proportion did not differ from chance levels given the vocalization rates of both partners and the duration of males' vocalizations (Z= 0.045, P = 0.964, r=0.004). But when considering only exchanges with at least one overlap (55.77% of the exchanges), females overlapped significantly more often their males' bouts than expected by chance (Z = -3.665, P = 0.0002, r=0.681).
Taken together, these results suggest that females matched males' tempo.

Effect of the context on the acoustic structure of female calls during vocal exchanges

The first composite score of acoustic structure of the female chattering calls (first principal component PC1 of the PCA) showed that females used calls with up-shifted frequency spectrum (higher mean, median, Q75 and average dominant frequency), wider frequency bandwidth (higher sd and IQR), larger modulations of frequency (higher sd of the dominant frequency), more spectral noise and lower amplitude in Exit than in Simple exchanges (Table 3, Fig.5a). PC2 showed that females used calls with up-shifted frequency spectrum (higher Q25 and average dominant frequency), narrower frequency bandwidth (lower sd and IQR) and higher amplitude in Feeding than in Simple exchanges (Table 3, Fig. 5a). The same differences on PC2 showed tendencies between Feeding and Exit exchanges, as well as between Exit and Simple exchanges.

Effect of the context on the temporal structure of vocal exchanges

Does the temporal structure of the three contexts of vocal exchange differ when compared within the same breeding stage? Within the same breeding stage, differences in acoustic structure could determine the outcome of the vocal exchanges (female exit in Exit exchanges, male entering and feeding in Feeding exchanges, or no movements of birds out/in the nest in Simple exchanges). To test for this hypothesis, we compared the structure of Simple, Exit and Feeding exchanges recorded during incubation, because it was the only breeding stage during which we recorded the three contexts of exchange. The first composite score of acoustic structure of the vocal exchange (first principal component PC1 of the PCA) showed that Feeding exchanges differed significantly from Simple and Exit exchanges (Table 3, Fig. 5b): Feeding exchanges were longer with more male and female vocalizations. The second composite score (PC2 of the PCA) showed that Exit exchanges differed significantly from Simple exchanges (Table 3, Fig 5b): female used longer bouts and more calls per bout during Exit exchanges than Simple exchanges.
Furthermore, Feeding exchanges tended to have more vocalizations bouts than Exit exchanges ($\chi^2 2=5.54$, P=0.063; posthoc Z=-2.31, P=0.021, r=0.44) and shorter IBI than Simple exchanges ($\chi^2 2=5.85$, P=0.054; posthoc Z=-2.33, P=0.020, r=0.45).

Thus, longer vocal exchanges in which birds produced more vocalizations at higher bout rate had a higher probability to end up in a feeding event, and the outcome of vocal exchanges with more female calls per bout was more likely to be an exit.

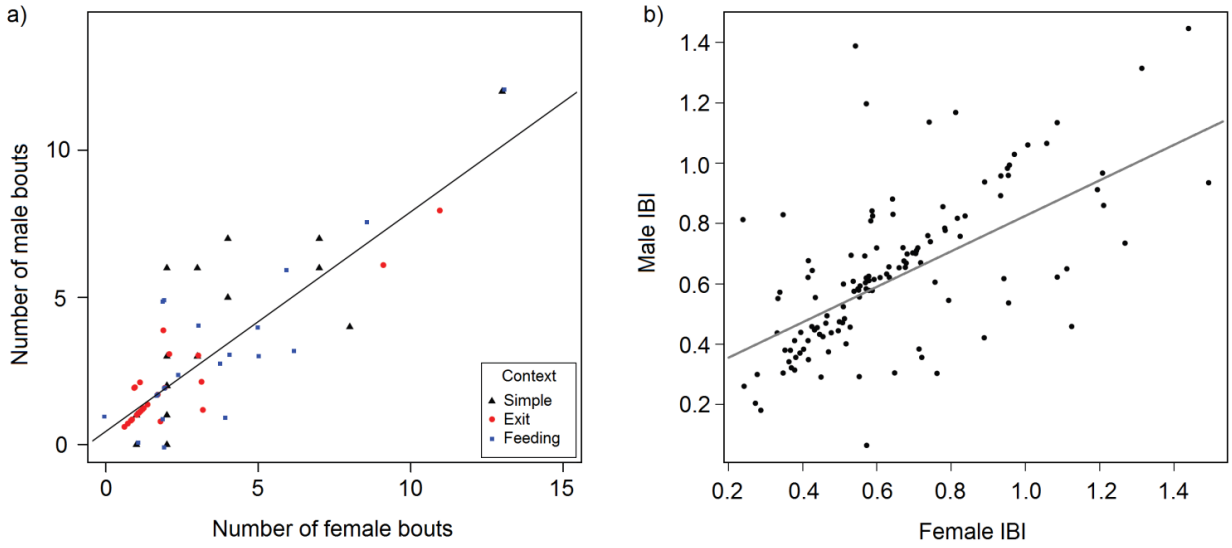


Figure 4. Temporal coordination between mates during vocal exchanges: linear regressions between the a) numbers of male and female bouts per exchange, b) male and female IBI in alternated sequences (see Methods). Regression lines result from the LMM analyses.

Table 3. Effect of the context on the acoustic structure of the female chattering calls and on the structure of the vocal exchange.

	Estimate	Std. error	CI 95%	z-value	Chi²	Df	P-value
PC1 of the PCA on chattering calls' structure (R2m=0.002, R2c=0.54)					6.98	2	**0.030**
Exit exchange	-0.7660	0.5530	[-1.98; 0.44]				
Feeding exchange	-0.6320	0.5510	[-1.84; 0.58]				
Simple exchange	-0.3990	0.5520	[-0.72; -1.61]				
Feeding exchange - Exit exchange	0.1337	0.1306		1.02			0.561
Simple exchange - Exit exchange	0.3669	0.1431		2.56			**0.028**
Simple exchange - Feeding exchange	0.2332	0.1231		1.89			0.140
PC2 of the PCA on chattering calls' structure (R2m=0.015, R2c=0.27)					24.49	2	**<0.001**
Exit exchange	0.1707	0.2512	[-0.37; 0.71]				
Feeding exchange	-0.0484	0.2486	[-0.59; 0.49]				
Simple exchange	0.4301	0.2503	[-0.11; 0.97]				
Feeding exchange - Exit exchange	-0.2191	0.1027		-2.13			0.083
Simple exchange - Exit exchange	0.2594	0.1125		2.31			**0.055**
Simple exchange - Feeding exchange	0.4785	0.0969		4.94			**<0.001**
PC1 of the PCA on acoustic structure of vocal exchanges (R2m=0.17, R2c=0.24)					16.47	2	**<0.001**
Exit exchange	-0.6020	0.3100	[-1.22; 0.02]				
Feeding exchange	1.0320	0.3350	[0.36; 1.70]				
Simple exchange	-0.3680	0.2940	[-0.96; 0.22]				
Feeding exchange - Exit exchange	1.6339	0.4319		3.78			**<0.001**
Simple exchange - Exit exchange	0.2341	0.4010		0.58			0.828
Simple exchange - Feeding exchange	-1.3998	0.4205		-3.33			**0.003**
PC2 of the PCA on acoustic structure of vocal exchanges (R2m=0.10, R2c=0.38)					11.35	2	**0.003**
Exit exchange	-0.4300	0.2790	[-0.99; 0.13]				
Feeding exchange	-0.0620	0.2960	[-0.66; 0.53]				
Simple exchange	0.5750	0.2680	[0.03; 1.12]				
Feeding exchanges - Exit exchange	0.3685	0.3292		1.12			0.502
Simple exchange - Exit exchange	1.0059	0.3034		3.32			**0.003**
Simple exchange - Feeding exchanges	0.6374	0.3187		2.00			0.112

Significant results are displayed in bold.

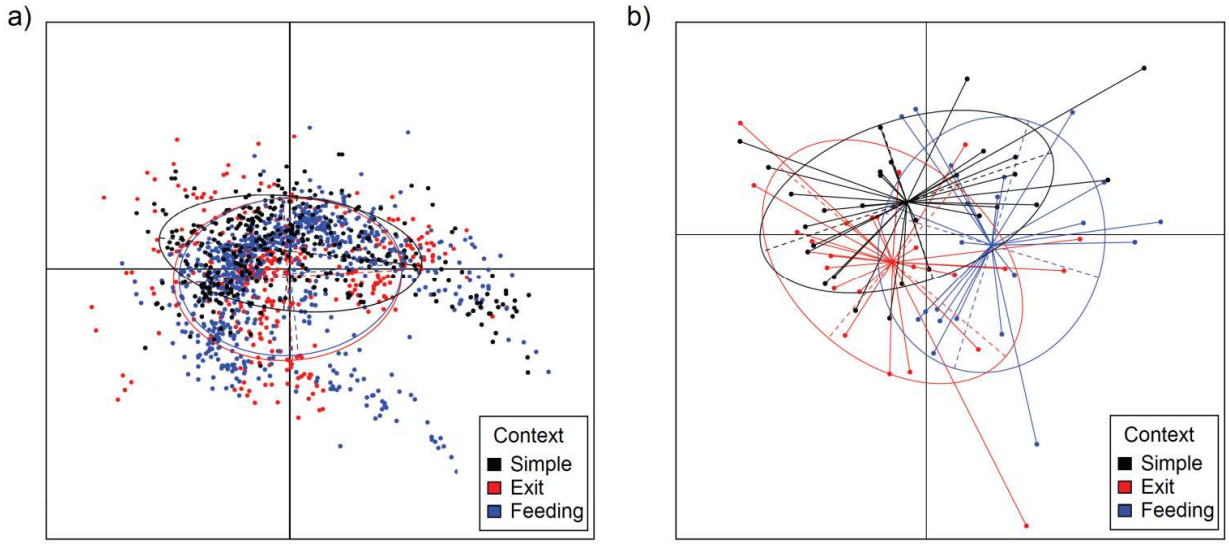


Figure 5. Effect of the context on a) the acoustic structure of female chattering calls and b) the temporal structure of vocal exchanges between mates. Results from the corresponding PCA analyses. Ellipses show 67% of the data points in each group.

Effect of the breeding stage on the temporal structure of vocal exchanges

Does the temporal structure of vocal exchange in a given context differ between breeding stages? The first composite score of acoustic structure of the vocal exchange (first principal component PC1 of the PCA) showed that Simple exchanges during laying were significantly longer, with more male and female vocalizations, longer female vocalizations bouts and more calls per female bouts, than during incubation (Z=-2.02, P=0.043, r=0.58). The breeding stage did not affect neither PC2, nor mean IBI per exchange, nor total number of vocalizations bouts per exchange (respectively Z=0.67, P=0.50, r=0.19; Z=0.40, P=0.686, r=0.13; Z=1.62, P=0.10, r=0.51).

DISCUSSION

To investigate the contexts and potential functions of intra-pair acoustic communication, we studied the characteristics of vocal communication around the nest between mates in the Great Tit. Over the studied period ranging from egg laying to the young nestlings stage, we found that interactive vocal communication around the nest between Great Tit mates takes place at all stages. This confirmed that this communication is not only a dawn chorus ritual (Gorissen et al. 2004, Gorissen and Eens 2005) and may have other functions than signaling females' receptiveness to copulate or mate guarding.

Males and females use different vocal repertoires during vocal exchanges at the nest

Our sample size was rather small, so the precise composition of the repertoire used during nest vocal exchanges remains to be fully studied using more recordings per pair. As Gorissen and Eens (2005), we found that chattering call was the most common female call and was used in all contexts (Simple, Exit and Feeding exchanges). Thus, this call type is not specifically associated with female exit from the nest, so is not only a departure call that aims at promoting vigilance and nest defense behavior by the male (Yasukawa 1989, McDonald and Greenberg 1991, Grunst et al. 2014), as suggested by Gorissen and Eens (2004).

Some females occasionally used songs during vocal exchanges. In female Great Tits, song is very uncommon and its function is poorly known (Hinde 1952, Gompertz 1961), although it is primarily reported during reproductive fighting or when the male is absent from the territory, which was not the case here. Because this vocalization had higher amplitude than other calls used by the female inside the nest cavity, female song may participate in extra-pair communication by signaling ownership of the nest cavity. The use of vocalizations at the nest as a territorial signal has been reported in other species. For instance, the nest calls of female Red-winged Blackbirds (Agelaius phoeniceus) might signal ownership of the nest and advertise the presence of a settled female (Small and Boersma 1990). The use of pair vocal exchanges for joint territory defence is also one of the main functions identified for the highly coordinated song duets of tropical bird species (Marshall-Ball et al. 2006, Molles and Waas 2006, Bradley and Mennill 2009, Koloff and Mennill 2011).

Finally, some males used alarm calls. Interestingly, females never emerged from their nest when males produced alarm calls. Our sample size concerning this vocalization type is rather small, but this result may suggest that vocal exchanges can be used as an anti-predator strategy: the male could signal to the female when it is safe or not to go out of the nest cavity. Japanese Great Tit (Parus major minor) produce acoustically distinct calls for different nest predators, which elicit appropriate predator searching and escape behaviors from both adults (Suzuki 2012) and nestlings (Suzuki 2011).

It remains to be studied whether male Great Tits use different alarm calls during vocal exchanges with the female at the nest.

Temporal structure of vocal exchanges between mates at the nest might determine the exchange's outcome

We observed vocal exchanges between the female inside the nest and her male outside in three contexts with different outcomes: (i) the female left the nest (Exit), (ii) the male entered the box with food (Feeding), giving rise to specific calling behavior by the female, (iii) mates stopped calling but birds did not move out/in the nest (Simple).

Within a given breeding stage, the outcome of the vocal exchange could be determined by differences in acoustic structure. During incubation, longer vocal exchanges in which birds produced more vocalizations at a higher tempo had a higher probability to end up in a feeding event in the nest (Feeding). The outcome of vocal exchanges with more female calls per bout was more likely to be an Exit. Moreover, females used chattering calls with up-shifted spectrum during Feeding and Exit exchanges. Female calls had narrower frequency bandwidth and higher amplitude in Feeding than in Simple exchanges, and wider frequency bandwidth, larger modulations of frequency, more spectral noise and lower amplitude in Exit than in Simple exchanges. All these results show that females could indicate their energetic needs to their mate both in the structure of their calls and in the temporal structure of the vocal exchange. The male would be able to decide to enter the nest to feed the female based on her calls and her vocal activity: if the female vocalizes more, at a high tempo, with high, loud and clear calls, he will enter the nest. If her motivation is a little lower (shorter exchange, no change in tempo but longer bouts, high but noisy calls), then he will wait for her to exit the nest. Indeed, female exits from the nest might lead to mate feeding because female Great Tits can often be fed by their mate outside the nest (Royama 1966), but it was difficult to observe male-female interactions outside the nest box in our experiment. In Northern Cardinals (Cardinalis cardinalis), females change the male's probability of coming to the nest with food by singing from the nest in response to his vocalizations (Halkin 1997). This could allow the male to limit his feeding visits to the nest to times when they are really needed and therefore to reduce flights to the nest that may attract predators. As acoustic signals produced by the female from the nest might also attract predators (Yasukawa 1989), the net benefit of mates' vocal exchanges might lie in the multiple functions of this communication.

Females might signal needs during vocal exchanges at the nest

Our results show that females might encode their need for food in the temporal structure of the vocal exchange and in the structure of their calls. We also found that Feeding exchanges, associated with feeding by the male in the nest, as well as Exit exchanges, which might lead to mate feeding outside the nest box (Royama 1966), were both absent during egg laying and started during incubation. Thus, these vocal exchanges do not only reflect female fertility and male mate guarding, as suggested by previous studies (Gorissen et al. 2004). In several passerine species, females signal their need for food with begging calls, whose intensity is condition-dependent and determines male feeding (Tobias and Seddon 2002, Otter et al. 2007, Moore and Rohwer 2012, Cantarero et al. 2014). Mate feeding in the early breeding stages has been suggested to have evolved as a compensatory energetic strategy in species in which the female incubates and builds the nest alone (Galván and Sanz 2011). In Great Tit, mate feeding starts at egg-laying, increases during incubation and lasts until the young leaves the nest. Thus, it takes place when the energetic demands are high for egg or heat

production and, more importantly, when female's foraging movements are limited during incubation (Hinde 1952, Royama 1966). Whether female nutritional state affects the rate of male feeding remains to be experimentally tested.

At the young nestlings stage, both the number of Feeding exchanges and the number of female exits without vocal exchanges increased. This is consistent with the fact that nestlings' nutritional needs are added to the female's needs, and in agreement with the possibility that females may use vocalizations to signal their own needs as well as the needs of their offspring (Halkin 1997, Ellis et al. 2009). Feeding vocal exchanges could thus play a role in the communication between mates to organize parental care.

Vocal exchanges between mates at the nest might function in pair bond maintenance

Simple exchanges were significantly longer with more male and female vocalizations, longer female vocalizations bouts and more calls per female bouts during laying than during incubation. This difference is in line with the peak of male song observed during the female fertility period (Mace 1987, Cramp and Perrins 1993). During laying, male vocalizations have been hypothesized to stimulate the female to lay eggs and to copulate, as well as decrease the risk of extra-pair copulations (Mace 1987). For instance, males can modify the acoustic features of their song according to female fertility and this capacity is correlated with female sexual fidelity: males who switch to a low-pitched version of their song at the peak of female fertility get cuckolded less often (Halfwerk et al. 2011). Thus, Simple exchanges observed in this study could participate in mate guarding and pair bond maintenance.

More generally, the vocal exchanges observed in the present study could all participate in pair bond maintenance, a primary function of songbird duets (Hall 2000, Benedict 2008). Indeed, we found that vocal exchanges at the nest share acoustic characteristics with song duets that are often considered as markers of pair bond strength and mates' commitment in pair bond activities. We observed that vocal exchanges are coordinated vocal emissions of the mates, as male and female alternate their vocalizations, and male and female tempos are correlated. Future studies could investigate to what extent the coordination of this communication relates to the coordination of pair members during breeding or to pair bond strength.

Here we show that Great Tit pairs use vocal interactions around the nest cavity along breeding, and these interactions take place in three contexts. More studies are needed to fully understand their functions, but they could possibly signal female's needs to the male, participate in predator vigilance and promote pair bond maintenance. With these possible functions in mind, the rich field of study on song duets appears as a theoretical background of choice to investigate mate's communication at the nest.

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Chapitre 4

Intra-pair vocal communication at the nest during incubation signals female's hunger: a feeding experiment in great tits

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Soumis à Animal Behaviour

Synthèse du chapitre 4

Question : les mésanges charbonnières femelles indiquent elles leur niveau de faim à leurs mâles pendant l'incubation ?

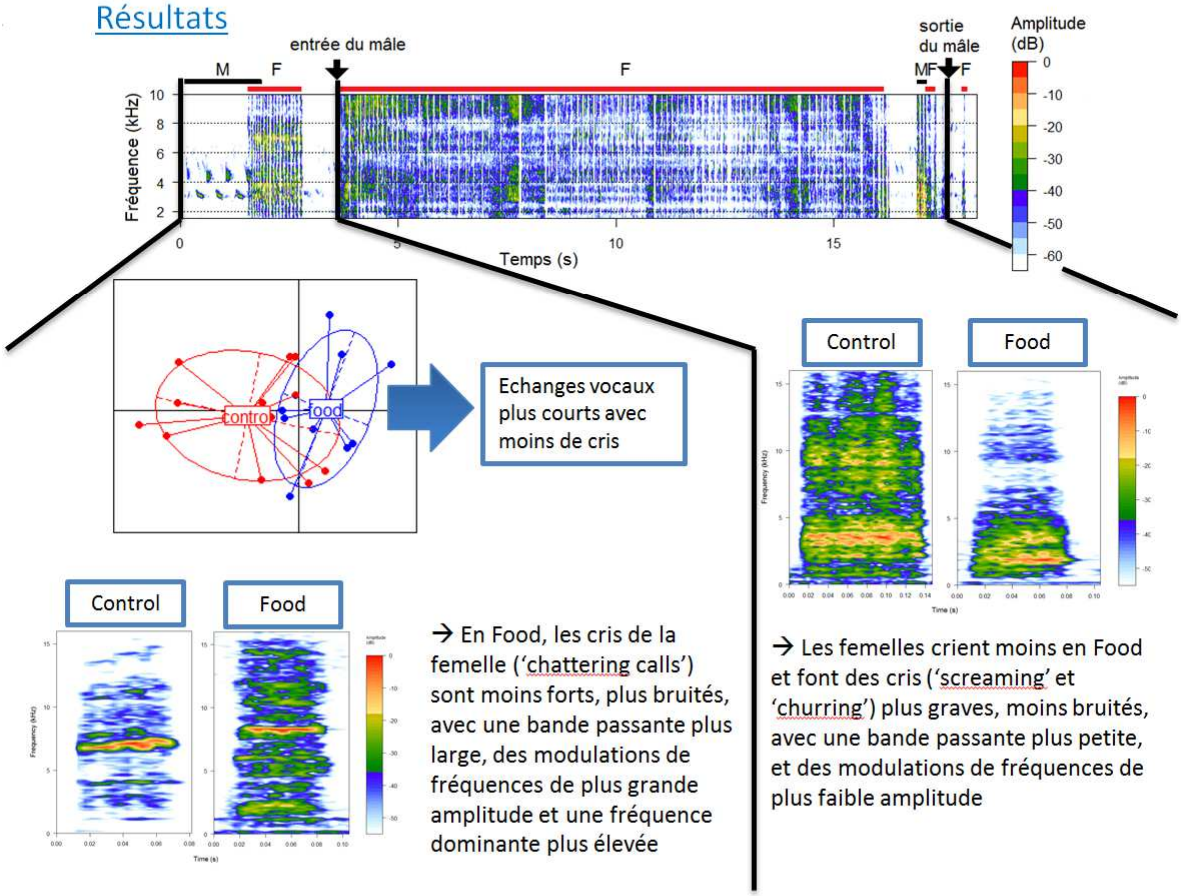
Méthodes

Couples enregistrés au nid dans deux conditions :

Food : mangeoire contenant 10g de vers de farine placée dans le nid

Control : mangeoire vide dans le nid

Résultats



→ En Food, les cris de la femelle ('chattering calls') sont moins forts, plus bruités, avec une bande passante plus large, des modulations de fréquences de plus grande amplitude et une fréquence dominante plus élevée

→ Les femelles crient moins en Food et font des cris ('screaming' et 'churring') plus graves, moins bruités, avec une bande passante plus petite, et des modulations de fréquences de plus faible amplitude

Conclusion

Les femelles indiquent leur niveau de faim aux mâles lorsqu'ils viennent les nourrir en criant plus et en modifiant la structure de leurs cris. La réponse des mâles face à cette information reste à déterminer.

ABSTRACT

Parental care is a key factor of breeding success and parents face evolutionary trade-offs between investment in current offspring and future reproduction and survival. Incubation in birds is energetically costly and the balance between parental and offspring's energetic needs is especially challenging when only one sex incubates, generally the female. In that case, males can contribute indirectly to incubation effort by feeding their mate and the female may use begging behaviours to signal her needs to her visiting partner. However, several visits to the nest may attract predators and pairs may gain to adapt male visits to the female's needs. The great tit (*Parus major*) is a good model species to test whether females use acoustic communication from the nest to signal their needs to their distant mate because the female vocally interacts with her mate during incubation in three contexts with different outcomes: (i) the female left the nest, (ii) the male entered the box to feed the female, (iii) mates stopped calling but did not move out/in the nest. Thus, females may use these vocal exchanges to communicate how hungry they are. To test this hypothesis, great tit pairs were recorded on two days during incubation: one day without food supplementation and one day with a feeder of mealworms into the nest box. First, food supplementation increased females' nest attentiveness, revealing a decrease in foraging activity and thus in needs of the females. Second, females signaled their need for food by increasing their calling intensity and by modifying the structure of their calls not only when their mate came inside the nest to feed them, but also during the vocal exchange preceding his visit. So females signal their needs from the nest to their distant male, whose response to this information remains to be determined.

Keywords: Acoustic communication - Bi-parental care - Female calls - Female begging - Incubation feeding - Intra-pair communication - Mate feeding - Nest attentiveness - Pair-bond - Solicitation calling

INTRODUCTION

Parental care is a key factor of breeding success and parents face evolutionary trade-offs between investment in current offspring and future reproduction and survival (Clutton-Brock, 1991). In birds, increased nest attentiveness during incubation – the percentage of time that parents spend on the nest – has a positive impact on fitness because it shortens the duration of the incubation period and consequently the exposition of parents and offspring to nest predators (Magrath, 1988; T E Martin, Scott, & Menge, 2000; Thomas E Martin, 2002), and it also improves hatching success (Carey, 1980; Lyon & Montgomerie, 1985; Reid, Monaghan, & Ruxton, 2002) and fledglings condition (Reid et al., 2002). Incubation in birds is an interesting model to study scaling of parental effort because it is energetically costly (Thomson, Monaghan, & Furness, 1998; Williams, 1996) and incubating parents must balance the thermal needs of the eggs with their own energetic needs. Moreover, this trade-off can be exacerbated by low food supply, which further limits the amount of energy an individual can obtain during a foraging bout (Chalfoun & Martin, 2007). This energetic trade-off might be especially challenging in species with single-sex incubation, because the eggs are left unattended when the incubating parent, generally the female, needs to forage (Deeming, 2002).

In many species of birds with female-only incubation, the males feed their mate during incubation and this might offset the trade-off between eggs' development and female's condition (Kendeigh, 1952; Lyon & Montgomerie, 1985; Silver, Andrews, & Ball, 1985). The female in most species is only marginally dependent on male incubation feeding because she also forages by herself, but the food brought by the male has been showed to have a significant nutritive value for the female (female nutrition hypothesis; Moreno, Redondo, Cantarero, Ruiz-de-Castañeda, & González-Braojos, 2011; Royama, 1966). This supplementation in energy resources can increase the pair's breeding success by improving female's body condition and thus her subsequent care, or increasing her nest attentiveness (Hałupka, 1994; Klatt, Stutchbury, & Evans, 2008; Lifjeld & Slagsvold, 1986; Lloyd, Andrew Taylor, Du Plessis, & Martin, 2009; Lyon & Montgomerie, 1985; Matysioková, Cockburn, & Remeš, 2011; Moore & Rohwer, 2012; Stein, Oh, & Badyaev, 2009). The rate of male incubation feeding differs both within and between species (Conway & Martin, 2000; Kendeigh, 1952), and can be influenced by various factors. Its intensity was found to increase with decreasing ambient temperature (Nilsson & Smith, 1988; Pearse, Cavitt, & Cully, 2004; Smith, Källander, Hultman, & Sanzén, 1989), higher male quality (Lifjeld, Slagsvold, & Stenmark, 1987; Siefferman & Hill, 2005), higher food supply on territory (Zanette, Doyle, & Trémont, 2000) and lower predation risk (Fontaine & Martin, 2006). Males were also showed to adjust their workload to the needs of their partner in experimental studies: they increased their feeding rate when their female's foraging ability was experimentally impaired by wing clipping, and decreased it when their female was supplemented in food (Cantarero, López-Arrabé, Palma, Redondo, & Moreno, 2014; Pearse et al., 2004; Smith et al., 1989). Thus, male incubation feeding responds at least partially to the female's needs and this suggests that communication within a pair might participate in this adjustment. Surprisingly, little is known on male-female communication during incubation.

During male incubation feeding, the female may use begging behaviours to signal her needs. Whereas nestlings' begging has been well studied (Horn & Leonard, 2005) and is considered as an honest signal of needs (Kilner & Johnstone, 1997), fewer studies have considered the role of female begging. In black-capped chickadees (*Poecile atricapillus*), begging call rate decreases in females

supplementally fed during the egg-laying period (Otter, Atherton, & van Oort, 2007). In the European robin (*Erithacus rubecula*), females that are well fed by their male during the fertile period have larger clutch and lower begging call rate (Tobias & Seddon, 2002). In the yellow warbler (*Setophaga petechia*) and the pied flycatcher (*Ficedula hypoleuca*), begging intensity increases when females have respectively not been fed by their mates for a long period or been experimentally handicapped (Cantarero et al., 2014; Moore & Rohwer, 2012). In both studies males responded to the increased begging rate of their female by increasing feeding rates accordingly. All these results suggest that females signal their needs to their male using begging behaviours during incubation feeding.

Because activity around the nest may increase nest predation risk (Magrath, 1988; T E Martin et al., 2000; Thomas E Martin, 2002), communication systems allowing the incubating female to signal her needs to her distant male before he comes inside the nest might be selected for. In Northern Cardinals (*Cardinalis cardinalis*), the female expresses her own needs for food or the need of her nestlings by singing from the nest and allows her male to adjust his feeding visits to the nest, thus saving flights that may attract predators (Halkin, 1997).

The great tit is a good model species to test whether females use acoustic communication from the nest to signal their needs to their distant mate. The female incubates the eggs alone and regularly leaves the nest to forage (Cramp & Perrins, 1993).The male often feeds the female and mate feeding starts at egg-laying, increases during incubation and last until the young leaves the nest (Hinde, 1952; Royama, 1966). The incubating female answers to her male singing outside the nest (Gorissen, Eens, & Nelson, 2004; Halfwerk et al., 2011; Halfwerk, Bot, & Slabbekoorn, 2012). Moreover, in a previous study (Boucaud et al., In press), we observed vocal exchanges, *i.e.* vocalization bouts alternated on the same tempo, between the female inside the nest and her male outside in three contexts with different outcomes: (i) the female left the nest, (ii) the male entered the box with food, and the female then used specific call types, (iii) mates stopped calling but did not move out/in the nest. Because birds vocalized more and at higher tempo during exchanges that ended up in male feeding inside the nest, it suggests that this communication could signal females' need for food to their mate (Boucaud et al., In press).

In this study, we tested whether interactive acoustic communication between the female inside the nest and her mate outside, as well as female calls produced during male feeding both signal females' needs for food. To do so, we experimentally manipulated females' needs during incubation using food supplementation. We recorded great tit pairs on two days: one day without food supplementation and one day with a feeder of mealworms inside the nest box. If females' acoustic communication at the nest is an honest signal of needs, females' calling intensity should decrease with food supplementation.

METHODS

Study Site and Species

Our study was conducted in spring 2014 in wooden areas close to the campus of Université de St-Etienne, in France (45°25'N, 4°25'E). 54 nest boxes (wood nest boxes, 150x150x250 mm, with an entrance hole of 32 mm diameter) were installed between winter 2012 and winter 2014. To

record occupation by a breeding pair, nest-boxes were checked once a week starting at the end of March, and when occupied, three times a week to monitor breeding.

Experimental Procedure

Data were collected in April-May 2014 on twelve great tit pairs. The acoustic activity of each pair was recorded during incubation on two successive days used as two experimental conditions (order randomly selected). In both conditions, a cardboard feeder (30x40x20 mm) was installed inside the nest box after sunset on the evening before the recording day. It was located on the balcony so it did not disturb birds' movement in and out of the nest box. The two conditions differed by the presence or absence of food in the feeder:

- Food condition: the feeder contained 10g of mealworms. At the end of the recording, remaining mealworms were weighted to measure the quantity of mealworms eaten by the female. Eleven out of 12 females ate the mealworms. Thus our final sample size was 11 pairs.

- Control condition: the feeder was empty.

When the feeder was installed on the evening before the recording day, a recorder (SongMeters SM2+, 16-bit, 44-kHz sampling rate; Wildlife Acoustics Inc., Concord, MA, USA) was positioned near the nest box on the tree trunk, connected to a microphone (SMX-II, Wildlife Acoustics Inc., Concord, MA, USA; omnidirectional, flat frequency response 20 Hz-20 kHz, sensitivity -36±4 dB) inside the nest box just below the ceiling (gain of +24 dB) and a microphone outside, fixed on the tree trunk at the height of the nest box (gain of +42 dB). This equipment did not perturb birds' activity at the nest. Pairs were recorded continuously between 05:30 and 14:00, so during 8.5h. Recorders also measured temperature every hour.

Definition of vocal exchanges

In all cases, the male initiated the vocal exchange by either singing or calling outside the nest-box and the female responded from inside. We considered that the vocal exchange started with the male song strophe or call preceding the first vocalization of the female, and ended with the last female vocalization (in some rare cases, male's last vocalization bout overlapped the one of the female and ended later; in those cases, the vocal exchange ended with this last male's vocalization bout). A vocal exchange stopped when the female stopped calling for at least 30 seconds, so two successive vocal exchanges were separated by at least 30 seconds. Sometimes, we observed females vocalizing outside the nest but these vocalizations were difficult to record with our setup, so only female vocalizations produced inside the nest box were considered here. We distinguished three contexts with different outcomes (birds make characteristic noises when entering or exiting the nest-boxes allowing us to detect precisely these events):

- Simple exchange: mates stopped calling but did not move out/in the nest;

- Exit exchange: the female left the nest and could be fed by the male outside;

- Feeding exchange: the male entered the nest to feed the female. Note that the male sometimes entered directly in the nest box, skipping the preceding vocal exchange. We named the interaction of the female and the male inside the nest box a Feeding sequence.

These three contexts of vocal exchange potentially occurred several times and in varied order in each recording session of 8.5h.

Spectrograms of recordings were visualized using SongScope software (Wildlife Acoustics Inc., Concord, MA, USA) to detect vocal exchanges. Furthermore, birds' exits and entrances as well as presence inside the nest were easily identified on spectrograms allowing measuring the time spent outside the nest by the female and distinguishing between the three different contexts of vocal exchanges.

Acoustic Analysis

In Food condition, the female had access to a feeder of mealworms but the time needed to find and eat a substantial amount of them could be variable between individuals. Thus, we extracted and analyzed the last two vocal exchanges from each context (3 contexts: Simple, Exit and Feeding exchanges) in each recording (2 recordings per pair: Control and Food) to increase the probability of analyzing vocal exchanges that occurred after the female ate the mealworms. Some pairs had missing exchanges in some contexts so 87 vocal exchanges were extracted for the 11 pairs (Food condition: 18 Simple, 12 Exit and 10 Feeding exchanges; Control condition: 18 Simple, 17 Exit and 12 Feeding exchanges). To study Feeding sequences (interactions of the female and the male inside the nest box), 16 more feeding events during which the male entered directly in the nest box without any preceding vocal exchange were extracted in Food condition and 8 more in Control condition. Thus, the sample size for this analysis was N= 18 in Control and N=20 in Food condition.

The temporal structure of vocal exchanges was manually analyzed using PRAAT software (http://www.fon.hum.uva.nl/praat/). Each vocalization (call or song motif) was labeled with the identity of the caller and the call type. Then, we measured temporal parameters for each vocal exchange: the duration of the exchange and, for each sex, the numbers of vocalizations, and the number of vocalization bouts (defined as series of notes from one caller separated with less than 1s). For females, we also measured the number and mean call rate of vocalizations in the bouts as well as the response latency to male vocalizations (duration between the start of a male bout and the start of the following female bout).

In Feeding sequences, we measured the time spent calling by the female (time between the first and the last calls) as well as the proportion of time spent calling by the female (duty cycle = sum of the durations of all female calls / total time spent calling). Finally, we measured the time spent by the male inside the nest.

For both vocal exchanges and Feeding sequences, an analysis of the acoustic structure of females' calls was performed using custom-written codes based on the Seewave library (Sueur, Aubin, & Simonis, 2008) implemented in R (R Development Core Team, 2014). For vocal exchanges, we analyzed chattering calls (described by Gorissen & Eens, 2005) which were the most frequent females' calls when the males were outside the nest. For Feeding sequences, all calls of good quality (not overlapped by male vocalizations or noises of birds' movements in the nest) were analyzed in a first step. These calls corresponded to screaming, churring and chattering calls described by Gorissen & Eens (2005). Chattering calls, which differed strongly in structure from screaming and churring and represented only 12.7% of total calls were automatically removed from the dataset by removing calls with a dominant frequency higher than 6kHz.

The duration of each call (in s) was measured between edges manually labeled using Praat software. After bandpass filtering (500Hz-20000Hz encompassing the spectral bandwidth of great tit

vocalizations, 'fir' function), the amplitude of the call was measured as the root-mean-square of the amplitude envelope of the call ('rms' function) and the following spectral parameters were computed using the 'specprop' function (FFT using a Hamming window and a window length of 512):

- As the frequency spectrum is a distribution, it can be characterized using classic descriptive statistics (all in Hertz). We used the median, the first (Q25) and third (Q75) quartiles of the spectrum of the call – which represent the frequencies below which lie respectively 50%, 25% and 75% of the energy of the call. The IQR (Inter Quartile Range) was defined as Q75- Q25. We also used the mean and standard deviation (sd) of the spectrum.

- The frequency of highest amplitude – the dominant frequency – can be measured over the duration of the call (obtained via the 'dfreq' function, window length of 512 and overlap of 50%) and characterized using its mean (average dominant frequency) and its standard deviation (in Hertz).

- The call's noisiness can be quantified using the Shannon spectral entropy. The Shannon entropy of a noisy signal tends towards 1 whereas it tends towards 0 for a pure tone.

For the statistical analysis of chattering calls' structure in vocal exchanges, we randomly selected 10 calls of each female per context and per condition. In 5 out of 46 cases, females had less than 10 chattering calls. In this case, all the calls available were selected. Finally, a total of 440 chattering calls (Food condition: Simple: 90, Exit: 58 and Feeding: 58 calls; Control condition: Simple: 90, Exit: 86 and Feeding: 58 calls) were analysed. For the statistical analysis of screaming and churring calls in Feeding sequences, 10 calls per female and per condition were randomly selected. Our sample size was of 100 calls in Food condition and 90 calls in Control condition.

Ethical Note

Experiments were performed under the authorization no. CU13N7-ENES-VIGNAL1 (issued by the Ethics committee of Université de Saint-Etienne) and were in agreement with the French and European legislations regarding experiments on animals.

Statistical Analyses

All statistical tests were performed using R software (R Development Core Team, 2014). Following all linear mixed models (LMM, 'lmer' function of the lme4 library), equivariance and distribution of the residuals were graphically checked using 'plotresid' function (RVAideMemoire library). For each model, marginal and conditional coefficients of determination are presented (r2m and r2c, 'r.squaredGLMM' function of the MuMIn library, Nakagawa & Schielzeth, 2013). P-values on models were obtained using Wald Chi-square tests ('Anova' function, car library).

Ambient temperature may have an impact on birds' behaviour, especially females' nest attentiveness (Kluijver, 1950; Matysioková & Remeš, 2010). First, we controlled that the two experimental conditions (Control and Food) did not differ in ambient temperature. To do so, we used a LMM with the condition as a fixed factor and the pair identity (11 levels) and the day (1st day or 2nd day) as random factors. We tested also the potential interaction between the condition and the time of day of the recording (scaled). As recommended for within subjects design with a covariate (Schielzeth & Forstmeier, 2009), the covariate was also included as a random slope as follows: Temperature ~ Condition * Time + (1 + Time | Pair identity) + (1 | Day). We found a significant effect of the time of day on the temperature (the temperature increased throughout the morning, Table 1)

Table 1. Results of the LMM testing the difference of temperature between the two experimental days.

	Estimate ± SE	R2m	R2c	Chi²	Df	P-value
Temperature (°C)		0.44	0.80			
(Intercept)	10.48 ± 0.87					
Condition				2.07	1	0.150
Food	0.43 ± 0.30					
Time	3.01 ± 0.35			90.54	1	**<0.001**
Condition * Time	-0.03 ± 0.30			0.01	1	0.918

Significant results are displayed in bold.

Table2. Variable loadings of the PCA on a) the number of vocal exchanges, b) the structure of vocal exchanges, c) the acoustic structure of female chattering calls, d) the acoustic structure of female screaming and churring calls in Feeding sequences.

a)

Variable	Transformation	PC1	PC2	PC3
Explained variance (%)		**44.21**	**32.35**	**19.98**
Number of Simple exchanges	square-root	-0.74	-0.49	0.45
Number of Exit exchanges	square-root	-0.36	0.72	0.58
Number of Feeding exchanges	square-root	0.69	-0.07	0.70
Simple exchange (proportion)	square-root	-0.74	-0.57	0.27
Exit exchange (proportion)	square-root	-0.37	0.91	0.06
Feeding exchange (proportion)	square-root	0.90	-0.15	0.32

b)

Variable	Transformation	PC1	PC2
Explained variance (%)		**53.56**	**24.32**
Duration of the vocal exchange	log10	-0.93	0.15
Number of male vocalization bouts	log10	-0.87	0.24
Number of female vocalization bouts	log10	-0.92	0.17
Mean duration of a female bout	log10	-0.24	-0.88
Mean number of calls in a female bout	log10	-0.24	-0.90
Female's response latency to male vocalizations	log10	0.18	0.34
Number of female vocalizations	log10	-0.93	-0.27
Number of male vocalizations	log10	-0.90	0.27

c)

Variable	Transformation	PC1	PC2
Explained variance (%)		**44.13**	**30.64**
Duration	none	-0.30	0.35
Mean	none	-0.75	-0.62
Sd	none	-0.83	0.43
Median	none	-0.67	-0.65
First quartile (Q25)	none	-0.06	-0.93
Third quartile (Q75)	none	-0.91	-0.22
Inter quartile range (IQR)	none	-0.83	0.40
Shannon spectral entropy	none	-0.87	0.23
Average dominant frequency	none	-0.35	-0.82
Sd of the dominant frequency	square-root	-0.68	0.25
Amplitude	log10	0.44	-0.62

d)

Variable	Transformation	PC1	PC2
Explained variance (%)		**60.83**	**11.68**
Duration	square-root	-0.50	0.31
Mean	none	-0.98	0.02
Sd	none	-0.76	0.51
Median	log10	-0.91	-0.27
First quartile (Q25)	none	-0.76	-0.46
Third quartile (Q75)	none	-0.95	0.17
Inter quartile range (IQR)	none	-0.88	0.35
Shannon spectral entropy	none	-0.85	0.18
Average dominant frequency	none	-0.70	-0.61
Sd of the dominant frequency	square-root	-0.61	-0.39
Amplitude	log10	0.17	-0.45

but no effect of the condition or the interaction (Table 1). Thus, the temperature was not included in models testing the effect of the experimental condition on behavioural and acoustic parameters.

The effect of food supplementation on females' number of exits and females' time off the nest was analysed using LMMs with the condition as fixed factor (two levels: Food and Control) and the pair identity (11 levels) and the recording day (two levels: 1st day and 2nd day) as random factors. LMMs were written as follows: Variable ~ Condition + (1|Pair identity) + (1|Day).

Four PCA were performed to test the effect of food supplementation on (i) the number of vocal exchanges, (ii) the acoustic structure of vocal exchanges, (iii) the acoustic structure of female chattering calls in vocal exchanges and (iv) the acoustic structure of screaming and churring calls produced during Feeding sequences. All variable loadings are given in table 2. Before being included in the PCA some parameters were transformed to reach a symmetrical distribution (Table 2). Principal components with eigenvalue above 1 (Kaiser criterion) were selected.

For the number of vocal exchanges, PC1, PC2 and PC3 were analyzed using a LMM with the condition (two levels: Food and Control) as fixed factor and the pair identity (11 levels) and the day (two levels: 1st day and 2nd day) as random factors. LMMs were written as follows: PC ~ Condition + (1|Pair identity) + (1|Day).

For the acoustic structure of vocal exchanges as well as for the acoustic structure of female chattering calls PC1 and PC2 were analyzed using a LMM with the condition (two levels: Food and Control), the context (3 levels: Simple, Exit, Feeding), the interaction between the condition and the context, and the time of day (in min, scaled) as fixed factors and the pair identity (11 levels) and the day (two levels: 1st day and 2nd day) as random factors. LMMs were written as follows: PC ~ Condition*Context + Time + (1|Pair identity) + (1|Day). When the interaction was significant, each context (Simple, Exit and Feeding exchanges) was analyzed separately using the following LMM: PC ~ Condition + Time + (1|Pair identity) + (1|Day).

The effect of food supplementation on the timing of the last vocal exchange was tested using a LMM with the condition (two levels: Food and Control), the context (3 levels: Simple, Exit, Feeding) as well as the interaction between the condition and the context as fixed factors. As previously, the pair identity (11 levels) and the day (two levels: 1st day and 2nd day) were used as random factors. Thus, the LMM was written as follows: Timing of the last vocal exchange ~ Condition * Context + (1|Pair identity) + (1|Day). Post-hoc tests were performed using the 'lsmeans' function (lsmean library) using Tukey contrasts.

The time spent calling by the female during Feeding sequences, the proportion of time spent calling by the female (duty cycle), and the time spent inside the nest by the male were tested using a LMM with the condition (two levels: Food and Control) and the time of day (in min, scaled) as fixed factors and the pair identity (11 levels) and the day (two levels: 1st day and 2nd day) as random factors. LMMs were written as follows: Variable ~ Condition + Time + (1|Pair identity) + (1|Day). The correlation between the time spent calling by the female and the time spent inside the nest by the male was tested using a spearman test because equivariance and distribution of the residuals were violated in the corresponding LMM.

RESULTS

Females' Periods Off the Nest and Vocal Events

In Food condition, 11 out of 12 females ate the mealworms. They ate on average 6.7 ± 2.3 g (mean ± sd) out of the 10g provided in the feeder. This food supplementation increased the time spent by the female in the nest as females exited significantly less often (27% decrease, Table 3; Fig.1A) and spent significantly less time off the nest (29% decrease, Table 3; Fig.1B).

The numbers and proportions of vocal exchanges of the three contexts were weakly influenced by food supplementation: The second principal component (PC2) revealed that Exit exchanges tended to be more frequent in Control condition (Table 4a), but conditions did not differ in the number of Simple and Feeding exchanges (Table 4a).

There was a great variability between nests in the timing of the last two vocal exchanges of each context as well as the last two exits of the female without vocal exchange, which occurred between 06:45 and 14:00. The last events occurred significantly later in Control than in Food condition (Table 5). The significant interaction between the condition and the context showed that this difference is driven by Exit exchanges, which stopped occurring 2 hours earlier in Food than in Control condition (Table 5).

In sum, with food supplementation, females were significantly less off the nest, were less likely to end vocal exchanges by an exit from the nest, and stopped doing so significantly earlier in the morning. Thus, food supplementation increased female's nest attentiveness.

Acoustic Structure of Vocal Exchanges

Whereas the acoustic structures of Simple and Exit exchanges were not affected by food supplementation (PC1 and PC2 from the PCA, Table 4b), Feeding exchanges were significantly shorter with less male and female vocalizations and vocalization bouts (higher PC1 values, Table 4b, Fig.2). With food supplementation, Feeding exchanges were not only shortened, but were skipped more often with direct male entrance in the nest without preceding vocal exchange (Food: 45.4% (10 out of 22) of direct male entrance, Control: 30% (6 out of 20)). In 81% of these direct male entrances (13 out of 16, Control: 5 out of 6, Food: 8 out of 10), the male vocalized before entering the nest but the female did not respond, and in the 3 others neither the male nor the female vocalized.

The acoustic structure of vocal exchanges was affected by the time of day: Simple exchanges performed later in the morning were longer, with more male and female vocalizations (lower PC1 values, Table 4b), whereas Exit exchanges performed late tended to be shorter with less vocalizations (higher PC1 values, Table 4b). Exchanges in all three contexts had shorter female vocalization bouts and less female calls per bout when performed late in the morning (higher PC2 values, Table 4b).

Table 3. Effect of food supplementation on females' periods off the nest.

	Estimate ± SE	R2m	R2c	Chi²	Df	P-value
Female number of exits from the nest (log)		0.17	0.40	5.97	1	**0.014**
(Intercept)	1.13 ± 0.06					
Food	- 0.17 ± 0.07					
Female time spent off the nest (min; log)		0.21	0.60	10.89	1	**<0.001**
(Intercept)	2.05 ± 0.06					
Food	- 0.19 ± 0.06					

Significant results are displayed in bold.

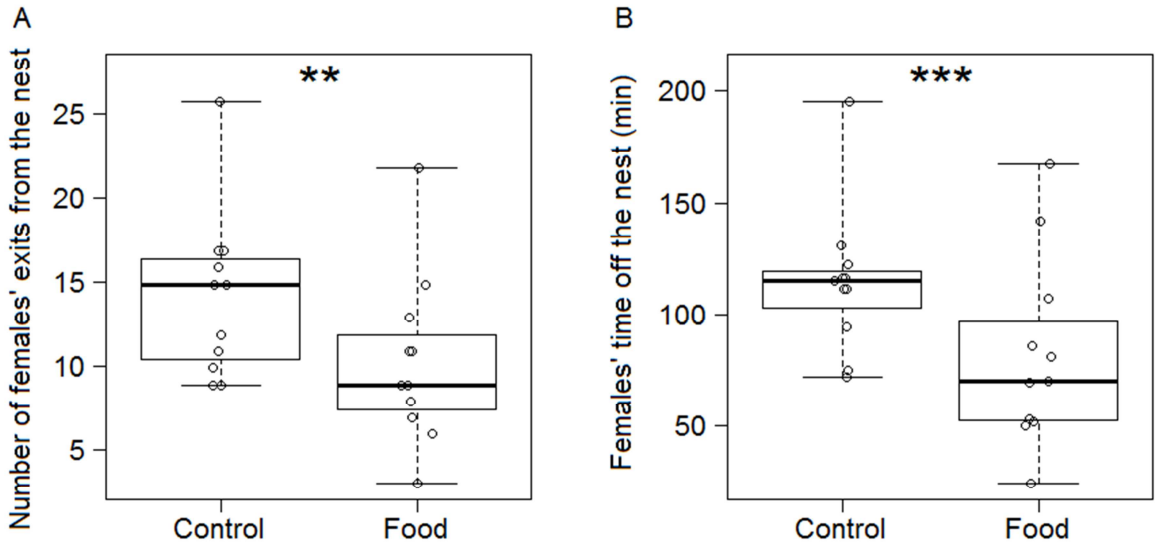


Figure 1. Effect of food supplementation on females' periods off the nest: A) number of exits and B) time off the nest. Boxes are 1st-3rd quartiles, lines are medians, whiskers are extreme values and each point represents a pair.

Table 4. Effect of food supplementation on a) the number of vocal exchanges, b) the temporal structure of vocal exchanges, c) the acoustic structure of female chattering calls, d) the acoustic structure of female screaming and churring calls in Feeding sequences.

	Estimate ± SE	R2m	R2c	Chi²	Df	P-value
PC1		0.01	0.80	1.47	1	0.225
(Intercept)	- 0.20 ± 0.52					
Food	0.39 ± 0.32					
PC2		0.07	0.54	3.13	1	0.077
(Intercept)	0.37 ± 0.45					
Food	- 0.75 ± 0.42					
PC3		<0.01	0.51	0.02	1	0.892
(Intercept)	0.02 ± 0.35					
Food	- 0.05 ± 0.34					

	Estimate ± SE	R2m	R2c	Chi²	Df	P-value
PC1 complete model		0.12	0.35			
(Intercept)	0.66 ± 0.69					
Condition				2.52	1	0.112
Food	- 0.60 ± 0.72					
Context				3.78	2	0.151
Feeding exchanges	- 2.18 ± 0.70					
Simple exchanges	- 0.88 ± 0.62					
Time (scaled)	- 0.21 ± 0.23			0.85	1	0.360
Condition * Context				6.80	2	**0.033**
Food Condition * Feeding exchange	2.85 ± 1.09					
Food Condition * Simple exchange	1.27 ± 0.93					
PC2 complete model		0.16	0.42			
(Intercept)	- 0.39 ± 0.58					
Condition				<0.01	1	0.997
Food	0.28 ± 0.46					
Context				8.39	2	**<0.001**
Feeding exchanges	0.08 ± 0.45					
Simple exchanges	0.99 ± 0.40					
Time (scaled)	0.51 ± 0.14			12.20	1	**<0.001**
Condition * Context				0.81	2	0.667
Food Condition * Feeding exchange	- 0.20 ± 0.70					
Food Condition * Simple exchange	- 0.53 ± 0.60					
PC1 Simple exchanges		0.29	0.40			
(Intercept)	- 0.14 ±0.46					
Condition				0.05	1	0.816
Food	0.14 ± 0.60					
Time (scaled)	- 1.11 ± 0.32			11.95	1	**<0.001**
PC1 Exit exchanges		0.08	0.42			
(Intercept)	0.39 ± 0.91					
Condition				0.53	1	0.468
Food	0.51 ± 0.70					
Time (scaled)	0.70 ± 0.36			3.77	1	**0.052**
PC1 Feeding exchanges		0.32	0.65			
(Intercept)	- 1.78 ± 0.60					
Condition				11.73	1	**<0.001**
Food	2.48 ± 0.72					
Time (scaled)	0.11 ± 0.42			0.07	1	0.797

	Estimate ± SE	R2m	R2c	Chi²	Df	P-value
PC1 complete model						
(Intercept)	0.44 ± 0.71					
Condition				0.04	1	0.849
Food	0.49 ± 0.31					
Context				9.87	2	0.070
Feeding exchanges	0.05 ± 0.30					
Simple exchanges	- 0.50 ± 0.26					
Time (scaled)	0.26 ± 0.11			5.70	1	**0.017**
Condition * Context				12.40	2	**0.002**
Food Condition * Feeding exchange	- 1.51 ± 0.45					
Food Condition * Simple exchange	- 0.29 ± 0.39					
PC2 complete model						
(Intercept)	0.03 ± 0.30					
Condition				0.09	1	0.763
Food	- 0.08 ± 0.29					
Context				4.22	2	0.121
Feeding exchanges	- 0.52 ± 0.28					
Simple exchanges	0.19 ± 0.25					
Time (scaled)	0.42 ± 0.10			17.20	1	**<0.001**
Condition * Context				2.82	2	0.243
Food Condition * Feeding exchange	0.59 ± 0.43					
Food Condition * Simple exchange	- 0.05 ± 0.37					
PC1 Simple exchanges		0.07	0.56			
(Intercept)	0.05 ± 0.66					
Condition				<0.01	1	0.977
Food	0.001 ± 0.27					
Time (scaled)	0.65 ± 0.24			7.43	1	**0.006**
PC1 Exit exchanges		0.00	0.58			
(Intercept)	0.03 ± 0.65					
Condition				0.19	1	0.664
Food	0.16 ± 0.38					
Time (scaled)	- 0.01 ± 0.19			0.00	1	0.968
PC1 Feeding exchanges		0.21	0.45			
(Intercept)	0.87 ± 0.40					
Condition				17.38	1	**<0.001**
Food	- 1.80 ± 0.43					
Time (scaled)	- 0.11 ± 0.28			0.17	1	0.682

	Estimate ± SE	R2m	R2c	Chi²	Df	P-value
PC1		0.08	0.42			
(Intercept)	- 0.82 ± 0.80					
Condition				13.95	1	**<0.001**
Food	1.31 ± 0.35					
Time (scaled)	- 0.45 ± 0.27			2.88	1	0.089
PC2		0.01	0.32			
(Intercept)	- 0.17 ± 0.25					
Condition				2.08	1	0.149
Food	0.25 ± 0.17					
Time (scaled)	- 0.01 ± 0.11			0.02	1	0.892

Significant results are displayed in bold.

Table 5. Effect of food supplementation on the timing of the last two vocal exchanges and last two female exits without exchange.

	Estimate ± SE	R2m	R2c	Chi²	Df	P-value
Time of day (min)		0.36	0.51			
(Intercept)	664.45 ± 25.23					
Condition				7.00	1	**0.008**
Context				74.61	3	**<0.001**
Condition * Context				8.74	3	**0.033**
Food	- 127 ± 36.19					
Exit without exchange	130.32 ± 30.92					
Feeding exchanges	54.09 ± 31.67					
Simple exchanges	- 5.68 ± 32.57					
Food * Exit without exchange	96.89 ± 46.20					
Food * Feeding exchanges	136.98 ± 46.87					
Food * Simple exchanges	81.98 ± 48.26					

Significant results are displayed in bold.

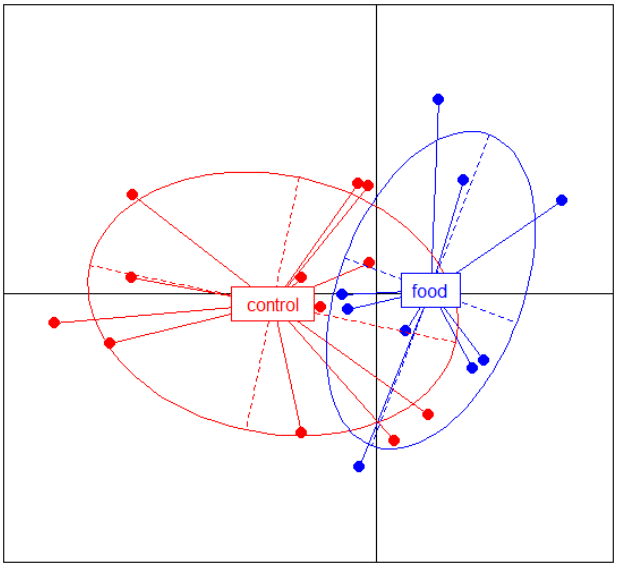


Figure 2. Effect of food supplementation on the temporal structure of Feeding exchanges between mates. Higher PC1 values indicate shorter vocal exchanges with less male and female vocalizations and vocalizations bouts. Higher values of PC2 indicate shorter female vocalizations bouts with less calls. Ellipses show 67% of the data points in each group.

Acoustic Structure of Female Chattering Calls during Vocal Exchanges

Contrary to female chattering calls in Simple and Exit exchanges, female chattering calls in Feeding exchanges were affected by food supplementation (PC1 from the PCA, table 4d). With food supplementation, female chattering calls showed lower PC1, i.e. wider frequency bandwidth (higher sd and IQR), larger modulations of frequency within calls (higher sd of the dominant frequency), energy in higher frequency bands (higher mean, median and Q75) and more spectral noise (higher entropy; Table 4c; Fig.3).

The structure of female chattering calls in all exchanges was affected by the time of day: calls of exchanges performed early in the morning were in higher frequency bands (higher mean, median, Q25, Q75 and average dominant frequency) (lower PC1 and PC2 values, Table 4c).

Acoustic Structure of Feeding Sequences

When the male entered inside the nest-box, the time spent calling by the female (i.e. the time between the first and the last calls) was 23% longer in Food than in Control condition (Table 6). This duration was highly correlated to the time spent inside the nest-box by the male (spearman correlation, r^2=0.92, N=38), which was also longer in Food condition (Table 6). Nevertheless, food supplementation decreased by 20% the proportion of time spent calling by the female (duty cycle = sum of the durations of all female calls / total time spent calling; Table 6; Fig.4).

Acoustic Structure of Female Screaming and Churring Calls Produced During Feeding Sequences

Female Screaming and Churring calls showed higher PC1 values with food supplementation i.e. energy in lower frequency bands (lower mean, median, Q25, Q75, dominant frequency), narrower frequency bandwidth (smaller sd and IQR), smaller modulations of frequency within calls (lower sd of the dominant frequency), and less spectral noise (lower entropy; Table 4d; Fig.5).

DISCUSSION

In this study we showed that providing food supplementation to incubating females enhanced nest attentiveness thus had an impact on females' parental care. This is consistent with the food limitation hypothesis (Chalfoun & Martin, 2007; T. Martin, 1987), and indicates that mate feeding may have an impact on breeding success in this species. Moreover, we found that food supplementation had an impact on pair communication, especially on vocal exchanges that ended with the male entering the nest-box to feed the female (Feeding exchanges) and subsequent Feeding sequences inside the nest.

When fed by their male inside the nest, the proportion of time spent calling by the female (duty-cycle) was shorter with food supplementation (thus with lower energy demand of the female). In addition, screaming and churring calls used by the female during these Feeding sequences inside the nest were in lower frequency bands, showed narrower frequency bandwidth, less modulation of frequency and less spectral noise. Similar modifications of vocal behaviour with hunger level have already been described in female begging calls (Cantarero et al., 2014; Moore & Rohwer, 2012; Otter et al., 2007; Tobias & Seddon, 2002), and in nestling begging calls (Cotton, Kacelnik, & Wright, 1996;

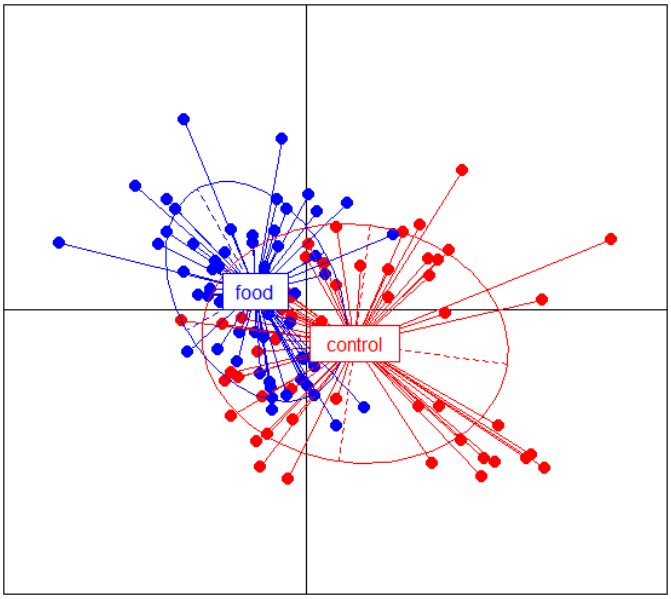


Figure 3. Effect of food supplementation on the acoustic structure of female chattering calls during Feeding exchanges. Lower PC1 indicates wider frequency bandwidth, larger modulations of frequency within calls, energy in higher frequency bands and more spectral noise. Ellipses show 67% of the data points in each group.

Table 6. Effect of food supplementation on male and female behaviours during Feeding sequences.

	Estimate ± SE	R2m	R2c	Chi²	Df	P-value
Time spent calling by the female (s; log10)		0.13	0.59			
(Intercept)	0.96 ± 0.08					
Condition				8.22	1	**0.004**
Food	0.21 ± 0.07					
Time (scaled)	- 0.05 ± 0.04			1.36	1	0.243
Time spent inside the nest by the male (s; log10)						
(Intercept)	0.15 ± 0.08	0.09	0.61			
Condition				6.08	1	**0.014**
Food	0.15 ± 0.06					
Time (scaled)	- 0.04 ± 0.04			0.97	1	0.324
Proportion of time spent calling by the female (%)		0.09	0.4			
(Intercept)	37.90 ± 3.64					
Condition				4.3	1	**0.038**
Food	- 7.81 ± 3.77					
Time (scaled)	- 1.59 ± 2.20			0.52	1	0.471

Significant results are displayed in bold.

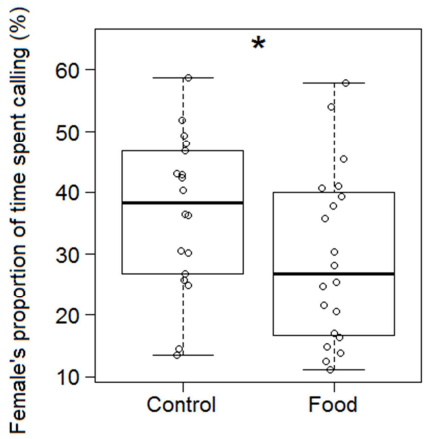


Figure 4. Effect of food supplementation on the proportion of time spent calling by females during Feeding sequences. Boxes are 1st-3rd quartiles, lines are medians, whiskers are extreme values and each point represents a pair.

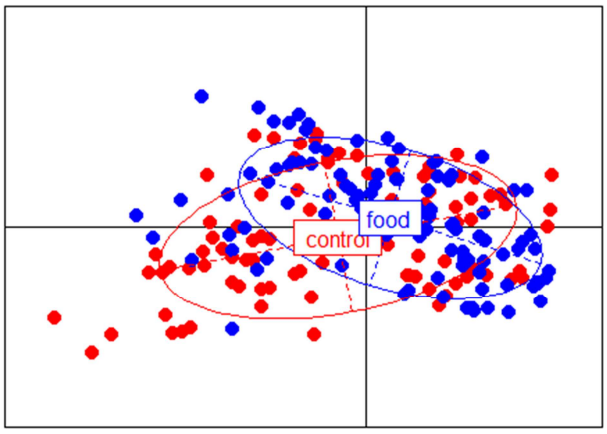


Figure 5. Effect of food supplementation on the acoustic structure of female screaming and churring calls produced during Feeding sequences. Higher values of PC1 indicate energy in lower frequency bands, narrower frequency bandwidth, smaller modulation of frequency within calls and less spectral noise. Ellipses show 67% of the data points in each group.

Horn & Leonard, 2005; Marques, Vicente, Márquez, Marques, & Vicente, 2009; Mondloch, 1995; Reers & Jacot, 2011). The calls used by great tit females during the Feeding sequences inside the nest recorded in the present study had been previously described (Gorissen & Eens 2005) and considered as different from female begging calls (zeedling calls observed during courtship feeding, Gorissen et al. 2004). Our results show that, even if these nest calls may have other functions, they act as begging displays during male visits and signal female's needs for food.

More interestingly, the vocal exchange between the female inside the nest and the male outside that preceded male feeding inside the nest was also modified by food supplementation. With food supplementation, vocal exchanges were shorter with less vocalizations from both partners. Moreover, female chattering calls used during these exchanges showed several modifications of their acoustic structure (wider frequency bandwidth, larger modulation of frequency and spectral noise, energy in higher frequency bands). Again, females signaled their needs for food, but they did it before the male entered the nest with a food item. This is consistent with our previous results showing that birds vocalized more during vocal exchanges ending up in feeding inside the nest (Boucaud et al., In press). These vocalizations thus signal the needs of the incubating female to her distant male outside the nest and might allow the male to adjust his feeding visits to the nest.

Surprisingly, the modifications of chattering calls in response to food supplementation were in the opposite direction to those of screaming and churring calls produced during Feeding sequences. However chattering calls have a very different acoustic structure from calls produced during Feeding sequences: chattering calls are higher pitched and with a clearer harmonic structure. This may explain why the same behavioural state triggered different acoustic modifications between these different call types. Another possibility is that these call types show different modifications in response to female needs because they have different sound propagation constraints. Chattering calls are used during exchanges with the male outside the nest and thus need to propagate on long distances compared to screaming and churring calls, which are used when partners are in close proximity inside the nest. When the female was hungry, her chattering calls were in lower frequency bands. Because sounds in low frequencies propagate on longer distances, this might improve sound reception by the male.

Simple and Exit exchanges were not modified by food supplementation indicating that they may have other functions than indicating female's hunger. We observed an effect of the time of recording on the structure of all exchanges, but the effect on Simple and Exit exchanges was opposite for some parameters (PC1 on temporal structure). Pairs that performed their last Simple exchanges early in the morning produced shorter Simple exchanges with less vocalizations whereas pairs that performed their last Exit exchanges early produced longer exchanges with more vocalizations. This might reveal the different functions of these two contexts of vocal exchanges.

Despite the modifications of female vocal behaviour in response to food supplementation, no difference was observed in the number of male feeding inside the nest. Several hypotheses can explain this result. First, males might either not perceive or not respond to the information given by their mate. If mate feeding has other functions like pair-bond maintenance (Lack, 1940), we expect this behaviour to depend on pair-bond strength or male quality. Indeed, Royama (1966) found that food items given to the female inside the nest were larger than those given outside. Those bigger food items must be difficult to find and mate feeding inside the nest may thus depends on male quality (Lifjeld et al., 1987; Siefferman & Hill, 2005) or food availability on territory (Matysioková & Remeš, 2010; Zanette et al., 2000). Second, males may already limit their visits to the nest to the minimum in order to reduce the likelihood of the nest being detected by a predator (T E Martin et al.,

2000; Muchai & Plessis, 2005; Raihani, Nelson-Flower, Moyes, Browning, & Ridley, 2010). To fully evaluate male response to female needs, mate feeding outside the nest must be considered as well. Royama (1966) estimated that the females was fed by her male about 160 times per day outside the nest during incubation. In our study, males came inside the nest on average 9 ± 1 times (mean ± SE) during a recording session (lasting 8.5h). Mate feedings inside the nest may thus represent a small part of total mate feedings and may not be sufficient to evaluate male provisioning rate. In the present study, Exit exchanges, i.e. vocal exchanges that ended up with the female exiting the nest to join her male outside, tended to be more numerous in control than with food supplementation. So mate feeding outside the nest may increase with higher female hunger and males adapted rates of feeding to supplemented females. Nevertheless, measuring the total amount of mate feeding remains necessary to fully conclude on the male's response to its female's needs. Moreover, the quality of food items must be of importance too, because study on nestlings provisioning showed that the quality of food items can be a better predictor of reproductive success than nestlings provisioning frequency (Schwagmeyer & Mock, 2008).

This study shows that incubating female great tits signal their needs during vocal exchanges with their male outside the nest and during subsequent Feeding sequences inside the nest by modifying calling rate, calling duration and call structure. Moreover, female nest attentiveness depends on female energy demand. Mate feeding may thus be of importance for breeding success in this species. Future studies should determine to what extent the male adjusts his feeding behavior both inside but also outside the nest based on the female modified vocalizations.

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DISCUSSION GENERALE

III. DISCUSSION GENERALE

III.1. Communication au nid des couples de diamants mandarins

III.1.a. Les duos permettent aux couples de se partager le temps d'incubation

Pendant l'incubation, les diamants mandarins font des duos dans trois contextes différents : lorsqu'un oiseau relève son partenaire qui incubait (relève), lorsqu'un oiseau rencontre son partenaire dans le nid et repart (visite) et enfin lorsqu'un oiseau se trouve à l'extérieur du nid et l'autre à l'intérieur, sans que l'un ou l'autre n'entre ou ne sorte du nid (sentinelle ; Chapitre 1). Parce que leur début prédit ce que les oiseaux vont faire par la suite (relève, visite ou sentinelle ; Chapitre 1), les duos pourraient permettre aux oiseaux d'indiquer à leur partenaire s'ils sont prêts à rester ou à partir du nid. L'ensemble de ces duos jouerait alors un rôle dans le partage du temps d'incubation en permettant aux parents de décider à quel moment la relève doit se faire. Dans le chapitre 2, je me suis intéressée plus particulièrement aux duos produits lors des relèves d'incubation. J'ai montré que lorsque le mâle était en retard pour faire la relève, la structure du duo était modifiée. De plus, les oiseaux indiquaient lors de cette relève le temps qu'ils allaient passer dans le nid par la suite. L'ensemble de ces résultats indique que les duos jouent un rôle dans le partage du temps d'incubation chez cette espèce et pourraient être à l'origine de la grande équité avec laquelle le mâle et la femelle se partagent cette tâche (El-Wailly, 1966; Delesalle, 1986; Zann & Rossetto, 1991; Gorman, Arnold, & Nager, 2005; Gilby, Mainwaring, & Griffith, 2013). C'est à ma connaissance la première fois qu'il est montré que la communication acoustique joue un rôle dans cette situation.

III.1.b. Quelles sont les autres fonctions possibles des duos ?

Les duos des diamants mandarins jouent un rôle dans le partage du temps d'incubation entre le mâle et la femelle. Mais parce qu'ils sont présents tout au long de la reproduction, depuis la construction du nid jusqu'à l'envol des poussins (Elie *et al.*, 2010), ils doivent également avoir d'autres fonctions. Le répertoire vocal des oiseaux dépend de leur position dans ou à l'extérieur du nid (Chapitre 1 ; Elie *et al.*, 2010), les duos pourraient donc permettre aux couples de rester en contact et de connaitre la position de leur partenaire (Logue, 2007; Mennill & Vehrencamp, 2008). Parce qu'ils sont plus produits lorsque les oiseaux doivent se séparer sur des brefs périodes, les duos pourraient également être un comportement de réunion renforçant le lien du couple (Elie *et al.*, 2010). Enfin, les duos pourraient avoir des fonctions différentes dépendant du contexte dans lequel ils sont émis. Les duos de visites sont souvent associés à un apport de matériaux de construction par le mâle ou par la femelle (Chapitre 1). Chez cette espèce, c'est en généralement le mâle qui apporte les matériaux mais la femelle peut participer à la construction du nid en leur donnant leur position finale (Zann, 1996). Au début de la construction, ce travail à deux est très ritualisé et les oiseaux produisent de nombreux cris et claquements de bec lorsqu'ils se retrouvent dans le nid (Zann, 1996). Lors de l'incubation les duos de visites pourraient être un rituel similaire. Alternativement, l'apport de matériaux pourrait être fonctionnel pour la construction du nid et les duos produits dans cette situation pourraient donc avoir un rôle dans la coordination des parents lors de cette activité. Les

duos de sentinelle ont été qualifiés ainsi car l'oiseau se trouvant alors à l'extérieur du nid semblait vigilant à son environnement (Elie *et al.*, 2010). En effet, chez cette espèce la prédation des nids est forte (jusqu'à 66 % ; Zann, 1994). De plus, parce que les diamants mandarins construisent des nids fermés dans lesquels ils accèdent par un petit tunnel (Zann, 1996), les adultes sont particulièrement vulnérables lorsqu'ils se font surprendre au nid car leurs possibilités de fuite sont limitées (Griffith, Pryke, & Mariette, 2008; Mainwaring & Griffith, 2013). Il a été montré chez cette espèce qu'un oiseau en train d'incuber sortait plus vite du nid et avait moins de chances de se faire surprendre par un observateur humain lorsque son partenaire se trouvait à proximité (Mainwaring & Griffith, 2013). Les diamants mandarins ne possèdent pas de cris d'alarme. Cependant leur niveau de stress peut être codé dans leurs cris de distance (Perez *et al.*, 2012) et être détecté par leur partenaire (Annexe 1). L'oiseau jouant le rôle de sentinelle pourrait donc signaler l'absence de danger lors des duos de sentinelle et utiliser des cris de distance codant un certain niveau de stress pour indiquer un danger à son partenaire.

III.1.c Le diamant mandarin est un modèle intéressant pour étudier la communication des couples en captivité

Les duos des diamants mandarins avaient été décrits en milieu naturel (Elie *et al.*, 2010). Dans mon travail de thèse, j'ai pu les étudier sur le terrain mais j'ai également montré pour la première fois qu'ils pouvaient être observés en captivité (Chapitre 2). Bien qu'une analyse soit nécessaire pour pouvoir affirmer que les duos produits en laboratoire ne sont pas différents des duos produits en milieu naturel, de nombreux points communs peuvent déjà être soulignés. Pendant l'incubation, on observe les duos dans les mêmes contextes que ce soit en volière où en milieu naturel (relève, visite et sentinelle ; Elie *et al.*, 2010 ; Chapitres 1 et 2). De plus, les oiseaux utilisent le même répertoire vocal (chant, « whines », « short calls », et bien que les claquements de bec n'aient pas pu être intégrés à l'analyse des duos dans l'étude réalisée en volière, ils étaient bien présents sur les enregistrements ; Elie *et al.*, 2010 ; Chapitre 1 et 2). Enfin, l'analyse des duos de relève montre que dans les deux cas leur structure est semblable et reste stable peu importe le sexe de l'oiseau qui revient au nid (Chapitre 1 et 2, ressource supplémentaire du chapitre 2).

Durant ma thèse j'ai aussi eu l'occasion d'enregistrer neuf couples se reproduisant dans des cages (Annexe 2). J'ai pu montrer que les duos de relèves produit par ces oiseaux étaient semblables à ceux observés en volière en terme de structure (durée, nombre de cris, taux de cris) de répertoire vocal utilisé et de participation relative des partenaires (proportion de cris du mâle sur le nombre total de cris dans le duo). La seule différence observée entre les couples se reproduisant en cage, en volière et en milieu naturel est le temps passé par un oiseau dans le nid entre deux relèves. J'ai observé une durée moyenne de 19 minutes en cage (Annexe 2), 38 minutes en volière (Chapitre 2) et 45 minutes en milieu naturel (Chapitre 1). Cette durée semble en fait liée à la distance à parcourir pour trouver de la nourriture et à la complexité du milieu. En effet, d'autres études ont montré que le temps passé dans le nid par un oiseau était de 20 minutes en volière, 40 minutes pour une population sauvage disposant de mangeoires artificielles (Zann & Rossetto, 1991) et jusqu'à 92 minutes chez une autre population sauvage (Immelmann, 1962).

Ces résultats indiquent que l'étude de la communication au nid des couples de diamants mandarins en captivité représente une bonne opportunité de mieux décrire et comprendre les fonctions de ce type de communication. En particulier, la volière que j'ai pu utiliser (Chapitre 2) a

permis de reproduire des éléments clefs du milieu naturel qui influent sur les relations sociales entre les oiseaux (Fig. 9). Elle a permis d'observer des comportements très proches de ceux des oiseaux en milieu naturel (observations personnelles). Un exemple est le temps passé au nid entre deux relèves par un oiseau pendant l'incubation, qui se rapproche plus de ce qui est observé en milieu naturel que de ce qui avait précédemment été décrit en volière ou de ce que j'ai pu observer en cage (voir plus haut).

III.2 Communication au nid des couples de mésanges charbonnières

III.2.a. Les femelles indiquent leurs besoins en nourriture

Chez la mésange charbonnière, les échanges vocaux entre la femelle se trouvant dans le nid, et le mâle à l'extérieur peuvent se terminer de trois façons différentes : la femelle sort du nid et peut alors être nourrie par le mâle à l'extérieur (sortie), le mâle entre dans le nid pour nourrir la femelle (nourrissage), ou l'échange se termine sans qu'un oiseau n'entre ou ne sorte du nid (simple ; Chapitre 3). Dans le chapitre 3, j'ai montré que la structure de l'échange vocal prédisait la façon dont l'échange allait se terminer (sortie, nourrissage ou simple), suggérant que la femelle pouvait indiquer au mâle si elle avait besoin d'être nourrie ou non. J'ai vérifié expérimentalement cette hypothèse dans le chapitre 4 en comparant une situation où je nourrissais les femelles à une situation contrôle. J'ai pu observer que ces dernières communiquent effectivement leurs besoins en augmentant l'intensité de leur production de cris. Mais je n'ai pas observé que le mâle ajustait le nombre de nourrissages qu'il faisait au nid en fonction de cette information. Une hypothèse est que le nombre de nourrissages au nid pourrait dépendre des ressources alimentaires du territoire. Cette hypothèse semble plausible si les proies apportées au nid sont plus grosses que celles données à l'extérieur du nid et donc probablement plus rares (Royama, 1966). De plus, des résultats préliminaires sur des couples enregistrés en ville et en forêt aux alentours de Dijon (en collaboration avec Bruno Faivre et Stéphane Garnier de l'Université de Bourgogne) montrent que le nombre de nourrissages au nid est plus important en forêt qu'en ville (Fig. 10). Hors, chez la mésange charbonnière, le succès de reproduction est généralement plus faible en ville (ce qui est bien le cas des couples enregistrés à Dijon) et une des raisons de cette différence est sans doute la disponibilité en nourriture (Horak *et al.*, 2000; Solonen, 2001). J'ai également observé que le nombre d'échanges vocaux se terminant par une sortie de la femelle du nid était légèrement plus faible lorsque les femelles étaient expérimentalement nourries (Chapitre 4). Le nombre de nourrissage par le mâle à l'extérieur du nid pourrait donc être plus faible dans cette condition. Malheureusement, estimer le nombre de nourrissages réalisés en dehors du nid semble une tâche difficilement réalisable aujourd'hui car les oiseaux sont très difficiles à observer lorsqu'ils se déplacent dans la canopée et cette espèce se reproduit difficilement dans les conditions plus contrôlées de la captivité. Toutefois, le fait que la femelle modifie sa communication en fonction de ses besoins et qu'elle passe plus de temps dans le nid lorsqu'elle a de la nourriture à disposition (Chapitre 4) suggère que la nourriture apportée par le mâle est probablement importante chez cette espèce et que la communication acoustique pourrait permettre d'ajuster l'effort du mâle aux besoins de la femelle.

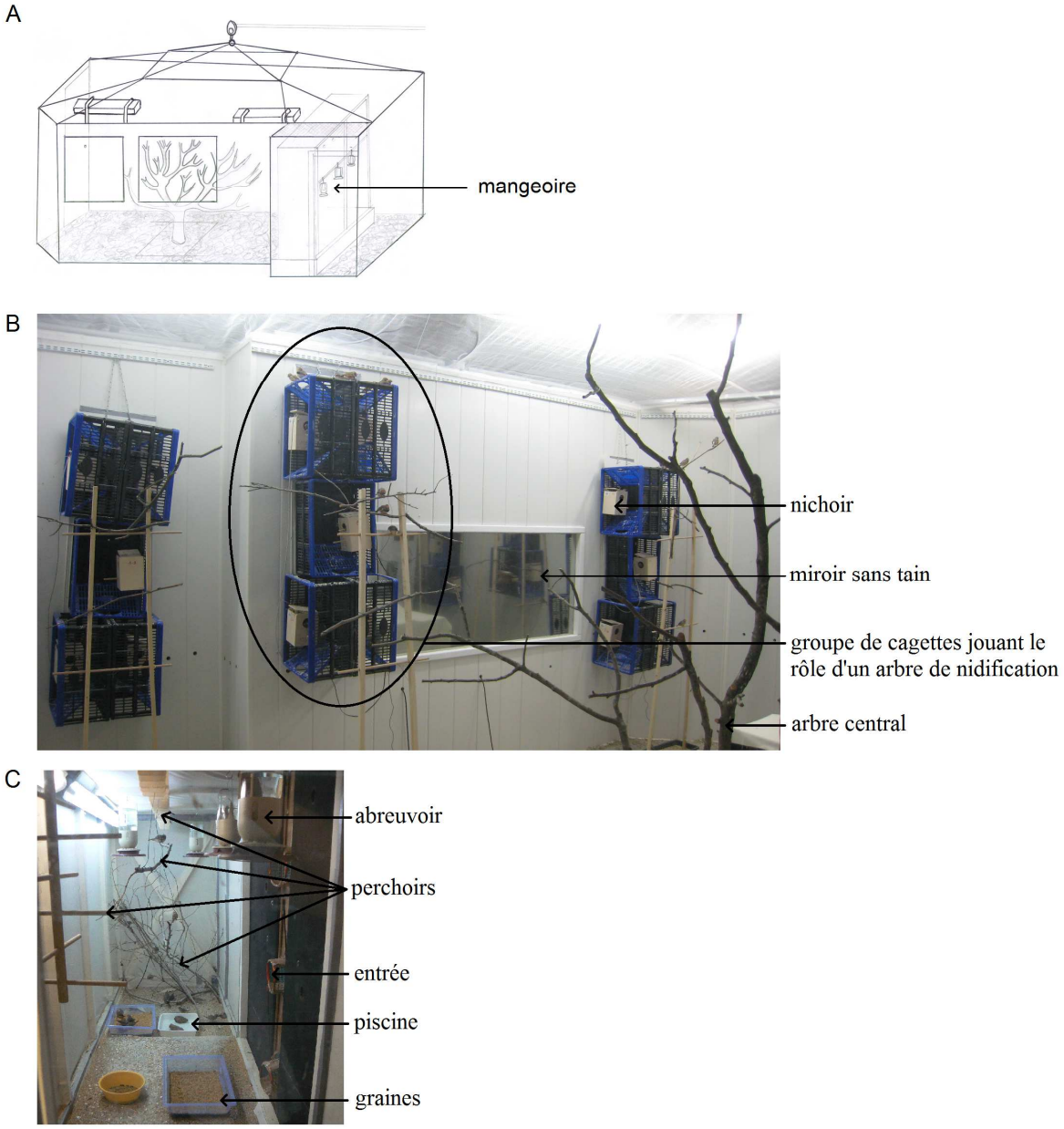

Figure 9. A) Dessin de la volière utilisée pour la reproduction des diamants mandarins (par Nicolas Boyer) et détails B) de la zone de reproduction et C) de la mangeoire. Cette volière recrée des éléments clefs du milieu naturel, susceptibles d'influer sur les interactions sociales chez le diamant mandarin. Un total de dix groupes de cinq nichoirs peut y être installé. Chaque nichoir est placé dans une « cagette » qui permet de délimiter une zone à défendre autour du nid et de placer les nids d'un même groupe proches les uns des autres tout en plaçant les entrées dans des directions opposées (les oiseaux devant contourner la cagette pour accéder à leur nid). Devant chaque groupe de nichoirs, des branches sont installées pour fournir des perchoirs aux oiseaux. Chaque groupe de nids simule donc un arbre de nidification dans la colonie. Un ou deux arbres supplémentaires sont mis au centre de la volière et jouent le rôle d' « arbres sociaux ». Enfin la nourriture et les zones de baignades sont installées dans une mangeoire isolée acoustiquement et visuellement de la zone de reproduction de façon à reproduire la séparation due à la distance en milieu naturel.

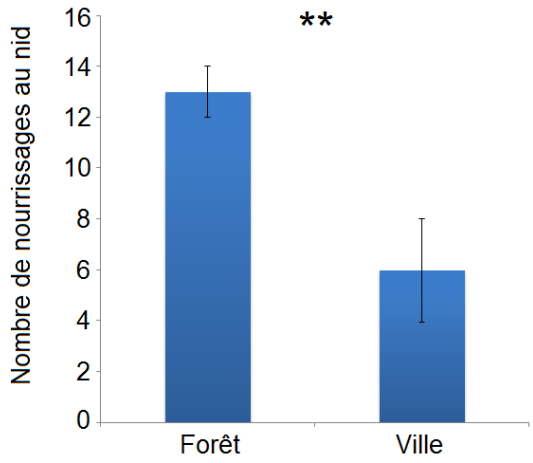

Figure 10. Nombre de nourrissages au nid de la femelle par le mâle réalisés entre 5h30 et 14h durant l'incubation : comparaison de deux populations de mésanges charbonnières enregistrées en 2014 dans les alentours de Dijon (29 couples en forêt et 21 couples en ville). Mann-Whitney : U = 441,5 ; p-value = 0.007.

III.2.b. Les femelles indiquent-elles les besoins en nourriture de la nichée ?

Juste après l'arrivée des poussins, la femelle reste encore très présente dans le nid pour les tenir au chaud. A cette période, le mâle augmente la fréquence de ses nourrissages qui sont alors destinés en partie à la nichée. Cependant, lorsque la femelle est présente lors des visites de son partenaire, elle émet des séquences de cris similaires à celles produites pendant l'incubation (chapitre 3). A cette période, la plus grosse partie de la nourriture apportée par le mâle doit pourtant être destinée aux poussins. Pour le vérifier, j'ai placé des caméras dans les nids de trois couples venant d'avoir des poussins en 2015. Chaque couple a été enregistré environ 5h et j'ai pu observer un total de 39 événements durant lesquels le mâle entrait dans le nid pendant que la femelle s'y trouvait. Dans 8% des cas, la femelle mangeait la nourriture apportée, dans 77% des cas la femelle récupérait la nourriture pour la redistribuer aux poussins, et enfin dans 15% des cas le mâle donnait directement la nourriture aux poussins. Dans cette dernière situation, la femelle n'était pas sur les poussins car elle était elle-même en train de les nourrir ou s'apprêtait à sortir. Les cris produits par la femelle à cette période de la reproduction pourraient donc indiquer au mâle les besoins de la nichée. Un tel système n'a jusque-là été observé qu'une seule fois chez l'oiseau. Chez le cardinal rouge (*Cardinalis cardinalis*), les mâles ont plus de probabilité d'apporter de la nourriture à la nichée si la femelle leur répond par des chants depuis l'intérieur du nid (Halkin, 1997). En effet, chez les espèces où la femelle couve seule les poussins, elle reçoit plus d'informations directes sur les besoins de la nichée, tout particulièrement si elle récupère la nourriture apportée par le mâle pour la distribuer après son départ. Elle pourrait alors jouer un rôle d'intermédiaire pour signaler les besoins des poussins. Chez la mésange à longue queue (*Aegithalos caudatus*), une étude a utilisé des playbacks de cris de poussins pour augmenter le taux de nourrissage d'un des deux parents (Meade *et al.*, 2011). Lorsque les poussins étaient encore couvés par la femelle (et donc que la quantité d'informations reçues sur les besoins de la nichée était plus importante pour la femelle) le mâle réagissait très fortement à une augmentation, même faible, du taux de nourrissage de la femelle en augmentant significativement le nombre de ses visites au nid. Mais lorsque c'est le taux de nourrissage du mâle qui était expérimentalement augmenté, la femelle ne modifiait pas son comportement. En revanche, lorsque les poussins n'avaient plus besoin d'être couvés (les deux parents disposaient alors de la même quantité d'information sur les besoins de la nichée), l'augmentation du taux de nourrissage d'un parent (mâle ou femelle) entrainait une augmentation du taux de nourrissage de son partenaire. Cette étude montre donc que les oiseaux peuvent utiliser les informations données par leur partenaire et que cette utilisation dépend de l'accès que chacun a aux besoins de la nichée. De plus, les auteurs notent de façon anecdotique que les adultes faisaient plus de cris durant les périodes de playback et que la communication acoustique pourrait donc jouer un rôle dans cette transmission d'information. De la même façon, chez le méliphage à sourcils noirs (*Manorina melanophrys*) qui pratique la reproduction coopérative, utiliser des playbacks de cris de poussins sur un adulte en particulier augmente son taux de nourrissage ainsi que le nombre de cris qu'il fait lors de ses visites au nid (McDonald, Kazem, & Wright, 2009). Les autres membres du groupe augmentent alors aussi la fréquence de leurs visites. Cependant, seul le taux de nourrissage de l'oiseau cible semble expliquer cet ajustement et le rôle des cris reste à déterminer. Une expérience utilisant un protocole similaire chez la mésange charbonnière permettrait de déterminer si le mâle peut utiliser les cris de la femelle comme une information sur les besoins de sa nichée et s'il adapte son comportement en conséquence.

III.2.c. Pourquoi les femelles chantent-elles ?

Sur 202 échanges vocaux de mésanges charbonnières analysés pendant ma thèse, 7% contenaient des chants de femelles. Cette proportion est constante sur les deux années d'enregistrement à St-Etienne (2013 et 2014, Chapitres 2 et 3) ainsi que sur les enregistrements faits à Dijon en 2014. Le chant n'est pas associé à un type d'échange vocal en particulier puisqu'on le retrouve à la fois dans les échanges simples, ceux se terminant par la sortie de la femelle du nid et ceux se terminant par l'entrée du mâle dans le nid. Les chants de femelles sont utilisés en réponse à des cris (21% des cas) ou à des chants du mâle (79% des cas). Lorsque le mâle et la femelle chantent tous les deux, la femelle utilise un chant proche ou identique à celui de son mâle dans 73 % des cas. Enfin, les femelles chantent par-dessus les vocalisations du mâle (chant ou cris) dans 19% des cas.

Bien que les chants de femelles puissent être associés à des cris (Fig. 11), l'échange de chant entre le mâle et la femelle rappelle dans sa forme les interactions entre deux mâles défendant leur territoire. Dans ce type d'interactions, le fait d'utiliser le même type de chant qu'un autre individu ou de chanter en même temps que lui peut avoir une signification particulière. Dans les interactions entre mâles on considère en général qu'il s'agit de signaux agressifs (Searcy & Beecher, 2009; Bradbury & Vehrencamp, 2011) mais les résultats de différentes études sur différentes espèces sont parfois contradictoires (Searcy & Beecher, 2009). Chez la mésange charbonnière, les mâles qui répondent à un playback en utilisant le même type de chants sont aussi ceux qui se montrent les plus agressifs en approchant le plus le haut-parleur (Krebs, Avery, & Cowie, 1981). Une autre étude, chez la mésange charbonnière, comparant la réponse des mâles à des oiseaux voisins ou étrangers semble également indiquer que le fait d'utiliser le même type de chant que son opposant est un signal d'agressivité (Falls, Krebs, & Mcgregor, 1982). Si les chants de femelles jouaient un rôle dans la défense du nid ou du territoire, le fait de chanter avec le mâle pourrait représenter une menace plus grande pour de potentiels intrus (Marshall-Ball, Mann, & Slater, 2006), et peut être d'autant plus si mâle et femelle utilisaient le même type de chant. Mais étant donné que le chant des femelles est ici observé pendant l'incubation, alors que les territoires sont établis, il pourrait être adressé non pas à des conspécifiques mais au partenaire. Chez le cardinal rouge (*Cardinalis cardinalis*), le mâle a plus de chance de rapporter de la nourriture au nid si la femelle lui répond en utilisant le même type de chant que lui (Halkin, 1997). Cependant, dans les enregistrements de mésanges charbonnières que j'ai pu analyser, les mâles ne rentraient jamais dans le nid après que leur femelle ait utilisé le même type de chant qu'eux. La quantité d'enregistrements que j'ai pu obtenir ne permet cependant pas de dire si la probabilité que les femelles fassent le même type de chant que leur mâle dépend du hasard on non et s'il y a un impact sur l'issue de l'échange vocal.

Dans les enregistrements que j'ai analysés, trois femelles seulement ont chanté par-dessus le chant de leur mâle. Chez la mésange charbonnière, les mâles sont sensibles au fait que leur chant soit recouvert par le chant d'un autre mâle et adaptent leur chant en conséquence (Dabelsteen *et al.*, 1996; Langemann *et al.*, 2000). Le fait que les femelles recouvrent leur chant pourrait également avoir une signification. Notre échantillon est ici très faible pour pouvoir tirer des conclusions, mais de façon intéressante, les 3 femelles qui ont chanté par-dessus le chant de leur mâle l'ont fait en utilisant un type de chant semblable au sien et certaines notes pouvaient même se superposer parfaitement avec celles du mâle (Fig. 11). Le fait que le mâle et la femelle puissent chanter ensemble n'est pas sans rappeler les duos de chant des espèces tropicales qui ont des fonctions multiples (voir introduction générale ; Farabaugh, 1982; Hall, 2004, 2009; Benedict, 2008; Dahlin & Benedict, 2013), dont la plus commune est la défense du territoire (Marshall-Ball *et al.*, 2006; Molles

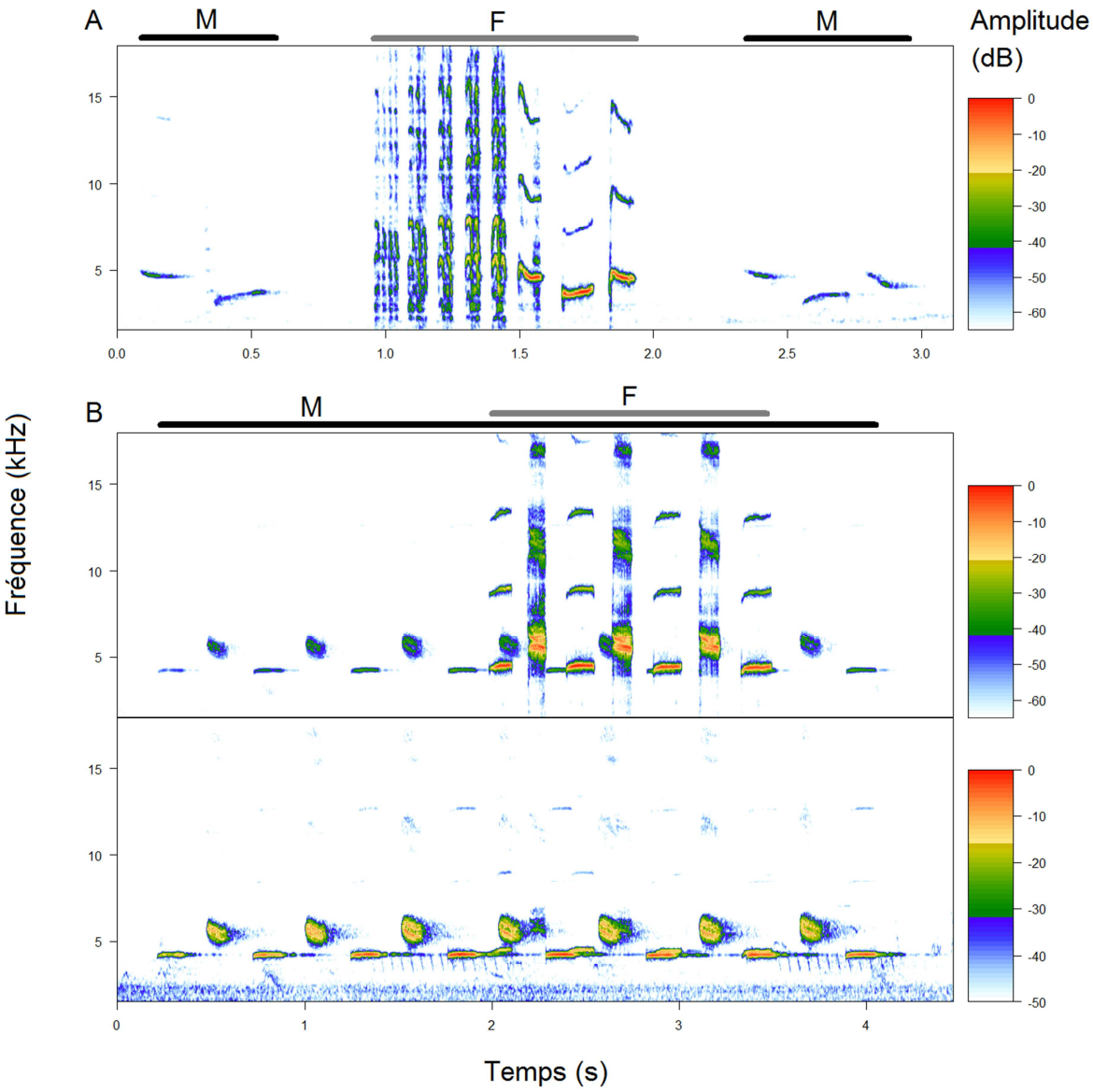

Figure 11. Exemples de chants de femelles produits depuis l'intérieur du nid en réponse aux chants de leurs mâles se trouvant à l'extérieur (M : chant du mâle ; F : chant et cris de la femelle). Mâle et femelle peuvent utiliser des chants similaires (A), et parfois même les superposer (B). Dans la partie B) de la figure, le sonagramme du haut montre l'enregistrement fait sur le microphone se trouvant à l'intérieur du nid tandis que le sonagramme du bas correspond au microphone se trouvant à l'extérieur. Ce dernier permet de mieux observer le chant du mâle qui est en partie masqué par celui de la femelle.

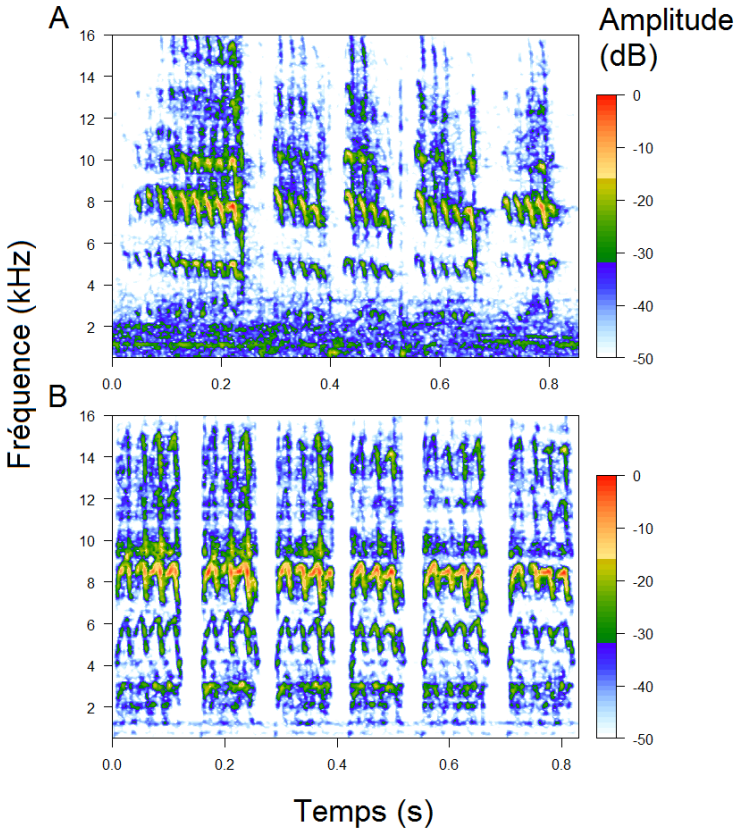

Figure 12. A) Cris produits par une femelle sur une mangeoire alors qu'un mâle se trouvait à proximité, et B) cris produits par une femelle depuis l'intérieur d'un nid pendant l'incubation ('chattering calls').

& Waas, 2006; Bradley & Mennill, 2009; Koloff & Mennill, 2011). Chez la mésange charbonnière, des études complémentaires sont nécessaires pour comprendre quelles peuvent être les fonctions du chant des femelles et s'il a une signification particulière lorsqu'il est émis en réponse au mâle.

III.2.d. Les nourrissages au nid sont-ils différents des « courtship feeding » ?

Chez la mésange charbonnière, lorsque le mâle nourrit la femelle en dehors du nid, le terme de « courtship feeding » est généralement utilisé (Hinde, 1952). Mais l'expression choisie est trompeuse puisque ces nourrissages n'ont pas lieu lors de la formation des couples, mais bien plus tard durant la période de reproduction (Hinde, 1952). La femelle produit alors des cris de quémande appelés « zeedling calls» (Hinde, 1952). La première étude sur les cris produits par les femelles de mésanges charbonnières au nid montrait que ces cris étaient différents des « zeedling calls» (Gorissen, Eens, & Nelson, 2004). Mais aucune étude récente utilisant des méthodes modernes d'acoustique n'a décrit précisément les séquences vocales produites par les femelles lors des « courtship feeding » et la comparaison reste donc difficile. Cependant, Hinde (1952) a observé des femelles utiliser des cris de quémande dans le nid durant sa construction et des enregistrements que j'ai réalisé en 2015 sur une mangeoire montrent des cris proches de certains cris pouvant être produit dans le nid (Fig. 12). Les nourrissages au nid pourraient donc être des comportements vocaux semblables aux « courtship feeding ». Il serait alors intéressant de voir si des différences existent car les femelles au nid pourraient par exemple adapter leur cris pour se faire entendre du mâle se trouvant encore à l'extérieur ou bien se montrer plus discrètes pour limiter les risques que le nid soit détecté.

III.3 Comparaison des deux espèces

III.3.a Les rôles des sexes dans les soins biparentaux se reflètent sur la communication

Le diamant mandarin et la mésange charbonnière ont été choisis car les couples de ces deux espèces ne se partagent pas les tâches de la même manière au cours de la reproduction. Chez le diamant mandarin, les tâches sont partagées de façon égale entre les partenaires tandis que chez la mésange charbonnière les tâches sont différentes selon le sexe. Cette différence interspécifique se retrouve au niveau de la communication des couples. Chez le diamant mandarin, le mâle et la femelle participent tous deux de la même manière au duo (Elie *et al.*, 2010 ; Chapitre 1). Elie et al. (2010) ont montré que le mâle et la femelle faisaient tous deux le même nombre de cris, alternaient leurs vocalisations sans que l'un ne semble se caler sur le rythme de l'autre et que le répertoire vocal utilisé ne dépendait pas du sexe de l'oiseau mais de sa position dans ou à l'extérieur du nid au début du duo. Dans le chapitre 1, j'ai décomposé les duos en plusieurs phases selon la position des oiseaux (dans ou à l'extérieur du nid) et montré que la participation relative des membres du couple pouvait varier tout au long des duos et qu'elle dépendait de la position des oiseaux. J'ai également retrouvé que le répertoire vocal dépend de la position des oiseaux : les « whines » étant utilisés exclusivement dans le nid alors que les « short calls » peuvent être utilisés dans le nid mais le sont surtout à l'extérieur de celui-ci.

Chez la mésange charbonnière, certaines vocalisations sont partagées par le mâle et la femelle comme les chants, les cris de contact et les cris d'alarme (Gompertz, 1961; Cramp & Perrins, 1993 ; Chapitre 3). Cependant, plusieurs cris utilisés par les femelles n'ont jamais été décrits chez les mâles (Gorissen & Eens, 2005). Mâle et femelle font le même nombre de séries de vocalisations dans un échange vocal mais c'est la femelle qui semble adopter le rythme imposé par le mâle en lui répondant (Chapitre 3).

Ici, les deux espèces étudiées présentent donc, au niveau de leur communication, des différences dans le rôle des sexes qui reflètent le partage des soins parentaux. Cependant, il n'est pas exclu que ces différences soient dues au fait que ces espèces sont phylogénétiquement éloignées et l'étude d'autres espèces serait nécessaire pour pouvoir généraliser cette conclusion. .

III.3.a Les rôles des sexes dans les soins biparentaux se reflètent sur les fonctions de la communication

Mon travail de thèse a permis de montrer que les diamants mandarins comme les mésanges charbonnières utilisent la communication acoustique dans l'organisation des soins biparentaux. Les fonctions de cette communication sont différentes entre les deux espèces et dépendent des rôles des mâles et des femelles puisqu'elle permet le partage du temps d'incubation chez le diamant mandarin (Chapitres 1 et 2), et l'approvisionnement de la femelle en nourriture chez la mésange charbonnière (Chapitres 3 et 4). Cependant, la présence d'échanges vocaux ayant lieu dans différents contextes et tout au long de la reproduction suggère que les fonctions possibles de la communication acoustique au nid sont multiples. Des premiers résultats laissent par exemple supposer que, chez les deux espèces, l'oiseau se trouvant à l'extérieur du nid pourrait avoir un rôle de sentinelle pour son partenaire (Chapitre 3 ; Elie *et al.*, 2010; Mainwaring & Griffith, 2013). La communication acoustique pourrait également jouer un rôle dans la coordination des parents pour le nourrissage de la nichée. Chez le diamant mandarin, chez qui les parents restent généralement ensemble pour aller chercher la nourriture et se rendre au nid (Mariette & Griffith, 2012, 2015), la communication acoustique permet sans doute aux oiseaux de garder le contact pour coordonner leurs déplacements (Zann, 1996). Chez la mésange charbonnière, la femelle pourrait indiquer les besoins de la nichée au mâle dans les premiers jours après l'éclosion (voir plus haut). Une fois que les poussins n'avaient plus besoin d'être couvés, j'ai parfois observé les parents entrant dans le nid avec de la nourriture communiquer avec leur partenaire resté à l'extérieur. Parce que le mâle et la femelle se coordonnent en alternant leurs visites au nid chez cette espèce (Johnstone *et al.*, 2014), un oiseau pourrait utiliser la communication acoustique pour signaler à son partenaire qu'il est passé nourrir la nichée.

III.4 Communication acoustique au nid chez les oiseaux

Une des hypothèses de départ de ce travail était que la plupart des espèces d'oiseaux à soins biparentaux devaient communiquer dans et autour du nid pendant la reproduction. Le diamant mandarin est l'espèce la plus étudiée en laboratoire mais la première étude décrivant des duos vocaux au nid pendant la reproduction (Elie *et al.*, 2010) est arrivée tardivement et a surpris même les spécialistes de cette espèce. La mésange charbonnière est également une espèce très étudiée mais le fait que la femelle puisse répondre à son mâle depuis l'intérieur du nid n'était que peu décrit (Gorissen *et al.*, 2004; Gorissen & Eens, 2005; Halfwerk, Bot, & Slabbekoorn, 2012) et les fonctions de

cette communication demeuraient jusque-là inconnues. Il ne serait pas surprenant que de nombreuses autres espèces utilisent des échanges vocaux au nid semblables à ceux des diamants mandarins et des mésanges charbonnières. En effet, les femelles de plusieurs espèces font des cris depuis l'intérieur du nid (Ritchison, 1983; Beletsky & Orians, 1985; Yasukawa, 1989; McDonald & Greenberg, 1991; Halkin, 1997). Chez la mésange bleue (*Cyanistes caeruleus*), les femelles font des cris très proches de ceux des mésanges charbonnières (Gorissen & Eens, 2005). Ces cris sont probablement émis en réponse aux mâles et pourraient avoir des fonctions semblables à ceux des mésanges charbonnières (Chapitres 3 et 4). Lors de l'étude réalisée à Fowlers Gap en Australie (Chapitre 1) j'ai enregistré un nid comportant des œufs de diamants mandarins ainsi que des œufs d'une autre espèce. Ce nid était en fait habité par des gérygones blanchâtres (*Aphelocephala leucopsis*) qui sont connues pour utiliser les nids de diamants mandarins abandonnés (Kikkawa, 1980) et sont régulièrement rencontrées dans les nichoirs installés à Fowlers Gap (Griffith *et al.*, 2008). La biologie de cette espèce est peu connue. S'il semble y avoir généralement des soins biparentaux, plusieurs cas de reproduction coopérative impliquant jusqu'à quatre adultes ont également été observés (Higgins & Peter, 2002). Lors de mon observation, trois individus étaient présents autour du nid et j'ai pu enregistrer un oiseau répondre depuis l'intérieur du nid à un autre se trouvant à l'extérieur (Fig. 13). Enfin des études récentes réalisées au sein de l'équipe à laquelle j'appartiens ont montré que des échanges vocaux au nid étaient présents chez le diamant à longue queue (*Poephila acuticauda*) et chez le cincle plongeur (*Cinclus cinclus* ; résultats non publiés).

Toutes ces observations semblent bien indiquer que la communication au nid entre les mâles et les femelles pourrait être très répandue chez les oiseaux. De plus, on la retrouve à la fois chez des espèces à soins biparentaux où mâles et femelles font les mêmes tâches (diamant mandarin, diamant à longue queue ; Zann, 1996; van Rooij & Griffith, 2011, 2013) que chez des espèces où la femelle seule couve les œufs et les poussins (mésange charbonnière, cincle plongeur, cardinal rouge ; Laskey, 1944; Hinde, 1952; Cramp & Perrins, 1993; Engstrand, Ward, & Bryant, 2002) et sans doute même chez des espèces à reproduction coopérative (gérygone blanchâtre ; Higgins & Peter, 2002).

III.5 Communication, soins parentaux et succès reproducteur

Si la communication acoustique joue un rôle dans l'organisation des soins biparentaux alors elle devrait avoir un impact sur le succès reproducteur des couples. Les femelles qui incubent seules peuvent être en partie nourries par leur partenaire. Dans ce cas, plusieurs études ont montré que les femelles pouvaient indiquer leurs besoins au mâle en utilisant des cris de quémandes comme le font les oiseaux juvéniles (Tobias & Seddon, 2002; Otter, Atherton, & van Oort, 2007; Moore & Rohwer, 2012; Cantarero *et al.*, 2014). L'apport en nourriture des mâles peut permettre aux femelles de passer plus de temps sur les œufs (Lyon & Montgomerie, 1985; Lifjeld & Slagsvold, 1986; Hałupka, 1994; Klatt, Stutchbury, & Evans, 2008; Lloyd *et al.*, 2009; Stein, Oh, & Badyaev, 2009; Matysioková, Cockburn, & Remeš, 2011; Moore & Rohwer, 2012). Or on sait que le pourcentage de temps passé à incuber a un impact sur le succès reproducteur des oiseaux puisqu'il influence le taux d'éclosion (Carey, 1980; Lyon & Montgomerie, 1985; Reid, Monaghan, & Ruxton, 2002) et la condition des juvéniles à l'envol (Reid *et al.*, 2002). De plus, une plus grande proportion de temps passé à incuber permet de diminuer la durée de la période d'incubation et donc de limiter l'exposition aux prédateurs (Magrath, 1988; Martin, Scott, & Menge, 2000; Martin, 2002). Une communication

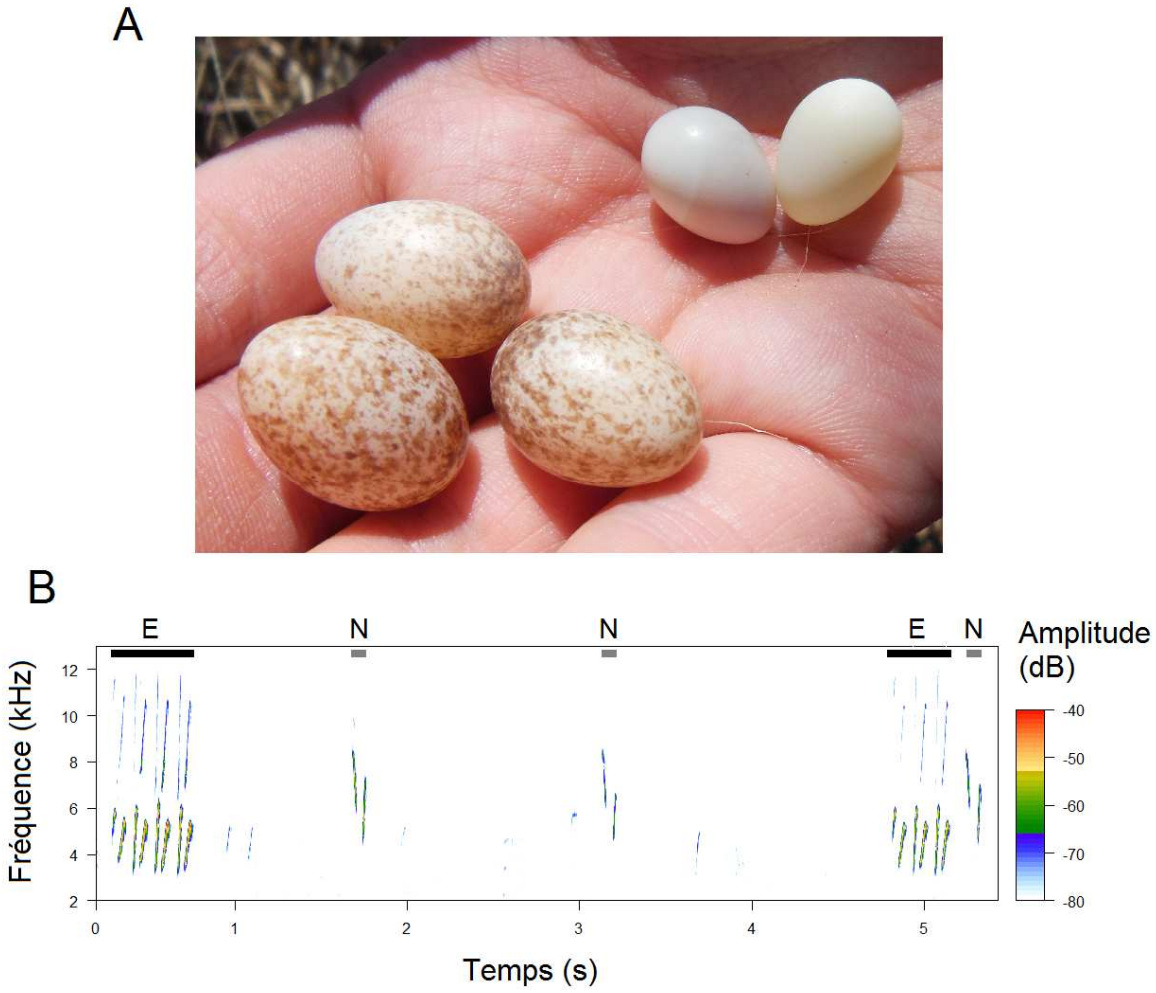

Figure 13. A) Ensemble des œufs trouvés dans un nid à la station de recherche de Fowlers Gap (Australie) en 2012. Les petits œufs blancs unis sont des œufs de diamants mandarins tandis que les plus gros et tachetés sont des œufs de gérygones blanchâtres. B) Extrait d'un échange vocal entre deux gérygones blanchâtres, une se trouvant dans le nid (N) et l'autre à l'extérieur (E).

efficace du couple permettant un approvisionnement optimum en nourriture pour la femelle devrait donc permettre aux couples d'améliorer leur succès reproducteur.

Lorsque mâle et femelle partagent les mêmes tâches, la communication acoustique peut leur permettre de se coordonner (Chapitre 1 et 2). La coordination entre le mâle et la femelle est très importante pendant l'incubation puisqu'un oiseau mettant trop de temps à revenir pour relever son partenaire ou une relève manquée pourraient avoir comme conséquences de laisser les œufs seuls anormalement longtemps (Ball & Silver, 1983; Chaurand & Weimerskirch, 1994). Après l'arrivée des poussins les adultes de certaines espèces à soins biparentaux ou à reproduction coopérative se coordonnent en se rendant ensemble au nid pour nourrir la nichée. Les nids ayant le meilleur succès reproducteur sont alors ceux des oiseaux les mieux coordonnés (Raihani *et al.*, 2010; Shen *et al.*, 2010; Mariette & Griffith, 2012). En effet, le fait que les adultes se rendent au nid ensemble pourrait permettre une distribution plus équitable de la nourriture entre les poussins (Shen *et al.*, 2010), et/ou de limiter l'activité autour du nid de façon à limiter les risques qu'il soit détecté par un prédateur (Martin *et al.*, 2000; Raihani *et al.*, 2010).

Durant ma thèse, j'ai mesuré le succès reproducteur des couples dans toutes mes études en prenant des paramètres tels que le nombre d'œufs, le nombre de poussins à l'éclosion, le nombre de poussins à l'envol et certaines mesures évaluant la condition des poussins. J'ai également prélevé du sang sur les adultes et leurs poussins et commencé à analyser l'ADN de façon à mesurer les taux de paternité extra-couple. L'analyse future de ces données permettra de déterminer si les couples qui ont un meilleur succès de reproduction, et/ou un taux de paternité extra-couple plus faible, présentent des particularités au niveau de leur communication acoustique. De plus, chez le diamant mandarin, j'ai fait une étude permettant de lier communication, coordination, expérience du couple et succès de reproduction. J'ai pour cela observé des couples de diamants mandarins sur deux reproductions successives. A chaque fois, j'ai enregistré les temps passés dans le nid par le mâle et par la femelle tout au long de l'incubation grâce à des transpondeurs implantés sur les oiseaux (Trovan ID100A) et détectés grâce à des antennes dans les nids (Fig. 14). J'ai également enregistré quatre duos de relève pour chaque couple et pris différentes mesures du succès de reproduction (voir plus haut). Un premier objectif de cette étude est de déterminer si la communication acoustique, l'organisation de l'incubation et le succès reproducteur sont liés. Mon hypothèse étant que les parents qui ont une communication plus efficace pourraient être plus coordonnés dans l'organisation de l'incubation (même temps passé dans le nid par le mâle et la femelle, peu de variabilité dans le temps qu'un oiseau passe dans le nid lors d'une session d'incubation etc.) et avoir un meilleur succès reproducteur. Le deuxième objectif de cette étude est de déterminer si les couples qui ont déjà une première expérience de reproduction ensemble sont plus efficaces que des oiseaux ayant déjà une première expérience mais avec un autre partenaire. En effet, les individus qui restent avec le même partenaire vont pouvoir bénéficier de leur expérience commune pour mieux ajuster leur comportement l'un à l'autre et donc être mieux coordonnés dans les activités partagées (« mate familiarity effect » ; Ball & Silver, 1983; Black, 1996). De plus, ils vont aussi pouvoir évoluer dans leur communication (Marshall-Ball *et al.*, 2006). Dans mon étude j'ai utilisé des oiseaux ayant tous le même âge (environ 1 an) et n'ayant encore aucune expérience de reproduction. Une moitié des oiseaux s'est reproduite deux fois avec le même partenaire tandis que la deuxième s'est également reproduite deux fois mais avec des partenaires différents. Cette étude va permettre de savoir si les couples de diamants mandarins bénéficient de leur expérience commune pour améliorer leur communication, leur coordination et leur succès reproducteur.

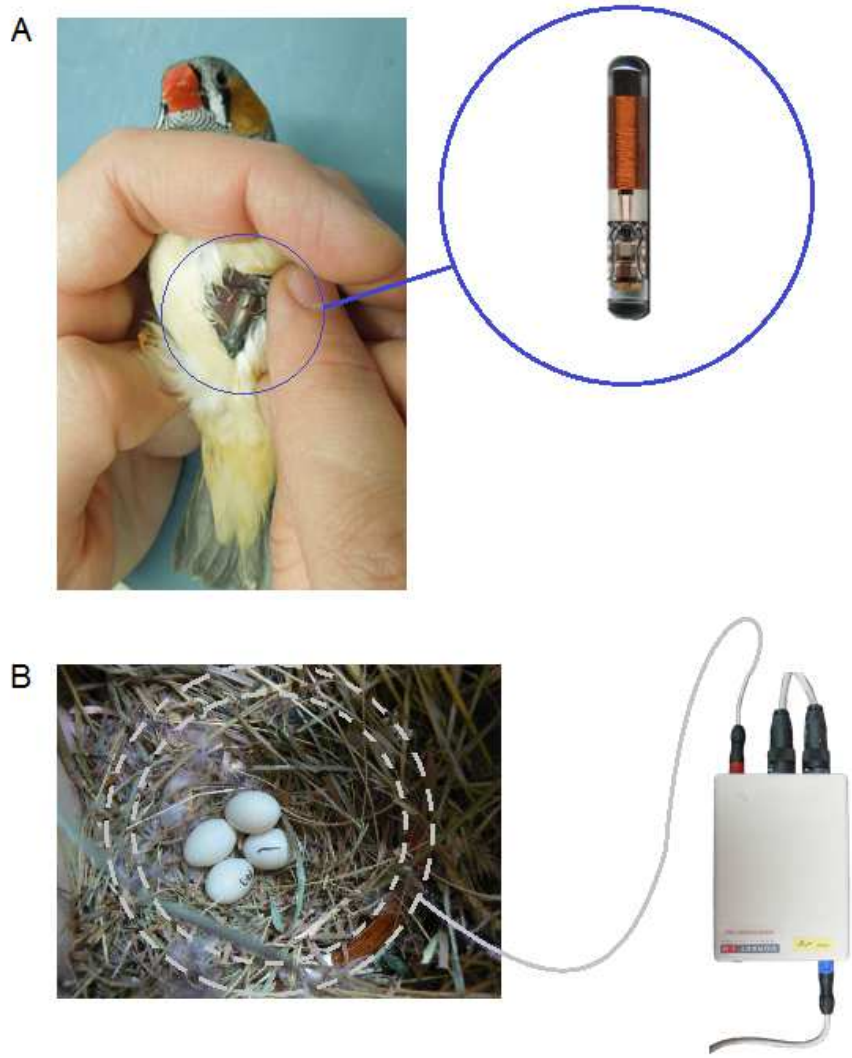

Figure 14. A) Transpondeur implanté au niveau du ventre d'un oiseau et, B) antenne placée autour des œufs et reliée à un décodeur permettant d'enregistrer en continu la présence des oiseaux dans le nid.

III.6 Conclusion et perspectives

Ce travail de thèse a permis de montrer que la communication acoustique des couples de mésanges charbonnières et de diamants mandarins pouvait jouer un rôle dans l'organisation des soins biparentaux. La communication de ces deux espèces est complexe et plus d'études sont nécessaires pour en comprendre toutes les fonctions et les subtilités. De plus, ce type de communication pourrait exister chez de nombreuses espèces d'oiseaux. La communication des couples d'oiseaux a été longtemps négligée (à l'exception des duos ; Farabaugh, 1982; Hall, 2004, 2009; Benedict, 2008; Dahlin & Benedict, 2013) car elle est souvent composée de signaux discrets et les individus ne sont pas toujours faciles à observer et à enregistrer. Cependant, de plus en plus d'études s'intéressent maintenant aux signaux acoustiques discrets chez les animaux (Anderson & Reichard, 2015). Mon travail a contribué à montrer l'intérêt de faire des enregistrements au nid. Cependant, s'intéresser uniquement à ce qu'il se passe au nid ne rend probablement compte que d'une petite partie de la communication des couples pendant la reproduction. De nouvelles technologies permettent aujourd'hui d'équiper des oiseaux, même petits, de microphones embarqués (Gill *et al.*, 2015). Ces microphones permettent d'enregistrer toutes les vocalisations émises par un individu, mais aussi de pouvoir savoir très facilement quelles vocalisations appartiennent à quel oiseau et ce même quand les individus sont très proches les uns des autres (Gill *et al.*, 2015). Cette technologie présente donc un fort potentiel pour étudier la communication des couples mais également des groupes d'oiseaux. Cependant, enregistrer les couples au nid présente également de nombreux avantages puisque cette solution reste pour le moment plus économique et permet d'enregistrer des couples sur le terrain sans avoir à les capturer.

L'utilisation ces différentes techniques pourrait donc permettre à l'avenir de mieux décrire la communication des couples. Cette communication pouvant être un élément clef dans l'organisation des soins parentaux et le succès reproducteur chez les oiseaux.

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ANNEXES

Annexe 1

Physiological resonance between mates through calls as possible evidence of empathic processes in songbirds

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ABSTRACT

Physiological resonance – where the physiological state of a subject generates the same state in a perceiver – has been proposed as a proximate mechanism facilitating pro-social behaviours. While mainly described in mammals, state matching in physiology and behaviour could be a phylogenetically shared trait among social vertebrates. Birds show complex social lives and cognitive abilities, and their monogamous pair-bond is a highly coordinated partnership, therefore we hypothesised that birds express state matching between mates. We show that calls of male zebra finches *Taeniopygia guttata* produced during corticosterone treatment (after oral administration of exogenous corticosterone and during visual separation from the partner) provoke both an increase in corticosterone concentrations and behavioural changes in their female partner compared to control calls (regular calls emitted by the same male during visual separation from the partner only), whereas calls produced during corticosterone treatment by unfamiliar males have no such effect. Irrespective of the caller status (mate/non-mate), calls' acoustic properties were predictive of female corticosterone concentration after playback, but the identity of mate calls was necessary to fully explain female responses. Female responses were unlikely due to a failure of the call-based mate recognition system: in a discrimination task, females perceive calls produced during corticosterone treatment as being more similar to the control calls of the same male than to control calls of other males, even after taking acoustical differences into account. These results constitute the first evidence of physiological resonance solely on acoustic cues in birds, and support the presence of empathic processes.

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Introduction

Several theoretical frameworks have tried to explain the origins of pro-social behaviours. While pro-social behaviours have been historically well studied and described in humans (Becker, 1931; Bierhoff, 2005; Davis, 1994; Hinde, 1986), more recent studies have acknowledged their existence in other primates (De Waal, 2012; Preston and De Waal, 2002; Puga-Gonzalez et al., 2014), rodents (Bartal et al., 2011; Bartal et al., 2014; Panksepp, 2011) and birds (Edgar et al., 2013; Seed et al., 2007; Wascher et al., 2008). Physiological resonance between individuals has been proposed as a proximate mechanism of pro-sociality (Bowlby, 2008; Buchanan et al., 2012; De Waal, 2008; Eibl-Eibesfeldt, 1971/1974; Preston and De Waal, 2002), as it promotes coordination of behaviours in groups and parental care. Physiological resonance refers to the match in internal state that occurs when one individual perceives the state of another, and often results in a match in behaviours. State matching also known as emotional contagion – where the affective state of the subject generates the same affect in the perceiver – is well described in human behaviour (De Waal, 2008; De Waal and Ferrari, 2010; Hatfield and Cacioppo, 1994; Preston and De Waal, 2002). For instance, cortisol response is contagious between observers and stressed speakers (Buchanan et al., 2012) and observing one's distress results in aversive arousal (Preston and De Waal, 2002).

According to the de Waal's Russian doll model, empathy – the capacity to be affected and share the emotional state of another individual as a result of perceiving the other's situation – has several layers (De Waal, 2009; Gomez, 2009). Emotional contagion, the most basic form of empathic processes, constitutes the central mechanism of emotional empathy and the core of more complex processes of cognitive empathy, such as consolation and perspective-taking. In this framework, state matching between individuals may be present through much of the

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evolutionary history of vertebrates (De Waal, 2012; Langford et al., 2006; Panksepp, 2011; Preston and De Waal, 2002; Singer, 2006) and should thus be found in most of the existing social species (Dougall, 1920; Panksepp and Panksepp, 2013). Achieved through acoustic (Watson and Caldwell, 2010), chemical (Bredy and Barad, 2009) or visual cues (Langford et al., 2006), state matching is more likely to occur with increasing familiarity or relatedness between protagonists (Bartal et al., 2014; Fraser et al., 2008; Jeon et al., 2010; Langford et al., 2006; Romero et al., 2010; Seed et al., 2007). If state matching is an evolutionary shared characteristic of highly social vertebrates, it might also exist in non-mammalian species. Birds show complex cognitive abilities (Boogert et al., 2008; Boogert et al., 2011; Clayton and Dickinson, 1998; Prior et al., 2008; Schloegl et al., 2012) and many group-living species of birds demonstrate elaborate social relationships (Brown and Farabaugh, 1997; Clayton and Emery, 2007; Dally et al., 2006; Emery et al., 2007; Paz-y-Mino et al., 2004). Therefore, the strong social partnership of birds' monogamous pair-bond is a likely candidate for state matching or physiological resonance in a non-mammalian species.

In zebra finches, mates pair for life and are highly coordinated social partners (Mariette and Griffith, 2012, 2015; Zann, 1996); they collaborate in chick rearing, nest guarding and many other life-critical behaviours. In this species, call-based individual recognition is used for pair-bond maintenance (Elie et al., 2010; Vignal et al., 2004; Vignal et al., 2008). A recent study showed that elevation of plasma levels of corticosterone in male zebra finches leads to several modifications of the acoustic parameters of their vocalisations (Perez et al., 2012). We thus hypothesised that females can perceive the corticosterone increase in the calls of their mate and consequently express a physiological resonance and corresponding behavioural changes. Previous studies of state matching in birds measured the response of bystander birds after being exposed to an entire stressful situation affecting another individual, i.e. from the stimulus causing the emotion to the entire reaction of the distressed parties (Edgar et al., 2013; Wascher et al., 2008). To demonstrate physiological resonance through acoustic cues only, we designed an experiment measuring the behavioural and physiological response of female birds to a single sensory cue attesting one aspect of the physiological stress of their male partner: his calls. We used control calls (regular distance calls emitted during visual separation from the partner) and calls produced during corticosterone treatment (distance calls emitted during visual separation from the partner, and after oral administration of exogenous corticosterone) of males as playback stimuli on their female partners. Each male was submitted to both treatments. To investigate if state matching could be achieved between any birds irrespective of their relationship, we also used control calls and calls produced during corticosterone treatment of unfamiliar males as playback stimuli on paired females. If state matching occurs specifically between mates, we expected females to show a physiological resonance and behavioural changes after hearing the calls produced during corticosterone treatment by their mate only. We thus monitored plasma levels of corticosterone after each playback session and followed the acoustic behaviour of the females during and directly after the playback.

To conclude on the existence of state matching in females, we ruled out alternative explanations. We first verified that females' responses were not simply due to acoustical features that are shared between all calls produced during corticosterone treatment (graded signal), but really to their specific value as calls produced during corticosterone treatment by the mate. To do so we checked whether females' corticosterone levels after the playback were affected by acoustic parameters of male calls used as stimuli. Then, we verified that females hearing calls produced by their mate during corticosterone treatment were not simply confused by an unexpected version of their mate call and thus failed to recognise their mate. To consider this possibility of failure of the call-based individual recognition system, we used a discrimination task in a conditioning paradigm: we specifically tested if females trained to discriminate caller identities would benefit from first learning the identity cues on control calls when successively tested on the calls produced during corticosterone treatment by the same individuals (Emotional Shift), compared to females that would be tested successively on two different sets of callers (Identity Shift). In both conditions, the calls used in successive days were acoustically distinct but the nature of these changes was different. We predicted that if the acoustical changes provoked by the corticosterone treatment did not alter the recognition of individualised cues, then females undergoing the Emotional Shift would perform better than females under the Identity Shift.

Methods

All statistical analyses were implemented in R (R Development Core Team, www.r-project.org, 2007).

Experiment 1: study of the perception by females of the physiological state of males using acoustic cues

Subjects and housing conditions

Subjects were 19 pairs of adult domestic zebra finches (*Taeniopygia guttata*) bred in our colony (ENES lab, University of Saint-Etienne). Pairs were obtained as follows: males and females freely formed pair-bonds in an aviary (dimensions: 200 × 200 × 300 cm) at least two months prior to the experiments. Identified pairs were then caught and kept in individual cages (dimensions: 24 × 29 × 39 cm) in the same room at least one month prior to the experiments.

Birds were maintained at a constant temperature of 22–24 °C and with a 14:10 light–dark cycle. Food and water were provided ad libitum except stated otherwise, with a supplement of salad once a week.

Separation protocol

We developed a protocol to separate the partners of a pair without handling to avoid unnecessary stress. The day before the experiment at 18:00, the partners of one pair were moved to the experimental room and placed together in a home-made separation cage (dimensions: 28.5 cm × 23 cm × 35 cm). This cage was provided with water and seeds ad libitum except stated otherwise. In the morning of the experiment, partners were physically separated without handling using two wood partitions placed in the middle of the experimental cage. One of the newly independent parts was then moved in a different room, rendering the partners physically, visually and acoustically separated from each other. The partners of each pair used in this study were separated six times using this device, i.e. twice to record the acoustic stimuli and four times to run the playback experiment (see below).

Recording of acoustic stimuli

Recordings were done using the separation protocol detailed above. On the day before the experiment, the separation cage was provided with only 3 g of seeds to ensure that males would be motivated to eat the seeds presented on the following morning (see below). On the day of the experiment, the female of the pair was moved to another room (doors closed to ensure social isolation) and the male was fed with either: a) 0.3 g of seeds containing exogenous corticosterone (12.5 μg i.e. 50 μL of a 0.25 mg/mL solution; Sigma Aldrich ref.: 27,840) so as to provoke an increase in plasma corticosterone (corticosterone treatment) (protocol previously described (Perez et al., 2012)) or b) 0.3 g of seeds alone (control). For both treatments (seeds alone or seeds with corticosterone in random order), birds were physically, visually and acoustically separated for ten minutes. After these ten minutes of complete separation (and thus social isolation), doors from the two rooms were opened, allowing the birds to be in acoustic contact only while still visually separated. This condition generated interactive calling between partners. Thus, this protocol allowed the recording of two types of calls: a) distance calls produced during corticosterone treatment (MATE CORT, UNFAMILIAR CORT) were recorded while the male was experiencing both visual and physical separation from the

partner and oral administration of exogenous corticosterone, b) regular distance calls (MATE CONTROL, FAMILIAR CONTROL, UNFAMILIAR CONTROL) were recorded while the male was experiencing visual and physical separation from the partner only (protocol previously described (Perez et al., 2012)).

Distance calls are one of many calls in the vocal repertoire of the zebra finch (Zann, 1996). Birds produce these calls to maintain or establish a contact at a distance with conspecifics and are easily elicited in mates upon separation and reunion (Zann, 1996). Note that this contact call is not exclusively uttered when birds experienced distressed situation (Zann, 1996). We previously showed that oral administration of corticosterone induces several modifications of behaviour also observed in social isolation (Perez et al., 2012, Fig. 1), a powerful stressor in zebra finches that triggers elevation of plasmatic corticosterone levels through activation of the Hypothalamic–pituitary–adrenal axis (Banerjee and Adkins-Regan, 2011; Perez et al., 2012; Remage-Healey et al., 2003). Even if administration of exogenous corticosterone induced an elevation of plasma corticosterone 10 times higher than social isolation, calls of corticosterone-treated males show similar changes in acoustic structure as calls produced in social isolation, i.e. higher frequencies (mean, dominant and fundamental frequencies), and thus represent a controlled behavioural output of male stress (Perez et al., 2012).

Preparation of acoustic stimuli

Male distance calls were extracted from the recordings and analysed as previously described (Perez et al., 2012; Vignal et al., 2008). Two sets of calls were selected for each male:

a) calls produced during corticosterone treatment (MATE CORT, UNFAMILIAR CORT): the ten calls with the highest mean frequency emitted after exogenous corticosterone administration (Mean = 3970 ± 41 Hz).

b) control calls (MATE CONTROL, FAMILIAR CONTROL, UNFAMILIAR CONTROL): the ten calls with the lowest mean frequency emitted in control condition (Mean = 3211 ± 49 Hz).

As previously demonstrated (Perez et al., 2012), distance calls emitted during elevated plasma concentrations of corticosterone show higher frequencies than control distance calls. Among acoustic parameters, the mean frequency was highly variable ranging from 2335 to 4749 Hz in control condition, and from 2643 to 4815 Hz in the CORT condition, but globally lower in control than in CORT condition for one given individual (Perez et al., 2012). Extreme samples of this variation were chosen here to maximise the difference between playback conditions.

Playback experiment

To test if females perceive the level of stress in their mate's distance calls, we used a playback experiment in which females heard their mate's control calls or calls produced by their mate during corticosterone treatment. In a first set of playback sessions, each female (n = 10) underwent four playback tests in random order, separated at least by a week. The female was stimulated by only one stimulus type per session: MATE CONTROL (the ten lower-frequency control calls of her mate), MATE CORT (the ten higher-frequency calls produced during corticosterone treatment by her mate), FAMILIAR CONTROL (the ten lower-frequency control calls of a familiar male i.e. the male of another pair, housed in the same colony room) to test for the level of familiarity and SILENCE (no playback) as a control of the effect of isolation on the female's physiology and behaviour.

To explore if the effects of calls produced during corticosterone treatment on females are specific to their mate or if the calls of any stressed male can impact females' behaviour, we ran a second set of playback sessions with new females: each female (n = 9) underwent four playback tests in random order, separated at least by a week. The female was stimulated by only one stimulus type per session: MATE CONTROL (the ten lower-frequency control calls of her mate), UNFAMILIAR CONTROL (the ten lower-frequency control calls of an unfamiliar male i.e. a male never housed in the same colony room as the subject), UNFAMILIAR CORT (the ten higher-frequency calls produced during corticosterone treatment by another unfamiliar male so as to avoid potential familiarity appearing between two successive experiments) and SILENCE (no playback). Two sets of females were preferred instead of one to run the two sets of playback sessions so as to avoid recurring stress due to handling, blood sampling and isolation (for more details, see section Ethics). As the two sets of females both underwent MATE CONTROL and SILENCE stimuli, the results of the two sets could be compared (see below).

Each playback test was conducted as follows. Using the separation protocol detailed above, the male of the pair was moved to another room and the female was completely isolated from him. After a 5-min resting period in the experimental room, the female was stimulated every ten minutes for one hour (i.e. six times). Each stimulation consisted of the playback of ten distance calls from the same male individual recorded in one of the above conditions (5-s stimulation, 10 calls

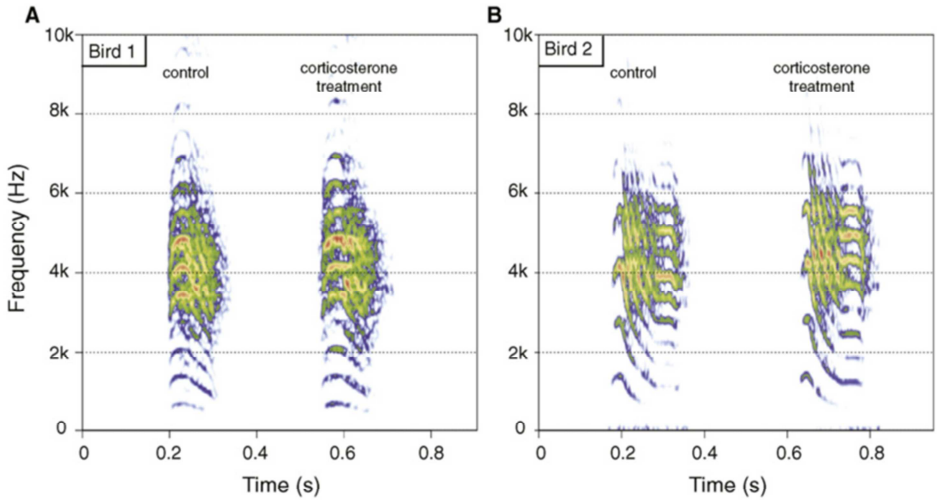

Fig. 1. Effect of corticosterone on male calls. Spectrograms of the calls of two male birds in the two experimental conditions (Control and Corticosterone treatment). Note the clear individual signatures in both conditions, the attenuation in the frequency band around 3 kHz and the enhancement of the energy in the frequency band between 4 and 5 kHz in the call produced during corticosterone treatment relative to the control call. These variations are consistent with a shift toward higher frequencies in the spectral envelope of the calls produced during corticosterone treatment. Spectrograms are calculated using the Seewave package implemented in R (overlap = 90%, window length = 16 ms, colour scale = 40 dB).

emitted in 5 sequences of 2 successive calls, with mean rate = 2 calls per second). At the end of the test (1 h), the female was caught for blood sampling. Good quality blood sampling failed in two females of the first set of experiments, so the sample size of hormone data is $n = 17$ ($n = 8$ and $n = 9$). A preliminary study of the effect of partner separation on plasma levels of corticosterone (Appendices A and B) showed that 1 h of separation significantly elevated stress level in females while plasma levels of corticosterone returned to baseline after 2 h of separation. To maximise the potential effect of the playback on females' plasma levels of corticosterone as well as to limit the duration of stressful partner separation, which could jeopardise the pair-bond over repeated playback sessions, we decided to perform the playback experiment during the first hour of separation, i.e. during the ascendant phase of plasma levels of corticosterone.

Blood sample collection and hormone assay

All blood samples (<150 μl) were obtained by puncturing the alar vein with a 25-gauge needle within three minutes so as to measure the corticosterone concentration induced by separation from the partner and the playback and not the corticosterone release due to handling (Romero and Reed, 2008). Blood was collected in heparinised tips, then plasma was separated under centrifugation (3800 g, 15 min) and stored at −20 °C for subsequent hormone assay. No bird was bled more than once per week to ensure that they had sufficiently replenished their blood volume between bleeds and hypothalamic–pituitary–adrenal axis function had recovered from previous sampling (Romero and Reed, 2008). Because circulating concentration of endogenous corticosterone shows circadian variations in many bird species with a peak just before the active period and a rapid decrease during the first hours of activity, all experiments were performed between 10:00 and 11:00, i.e. between 3 and 4 h after daylight (Tarlow et al., 2003). As previously described (Perez et al., 2012), hormone assays were performed using the ELISA method (Kit Corticosterone EIA, Cayman, #5,000,561) according to the manufacturer's recommendations. All samples were run in duplicate in four different assays. The detection limit was 0.2 ng/mL, intra-assay coefficient of variation was 0.058, and inter-assay coefficient of variation was 0.146.

Hormone data were analysed as follows: because corticosterone levels across birds did not follow a normal distribution, we used non-parametric statistics to test for significant differences. The two different sets of females were first analysed separately. Differences across playback conditions were examined using a Friedman test, the non-parametric equivalent of the repeated measures ANOVA. The results of this non-parametric statistical test are identical for raw values or transformed values (e.g. log) of the corticosterone concentrations since it only depends on the relative rank of the measured values. When the Friedman test showed significant differences across playback conditions, we performed a post-hoc analysis for significant pairwise comparisons using the non-parametric Wilcoxon Signed Rank test. Here we used the logarithm of the raw data to rank the observed pairwise differences of corticosterone concentrations in terms of ratios instead of differences, because $\log(A) - \log(B) = \log(A/B)$. Log-transformation is classically used for its pseudo-scaling effect (differences between large and small values in the data set are reduced after log-transformation), because it allows investigating within-subjects effect of a treatment on biological parameter showing high inter-individual variation in both control/basal levels and in response to stressors, as it is the case for corticosterone concentrations (Cockrem and Silverin, 2002; Cockrem et al., 2009; Cockrem, 2013; Wada et al., 2008).

As the two sets of playback sessions were performed on two different groups of females, we performed a second analysis to verify that the change of corticosterone plasma level in response to playback was not different between the two groups. To do so, the two groups were compared using data of the mate's control calls condition (MATE CONTROL) i.e. the acoustic stimulus that the two sets had in common. As significant individual variation in adrenocortical response has been described in this species (Evans et al., 2006; Wada et al., 2008), here we normalised values of corticosterone levels using a ratio: each value in MATE CONTROL was divided by the value in SILENCE condition. The two groups of females were then compared using a Mann–Whitney–Wilcoxon test for independent samples.

Analysis of the behavioural response

During the whole experiment, the behaviour of the female was monitored using a webcam (Hercules® Webcam Deluxe) and a microphone (Sennheiser MD42) located 50 cm above the subject and connected to a recorder (Marantz PMD 670, sampling rate: 44.1 kHz). The vocal activity (number of calls) of the female was scored on acoustic recordings of playback sessions by an observer blind to experimental conditions (TC). Recordings were scored in random order regarding conditions and identity of the birds using Etholog software (created by Ottoni). Two different types of vocalisations were emitted by the female during the experiment: distance calls commonly used by zebra finches when they lose visual contact and tet calls i.e. short calls commonly used during close interactions (Zann, 1996). These calls were pooled in one parameter of calling activity (see Appendices C and D to listen to the vocalisations emitted by a female in response to the playback of the control calls of its mate, and see the corresponding spectrogram).

Each playback session was divided into three periods of time: a five-second playback period (5-s stimulation, 10 calls emitted in 5 sequences of 2 successive calls, with mean rate = 2 calls per second, repeated six times per session), followed by a one-minute post-playback period (repeated six times), followed by a 8 min 55 s inter-playback period (repeated only five times). Vocal activity was quantified both during playback and during post-playback periods and was analysed as a binary variable (0: no response, 1: response). These data were analysed using a generalised linear mixed effect model fitted using a binomial distribution and the logit function (lmer, lme4 package, R). The effect of the playback condition (6 levels) on the vocal activity of the female was assessed with the female identity (19 levels) and the playback set (2 levels) as random factors. Multiple comparisons of means were performed on the models using the glht function from the multcomp package using Tukey contrasts.

Analysis of acoustic stimuli for regression analysis between call acoustic structure and female playback-induced corticosterone concentrations

All the distance calls selected as stimuli for the playback tests ($n = 380$) were analysed using the Seewave package (rug.mnhn.fr/seewave/) in R. Each call was first bandpass filtered (0.25–10 kHz, encompassing the range of maximum sensitivity of hearing in zebra finches). Then thirteen acoustic parameters were measured.

- the duration (in seconds) using 'timer' function, which computes the energy from the signal envelope and uses the peak maximum as an energy reference to define the start and the end of the call (threshold: 6%).
- the call dominant frequency and its dispersion (in Hertz). The frequency of highest amplitude is first assessed (via the 'dfreq' function) on time bins using a window length of 512 samples (11.6 ms) and the series of values obtained over the duration of the call is used to calculate the mean (the dominant frequency) and the standard deviation (the dispersion of the dominant frequency).
- the call fundamental frequency and its dispersion (in Hertz). The fundamental frequency values are tracks of the fundamental frequency through a short-term cepstral transform (obtained via the 'fund' function). The series of values obtained over the duration of the call is used to calculate the mean (the fundamental frequency) and the standard deviation (the dispersion of the fundamental frequency).
- eight parameters of the call spectrum (using 'specprop' function): the mean, the median, the mode, the first (25%) and third (75%)

quartiles, the skewness, the kurtosis and the spectral flatness.

As ten different calls of the same individual were used as stimuli in each playback test, the average value of each acoustic parameter was calculated on these ten calls and this average value was used to investigate the relationship with the female's corticosterone concentration. To examine the link between the presentations of calls produced during corticosterone treatment and the physiological response of females, we examined whether the corticosterone concentrations measured in females after playback was affected by the acoustic parameters of the calls used as stimuli. This relationship was analysed for each acoustic parameter using a linear mixed effect model (lmer, lme4 package, R) with the log of the concentration of corticosterone as a response variable, the acoustic parameter, the status of the caller (2 levels: mate versus non-mate) and their interaction being set as predictor variables. The female identity (17 levels), the playback set (2 levels) and the stimulus identity (23 levels) were specified random factors.

To account for the multidimensional nature of the stimuli, the same model was also run on the first principal component PC1 of a Principal Component Analysis (PCA, dudi.pca function, ade4 library, R) on all acoustic parameters, and on the first discriminant function DF1 of the DFA estimated to separate calls produced during corticosterone treatment from control calls (lda function, MASS package, R). The statistical significance of the effect of each parameter was calculated using a likelihood ratio test (Anova, car package, R).

Experiment 2: study of the recognition of male emitters by females based on control calls and calls produced during corticosterone treatment

One way to control for the possibility that females are simply confused by an unexpected version of their mate call and thus fail to recognise their mate is to investigate whether control calls and calls produced during corticosterone treatment by the same male are recognised as calls from the same individual. We explore that question using an operant conditioning paradigm where unpaired females were assigned to a caller discrimination task.

Subjects and housing conditions

Subjects were seven unpaired females of adult domestic zebra finches bred in our colony (Theunissen Lab, University of California, Berkeley) and were kept in unisex cages. Birds were maintained at a constant temperature of 22–24 °C and with a 12:12 light–dark cycle. For the duration of the shaping and testing days in the operant chamber, females were housed as pairs of females in individual cages in the colony room and were only isolated in the soundproof chamber for the duration of the shaping and testing (5 to 6 h) every experimental day. During this period, females were also fasted: they were given daily only 1.5 g of mixed seeds for finches per individual at the end of each day.

Protocol outline

Females were tested in a computer-assisted test chamber where they had to peck a pad to induce the playback of calls from two previously unknown males. Calls from one of the individuals were infrequent (20%) and rewarded by seeds at the end of the sequences of calls (Re Individual) while calls from the second individual were probable (80%) but not rewarded (NoRe Individual). After triggering the playback, the female could either wait until the end of the playback (6 s) or interrupt it by pecking again to trigger another stimulus. Females were encouraged to interrupt the NoRe Individual to obtain more Re Individual stimuli and increase their access to seeds. Discrimination between the stimuli was assessed by comparing the number of interruptions of the NoRe Individual to those obtained for the Re Individual. In contrast to other conditioning paradigms used to test discrimination capacities of birds (Cynx, 1990; Gess et al., 2011), this paradigm avoids using punishment for incorrect responses and allows for quick learning (Appendix E).

Chamber apparatus

Fasting females were tested in a computer-assisted extra tall modular test chamber (Med Associates Inc., St. Albans, VT 05478, USA; size: 30.5 cm × 24.1 cm × 29.2 cm) placed in a soundproof booth (Acoustic Systems). Acoustic stimuli were broadcasted by the computer using Matlab (The MathWorks Inc., Natick, Massachusetts) connected to an amplifier (Technics, Matsushita Electronices, SA-EX140) and a loudspeaker (Bose Model 141) placed at 20 cm from the test chamber. The behaviour of the bird was monitored both by the computer and by a webcam (Logitech) placed inside the soundproof booth.

Code and Procedure

A homemade code written in Matlab allowed the bird to run three sessions of 30 min each per day with a minimum inter-session delay of 90 min. The code detected the beginning of each session (when the bird pecked the key pad for the first time) and ended the session 30 min later. During each session, it monitored the pecking behaviour of the bird by recording the status of the key pad every 0.1 s and broadcasted a different 6 s sequence of calls each time the key pad was depressed by the bird. Interruption of stimuli was permitted: the bird could peck while the computer was playing a 6 s stimulus and promptly get another stimulus before the end of the 6 s. The probability to get a Re stimulus (rewarded by a 15 s access to seeds) was set at 20%. Note that the reward was only accessible to the bird at the end of the Re stimulus and that interruption of the Re stimulus would cancel the access to the reward. Each acoustic stimulus consisted of six band-pass filtered (0.25–12 kHz) distance calls of the same individual, randomly assigned within a 6 s window. The average inter-call interval was 995 ± 7.2 ms (mean ± 2SE). Before each trial, the computer was randomly constructing 80 Re stimuli and 320 NoRe stimuli using a call bank of 20 different calls per individual. Each stimulus was only played once during the session.

Shaping

Birds were shaped in two days. On the first day the female first had a period to acclimate to the cage and find the rewarding feeder. Once it had found the feeder and had three 10 s opportunities to eat, the female could run a first trial with the probability to get a Re stimulus set at 1. After several successful sequences of key-activation and feeding at the feeder (typically 5–10), the female could run a long trial (1 to 3 h) with the probability to get a Re stimulus set between 0.5 and 0.9 depending on the female motivation to activate the key (rate of activation when the rate of Re stimulus was set to 1). During this trial, the females learn two associations: 1) that pecking the key pad triggers a vocalisation, 2) that one of the two vocalisations is followed by a brief access to the feeder. On the second day, three regular sessions of 30 min were conducted with a probability to get the Re stimulus of 0.2 (Appendix E), during which the bird was expected to learn to interrupt the stimuli. A subject was considered ready to run the experiment if the female interrupted at least 20% of the NoRe stimuli and if the difference in percentage of interruption between NoRe and Re stimuli (NoRe − Re) was at least 20%. Stimuli used for shaping were two 6 s sequences of songs recorded from two different males from our colony.

Random test

As a control for any arbitrary preference in this discrimination task, the performance of females at the discrimination task was estimated (Appendix E) when 20% of the stimuli were rewarded irrespective of the stimulus being played. Stimuli used for the random test were the two songs used for shaping.

Conditioning

Two protocols were conducted: Identity Shift and Emotional Shift. In both protocols, females ($n = 7$) run three sessions per day and were tested on the first day with control distance calls of previously unknown males (four pairs of individuals were used for this experiment). On the second day, three females were re-tested with control distance calls of new pairs of previously unknown males (Identity Shift protocol), while four females were re-tested with calls produced during corticosterone treatment by the same males as the previous day (Emotional Shift protocol). We thus examined whether females would benefit from having learned the identity cues on control calls when tested with calls produced during corticosterone treatment by the same individuals. Our predictions were that if the change in the acoustic structure of the calls due to corticosterone treatment was deteriorating all individualised acoustic cues of the calls, preventing recognition by females, then females undergoing the Emotional Shift would not benefit from the training received in the first day and would show similar performances to females under the Identity Shift. Conversely, if females did benefit from the first day of test with the control calls, then corticosterone treatment of callers is unlikely to disturb the recognition of all individualised acoustic cues.

Statistical analysis of the ability of females to individualise both control calls and calls produced during corticosterone treatment

The effects of the type of stimulus (Re or NoRe) and of the type of distance calls (control calls or calls produced during corticosterone treatment) on the number of interruptions performed by females were analysed using logistic regression with the female identity nested in the day number (first, second) as random factors. Because calls produced during corticosterone treatment were only used on the second day of the experiment, we confirmed the results of the previous regression by performing the same logistic regression using the dataset restricted to values obtained for the second day and using only the female identity as a random factor. Results obtained were similar: the effect of the type of stimulus was highly significant in both regressions ($P < 0.001$, see Table J of Appendix F) and increases in odds of interrupting (the ratio of the probability of interrupting a stimulus and the probability of not interrupting) were of the same range (2.5 [1.9–3.2] for control calls and 7.1 [5.9–8.5] for calls produced during corticosterone treatment).

Statistical analysis of the ability of females to categorise control calls and calls produced during corticosterone treatment by the same individual as calls from the same bird

The combined effects of the type of stimulus (Re or NoRe), of the protocol (Identity Shift versus Emotional Shift) and of the day number (first, second) on the number of interruptions performed by females were analysed using logistic regression with the female identity nested in the type of protocol (Identity Shift versus Emotional Shift) and type of stimulus (Re or NoRe) as random factors.

To evaluate the difficulty of the discrimination task in each protocol (Emotional or Identity Shift) and include that parameter in the model, we calculated the average acoustic distance of calls between the first and the second day of the experiment. The average acoustic distance of stimuli is a measure of similarity of calls between the first and second day. It was calculated as follows: first, the centroid of each set of 20 calls from the same caller in a given treatment (Corticosterone or Control) was calculated in the three dimensional space defined by the three first discriminant functions of the DFA designed to discriminate calls' individual identity in control condition based on the 11 acoustic features described above; then, for each subject of the operant conditioning experiment, the acoustic distance of Re stims on the one hand and of NoRe stims on the other hand between the first and second day was calculated using the Euclidian distance between the centroids of these sets of calls; then these two distances per subject were averaged to give an average distance of stimuli per subject. Because the similarity between calls in the first day and calls in the second day was different in the Emotional Shift protocol (average acoustic distance: 2.5 ± 1.4; mean $\pm$ sd over subjects) from that of the Identity Shift protocol (average acoustic distance: 4.6 ± 0.7), we confirmed the results of the previous regression predicting the number of interruptions performed by females by using the same logistic regression but adding two fixed effects: 1) the interaction between the day number (first or second) and the z-scored average acoustic distance of stimuli; 2) the triple interaction between the type of stimulus, the day number and the z-scored average acoustic distance between stimuli. The z-scored average acoustic distance of stimuli was obtained by z-scoring across subjects the average distance of stimuli per subject.

For all the regressions, effect sizes were quantified by calculating the increase in odds of interrupting (the ratio of the probability of interrupting a stimulus and the probability of not interrupting) (Parzen et al., 2002). The statistical significance of the effect of each parameter was calculated using a likelihood ratio test on nested logistic models (lmer, lme4 package and Anova, car package in R). All values displayed are means $\pm$ s.e.m. except variations in odds of interrupting which are given with their confidence intervals [5%–95%]. All significant differences are reported for $P \leq 0.05$.

Ethics

Experiment 1 was performed under the authorisation no. 42-218-090-38SV09, experiment 2 was approved by the Animal Care and Use Committee at the University of California at Berkeley.

Isolation and separation from the partner are known to increase plasma level of corticosterone in the zebra finch (Perez et al., 2012, Remage-Healey et al., 2003). Protocols were thus performed so as to minimise unnecessary stress. For the playback experiments, (experiment 1) birds were separated for less than 30 min when recording the acoustic stimuli, and no more than 1 h 30 min when running the playback experiment. After each separation, birds were immediately put back together in their home cage and monitored every hour for 2 h to verify their health and behaviour. To avoid health issues related to blood sampling (Romero and Reed, 2008), no bird was bled more than once per week. Repetitive stressful situations can induce chronic stress known to have deleterious effects (Buchanan, 2000); the playback experiments were thus run on two different sets of females so as to avoid recurring separation from the partner and recurring blood sampling.

Several hours of complete fasting or food deprivation can also have deleterious effects on plasmatic levels of steroids and body mass, with parts of the effects quickly normalised with refeeding (Fokidis et al., 2013). In experiment 1, males were fasted so as to be motivated to eat the seeds presented to them, but they were only fasted for a couple of hours before the beginning of the experiment, as they received enough food the day before the experiment to eat before the night. In experiment 2, females were food deprived to increase their motivation to run the test. Nevertheless, they received 1.5 g of seeds every day. This food supplement added to what they were gaining during the test as rewards ensured that they would get an average of 3 g. From pilot measurements, 3–3.5 g is the average amount of food a zebra finch would eat per day when given ad libitum access.

Results

Females perceive the physiological state of their mate using acoustic cues only.

Females from the first set of playback sessions (four conditions: MATE CORT, MATE CONTROL, FAMILIAR CONTROL, SILENCE) had significantly higher plasma corticosterone concentrations after hearing the calls produced by their mate during corticosterone treatment than after hearing the control calls of their mate (Friedman $\chi^2 = 8.55$, $df = 3$, $P = 0.036$, posthoc Wilcoxon signed rank test MATE CORT versus

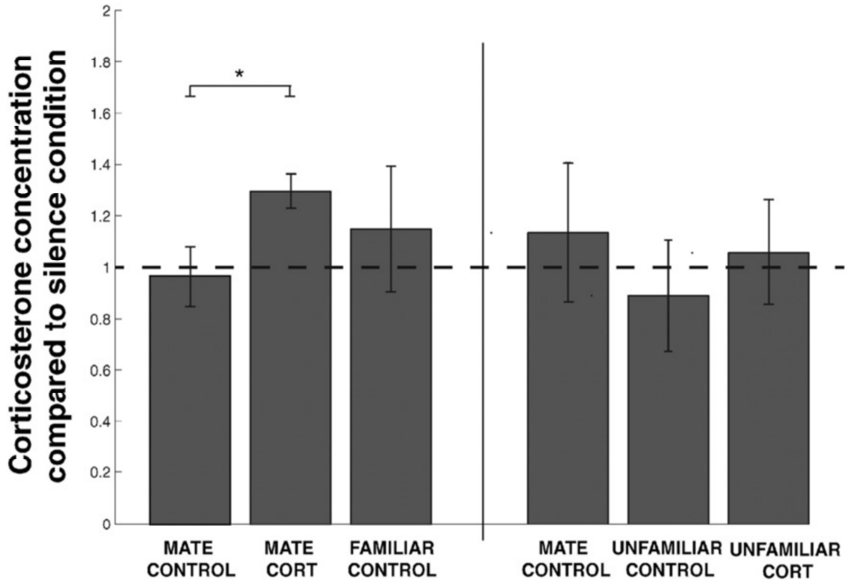

Fig. 2. Effect of the calls produced during corticosterone treatment by male zebra finches on corticosterone plasma concentrations of their female partner. For display purpose only, raw data are normalised by dividing by individual levels during the silence condition. Friedman statistical tests were performed on raw data. Boxes are means and error bars are standard errors. * = $P < 0.05$. First set of playback sessions (left): $n = 8$; Second set of playback sessions (right): $n = 9$.

MATE CONTROL $n = 8$ and V = 3, $P = 0.039$) or than following the silence condition (posthoc Wilcoxon signed rank test MATE CORT versus SILENCE $n = 8$ and V = 36, $P = 0.008$). Seven out of eight females had higher corticosterone concentrations after hearing the calls produced by their mate during corticosterone treatment than after the control calls of their mate and this increase was on average of 29.6% compared to levels in the silence condition (Fig. 2). Corticosterone concentrations after the control calls of a familiar male (FAMILIAR CONTROL) were not significantly different from the other conditions (any comparison $P > 0.5$, Fig. 2 and Appendix F).

The effect of the calls produced during corticosterone treatment was only observed for mate calls as none of the stimuli used on the second group of females to investigate the effect of familiarity elicited significant differences in plasma corticosterone concentrations (four conditions: UNFAMILIAR CORT, UNFAMILIAR CONTROL, MATE CONTROL, SILENCE, Friedman $\chi^2 = 3.2667$, df = 3, n = 9, $P = 0.352$, Fig. 2 and Appendix F). The two groups of females did not differ in the normalised concentration of corticosterone in response to their mate's control calls (Wilcoxon n1 = 9, n2 = 8, W1 = 37, $P = 0.963$, for dataset see Appendix G).

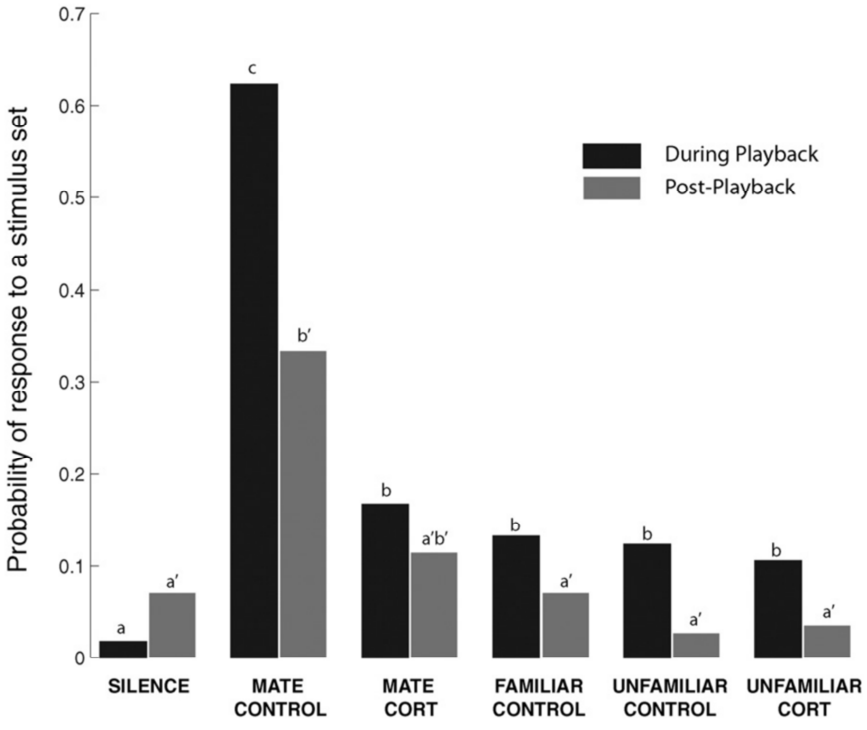

Fig. 3. Effect of the calls produced during corticosterone treatment by male zebra finches on the behaviour of their female partner. Probability of vocal response to one stimulation during (dark bars) and after the playback periods (light bars) (n = 19, six stimulations per test). Different letters indicate a significant difference between pairs at the $P < 0.05$ level.

During the playback period of any stimulus, the probability of calling was higher than during silence (multiple comparisons of means using Tukey contrasts, all differences with SILENCE significant at $P \leq 0.01$, Fig. 3 and Appendix F for complete models and posthoc comparisons). Thus, call playbacks were salient to females. Among stimuli, the control calls of the mate had a significantly higher probability of response than any other stimulus (multiple comparisons of means using Tukey contrasts, all differences with MATE CONTROL significant at $P \leq 0.01$, Fig. 3 and Appendix F for complete models and posthoc comparisons). During the one-minute post-playback period, the control calls of the mate were also the only stimuli to elicit vocal responses above basal levels (multiple comparisons of means using Tukey contrasts, all differences related to MATE CONTROL $P \leq 0.01$ except for MATE CONTROL versus MATE CORT $P = 0.28$; any other comparison $P > 0.05$; Fig. 3, Appendix F; for dataset see Appendix H). Thus, the mate's control calls elicited the strongest vocal response in females while the calls produced by the mate during corticosterone treatment were less stimulating for female vocal behaviour than mate's control calls.

Females' physiological responses differ between mate and non-mate calls but are also affected directly by some calls' acoustic features.

The females' corticosterone concentrations significantly increased with some acoustic features of the calls used as stimuli in the playback experiment: the fundamental frequency (LMM: $\chi^2(1) = 4.48$, $P = 0.034$, slope estimate ± std. error: 0.97 ± 0.57 in units of log(Cort)/kHz; for more details on statistics, see Appendix F and for dataset see Appendix I) and the dispersion of the dominant frequency (LMM: $\chi^2(1) = 4.13$, $P = 0.042$, slope estimate ± std. error: 0.46 ± 0.22). Among the two linear combinations of the 13 acoustic parameters measured on calls used as stimuli (PC1 obtained from a PCA that explained most of the variance and DF1 obtained from a DFA that best discriminates calls produced during corticosterone treatment from control calls), only DF1 yielded an acoustic measure that was predictive of corticosterone concentrations in females (LMM: $\chi^2(1) = 9.09$, $P = 0.003$, slope estimate ± std. error: −0.09 ± 0.03). Finally, corticosterone concentrations in females were significantly affected by the interaction between the dispersion of the dominant frequency and the status of the caller (mate versus non-mate) (LMM: $\chi^2(1) = 4.34$, $P = 0.037$): here, the slope of the relationship is effectively zero for mate (−0.06 ± 0.34) and positive for non-mate (0.93 ± 0.32).

Corticosterone treatment of calls is less of a challenge than identity change for females engaged in an individual discrimination task.

Irrespective of the type of calls used (control calls or calls produced during corticosterone treatment), females interrupted more the NoRe than the Re Individual ($\chi^2 = 159.4$, df = 1, $P < 0.001$, Fig. 4A, Appendices J and F). Thus, females could successfully individualise both types of calls.

Females undergoing the Emotional Shift performed significantly better than females under the Identity Shift (significant triple interaction $\chi^2 = 6.31$, df = 1, $P = 0.012$, Appendix F): on the second day, females under the Emotional Shift maintained a high rate of successes (49.4 ± 11.3% interruptions of NoRe on the second day versus 50.2 ± 9 on the first day) and decreased the percentage of errors compared to the first day (13.7 ± 8.1% interruptions of Re on the second day versus 23.4 ± 9.9% on the first day, Figs. 4A, 5 and Appendix J). This significant increase in performances from the first to the second day (significant interaction of day and Individual Re/NoRe, $\chi^2 = 5.66$, df = 1, $P < 0.02$) translated to a 61% decrease in the odds of interrupting the Re Individual (ratio of the probabilities of interrupting and not interrupting) (Fig. 4B; value [5%–95%] confidence interval: −61% [−74.8 to −39.6%]). On the contrary, females under the Identity Shift slightly decreased the percentage of successes between the first and the second day (47.32 ± 7.2% versus 40.7 ± 9.8%) and maintained the same level of errors (20.1 ± 8.6% versus 19.8 ± 9.8%) (Figs. 4A, 5 and Appendix J). This non-significant decrease in performances (non-significant interaction of day and Individual Re/NoRe, $\chi^2 = 0.31$, df = 1, $P = 0.58$) translated to a 19.9% decrease in the odds of interrupting the NoRe Individual (Fig. 4B; −19.9% [−48.2–24%], for dataset see Appendix K). Finally, the recognition performance demonstrated by females in the Emotional Shift protocol could not only be explained by the acoustic similarity of the calls they were tested on. Although the similarity of the calls between the first and the second day was higher in the Emotional Shift than in the Identity Shift protocol (average acoustic distance: Emotional Shift, 2.5 ± 1.4; Identity Shift, 4.6 ± 0.7; mean ± sd), females undergoing the Emotional Shift performed significantly better than what would be expected solely from acoustical similarities between the stimuli used in the first and second days (significant triple interaction: $\chi^2 = 13.34$, df = 1, $P < 0.001$).

Discussion

In this study, we show that the plasma corticosterone concentration of female zebra finches is affected by the acoustic characteristics of male

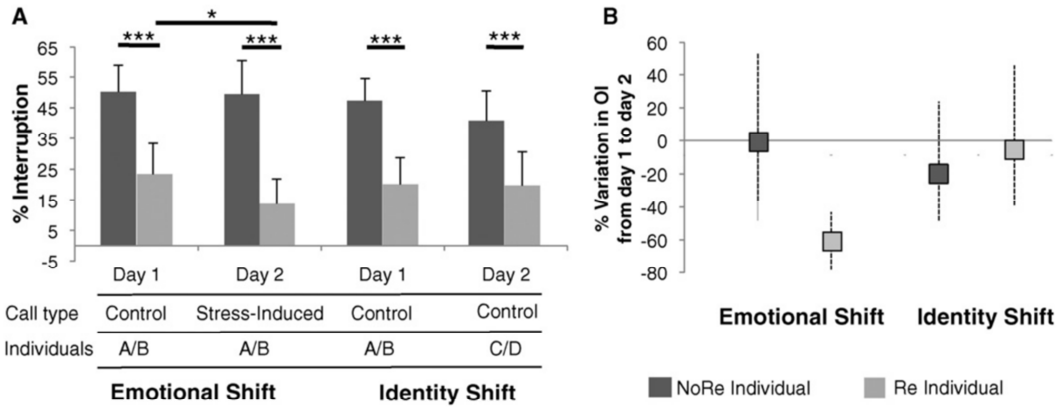

Fig. 4. Recognition of male individuals by females based on control calls and calls produced during corticosterone treatment. Interruption behaviour of females depending on the type of emitter (Re or NoRe) and the type of protocol (Identity Shift, i.e. tested with different pairs of individuals on the first day and the second day, $n = 3$; or Emotional Shift, i.e. tested with the same pair of individuals using calls produced during corticosterone treatment on the second day, $n = 4$). A) Percentage of call sequences interrupted by females (mean ± s.e.m). *** = $P < 0.001$, * = $P = 0.012$. B) Percentage of change in the Odds of Interrupting (OI) between the first and the second day (value [5%–95%] confidence interval).

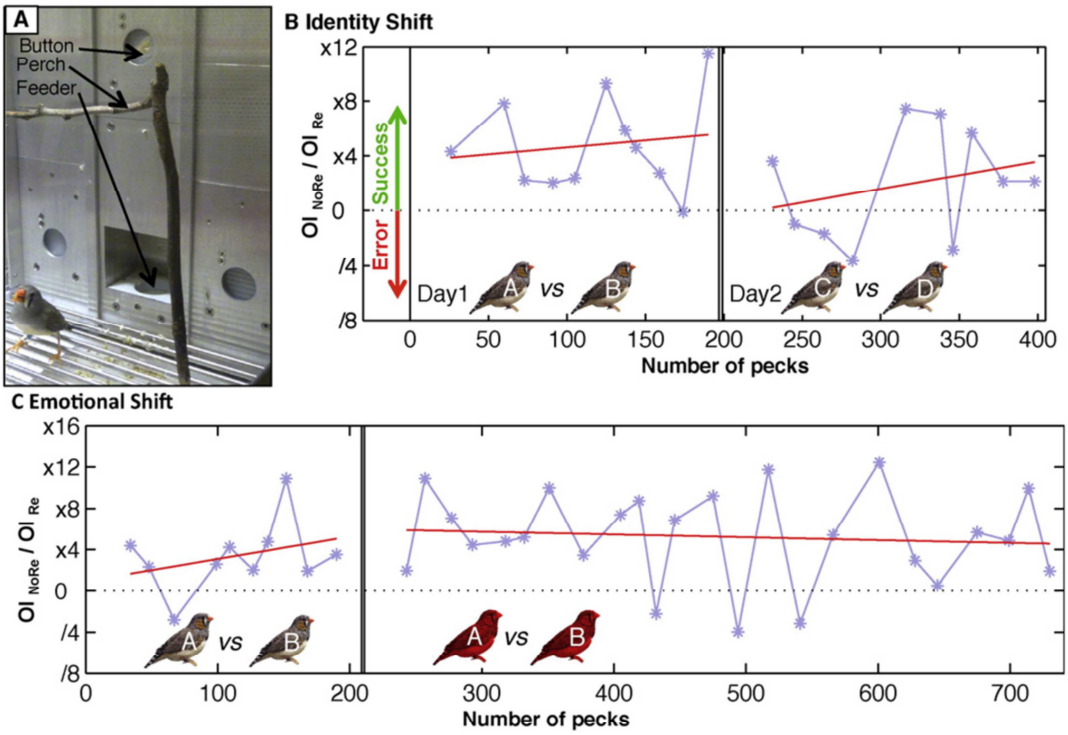

Fig. 5. Recognition by females of individualised acoustic cues in the control calls and the calls produced during corticosterone treatment by male emitters. A) Pecking test chamber. B) Performances of a subject under the Identity Shift protocol (i.e. tested with different pairs of individuals on the first and the second day). C) Performances of a subject under the Emotional Shift protocol (i.e. tested with the same pair of individuals using calls produced during corticosterone treatment on the second day). In B–C), letters on birds indicate identity while their colours grey/red match the experimental condition control/corticosterone. Performances are measured as an odds ratio: the ratio of the odds of interrupting the NoRe Individual (OI_{NoRe}) and the odds of interrupting the Re Individual (OI_{Re}). OI was obtained from responses to 4 Re stimuli and all the NoRe stimuli that occurred during the same time period (a varying number). This time window was chosen in order to get the minimum number of Re stimuli to estimate the OI and, in this manner, follow the time course of learning. To correct for the biases due to small numbers, the probabilities used in the calculation of the OI were estimated using the median unbiased estimate of the probabilities of interruptions for the Re and NoRe conditions as proposed by Parzen et al. (Parzen et al., 2002). Red lines: linear regressions calculated on each daily data set.

calls heard during playback. Indeed, irrespective of the status as mate or non-mate of the caller, the calls' acoustic properties that characterise stressed calls were predictive of female corticosterone concentration after playback: the linear combination of 13 parameters that best discriminates calls produced during corticosterone treatment from control calls (i.e. DF1 from the DFA) was correlated with corticosterone concentrations while the linear combination of the same parameters that captured most of the variance across calls (i.e the PCA1) did not. Among individual acoustical parameters, the fundamental frequency and the dispersion of the dominant frequency had the most significant contribution to this effect. Thus, male calls acted as graded signals on females' physiological response irrespective of males' identity. In addition, we show that female zebra finches reacted with elevated plasma corticosterone after hearing calls produced by their mate during corticosterone treatment as compared to their control calls (physiological effects higher at the group level in response to mate calls than non-mate calls), and behavioural results show that the calls produced by the mate during corticosterone treatment were less stimulating than the mate's control calls in eliciting vocal response from the female. This behavioural effect was not observed with unfamiliar calls. Thus, females show both behavioural and physiological changes only after hearing calls produced by their mate during corticosterone treatment and not calls produced during corticosterone treatment by other males. Moreover, female corticosterone concentration depended on the interaction between one acoustic parameter (the dispersion of the dominant frequency) and the status of the caller as mate or non-mate. Thus, females' physiological response to acoustical features might be affected not only by features that are characteristic of stressed calls but also by the specific status of the caller, i.e. mate or non mate.

Furthermore, to strengthen our results, we investigated how the corticosterone treatment of callers affected the perception of the caller identity by females. Indeed, one could argue that the increase in corticosterone level experienced by females after hearing their corticosterone-treated mate calls could be due to a failure of the individual recognition vocal based system. Females could be more aroused by hearing calls that they cannot identify as their mate calls. The results of the operant conditioning experiment on unpaired females revealed that females under the Emotional Shift protocol performed better on the second day than females experiencing the Identity Shift: they benefited from learning the acoustical specificities of the control calls of the pair of males that they had to discriminate on the first day when tested with the calls produced during corticosterone treatment by the same males on the second day compared to a new pair of males. This benefit was not only explained by the stronger acoustic similarity of stimuli experienced by females submitted to the Emotional Shift compared to females submitted to the Identity Shift, but also by the fact that the identity of the pair of callers was unchanged in the Emotional Shift protocol and changed in the Identity Shift. Thus females are likely able to identify the caller irrespective of its level of corticosterone. Of course, a nice confirmation of this result would be obtained from the same operant conditioning experiment in which females would experience the same acoustic similarity between stimuli of the first and the second day irrespective of the type of shift (Emotional or Identity).

Taken together, the results in this study show that females experience physiological responses to calls produced during corticosterone treatment by all individuals but a stronger state matching that includes both a physiological and behavioural response with familiar individuals, here their mate. Our results are therefore consistent with a dual mechanism for state matching: as it is also the case for fear related emotions (LeDoux, 1992), state matching could involve a fast reflexive response to the auditory cue yielding the physiological stress responses for both

mate and non-mate calls, followed by a slower mechanism that involves individual recognition of the mate call for the production of the appropriate behaviour that reflects the complete shared experience (Preston and de Waal, 2002). The initial graded response expressed irrespective of the caller identity can be then overridden by the particular significance of the mate calls. The ability to share affective experience could correlate with the strength of the social bond, as shown in hens (Edgar et al., 2011) and geese (Wascher et al., 2008). Since zebra finches form life long pair-bonds, the age of the pair could predict the level of emotional contagion. In this study, we did not assess the influence of the relationship's strength or the specificity of the pair in state matching. To do so would require playback of the calls produced during corticosterone treatment by males with varying degrees of familiarity to the females. Nevertheless, females only show stronger state matching when hearing the distance calls of their mate compared to unfamiliar ones. We thus show a bias for the familiar individual, i.e. the mate in our study. This preference for the familiar individuals has been previously demonstrated in other studies of state matching, emotional contagion or altruistic behaviours: mice show significantly more pain-related behaviours when seeing a cage mate in pain than when seeing a stranger individual (Langford et al., 2006). Rats normally do not help to rescue trapped individuals of a different strain, except when previously housed with the trapped animal (Bartal et al., 2014). In chimpanzees, post-conflict consolation is more likely to occur between individuals sharing more valuable relationships (Fraser et al., 2008; Romero et al., 2010), and rooks exclusively express post-conflict consolation toward their social partner (Seed et al., 2007).

In zebra finches, calls produced during corticosterone treatment are similar in their acoustic structure with calls emitted during social isolation, i.e. during a stressful context (Perez et al., 2012). In the present study, both control calls and calls produced during corticosterone treatment were recorded in a situation of separation from the partner after 10 min of social isolation, i.e. during a stressful context thus possibly a context of negative valence for the emitter (Briefer, 2012). The only difference between the contexts of utterance of the two types of calls is the male's plasma levels of corticosterone, i.e. the physiological arousal of the bird. Therefore, calls produced during corticosterone treatment can be interpreted as high arousal calls, in comparison with the control calls of lower arousal. Females were thus stimulated with calls of two different levels of arousal, in a context of negative valence for the emitter, a context of separation from the partner after 10 min of complete isolation. Females, as receivers, experienced a state of negative valence (by the context of social isolation during the playback) and showed high arousal in response to their mate's calls of high arousal. Females thus experienced state matching after hearing the calls of high arousal, showing a physiological resonance as measured by the elevation of plasma concentrations of corticosterone, and an inhibition of the usual vocal response to mate's calls. Such inhibition of the vocal response to the mate contact calls could be compared to the common antipredator behaviours of freezing and vigilance in birds (Zann, 1996).

Social isolation during the playback session represents a stressor for the female (Remage-Healey et al., 2003). One could expect females to experience some relief when hearing the calls of low arousal of their mate (control calls) and thus show a reduction in their plasma level of corticosterone. Indeed, previous studies have shown that individuals better recover from a stressful experience when exposed to the vocalisations of a social partner (Kikusui et al., 2006; Rukstalis and French, 2005). This effect, known as vocal buffering, is a particular version of the more general social buffering effect in which an individual shows a better recovery from a stressful experience due to the presence of a social partner or only a clue informing about the presence of the social partner. In vocal buffering, the soothing effect of the social partner is only mediated by its vocalisations. Vocal buffering might be expected in the female birds of the present study who are exposed to the calls of their mate while experiencing the stress of social isolation. Contrary to this hypothesis, we did not find any reduction of the females' corticosterone concentrations in response to mate control calls compared to silence condition. The lack of positive effect might be due to the fact that our mate control calls were still emitted by socially stressed individuals, since males where visually and physically separated from their mates when their calls were recorded. Control calls could thus be considered as calls of intermediate arousal, with potentially less vocal buffering effect than expected. In addition, other studies suggested that social buffering is more likely to occur with the physical presence of a conspecific: in rodents, primates and birds, recovery from a stressful event is associated with a close physical proximity between the stressed individual and conspecifics (Fraser and Bugnyar, 2011; Kiyokawa et al., 2007; Levine and Wiener, 1988). As zebra finches exhibit physical affiliative behaviours between mates such as allopreening and clumping, social buffering in this species could require multisensory integration.

Because control calls and calls produced during corticosterone treatment belong to the same call category (distance contact calls) and differ only by subtle differences in acoustic structure, such as an increase energy in higher frequency bands (Perez et al., 2012), the perception by the female of the arousal of their mates is comparable to the perception of affective content in speech through prosodic cues in humans (Iredale et al., 2013; Morton and Trehub, 2001). Here, we show for the first time that a songbird seems to perceive what can be qualified as the emotional content in the calls of conspecifics and react to them through physiological resonance.

Male and female partners in zebra finches are nearly always physically associated and therefore undergo the same threats and risks. Females could learn that a stress for their mate is also, with high probability, a threat to themselves. A mechanism of associative learning could thus explain why hearing a call produced during corticosterone treatment by the mate is stressful for the female. Calls produced during corticosterone treatment by the partner could potentially act as an alarm call within the pair, as alarm calls are known in other bird species to evoke vigilance and predator avoidance responses in other individuals (Leavesley and Magrath, 2005; Wood et al., 2000). Nevertheless, the specific calls used in this study, distance calls, are used by zebra finches when they lose visual contact with their partner or during flight, but are not produced against predators (Zann, 1996). Wild zebra finches do not mob or try to distract predators, and they do not show any anti-predator calls that would elicit help from conspecifics for collective mobbing. Their typical anti-predator behaviour is to stop calling, fly away or stay hidden in the vegetation (Zann, 1996). However, mates can act as sentinels for each other to warn against predators at the nest (Mainwaring and Griffith, 2013), and the proposed primary function of this sentinel behaviour is to maintain the partnership by preserving the life of the mate. Thus, it is more likely that the calls used in our study represent a within-pair communication system that can be fine-tuned by the precise context experienced by the bird, and could promote pair-bond coordination and maintenance.

As it is often emphasised in human studies of empathic processes (Buchanan et al., 2012), we cannot rule out the possibility that the physiological resonance observed in females represents a self-oriented response to the partner's state, with a potential motivation of reducing one's own distress. In animals, it is definitely hard to disentangle self-oriented behaviours from behaviours driven by the concern of the well-being of others. The difficulty to objectively measure the cognitive part of empathic processes will always be a limit of animal studies, as the possibilities of investigating animals' theory of mind and capacity of perspective-taking are very limited in most non-human species (Shamay-Tsoory, 2011). If evidence for empathy requires showing that individuals possess a representation of the receiver's emotional state (Vasconcelos et al., 2012), then it certainly remains unproven in our study. However, if empathy starts in its primal form with physiological resonance and state matching (De Waal, 2009), then our study provides evidence for basic empathic processes in a songbird.

Conclusion

In this study, we show that females experienced a state matching when hearing the calls produced by their mate during corticosterone treatment through an elevation of their plasmatic corticosterone and a modified vocal behaviour. Females' physiological resonance was explained by both the acoustic properties of calls acting as graded signals irrespective of the caller identity, and the specific value of mate calls. As females were able to identify the caller irrespective of its level of corticosterone in a discrimination task, these results cannot be explained by a failure in the mate recognition system. Taken together these results constitute the first evidence of physiological resonance solely on acoustic cues in a bird species. The zebra finch is a widely used model in biological sciences (Brainard and Doupe, 2013; Griffith and Buchanan, 2010) and its significance is likely to further increase since the publication of its genome sequence (Warren et al., 2010). The demonstration of physiological resonance and state matching in this bird offers new opportunities to study the neuronal substrates underlying the ability to detect and react to the emotional states of social partners.

The datasets supporting this article have been uploaded as Appendices. Videos and acoustic recordings related to behavioural data from the playback experiment can be accessible on demand by emailing the corresponding author: perezemilie42@yahoo.fr. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.yhbeh.2015.09.002.

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Annexe 2

Comparison of the structure of zebra finch duets in cage and in aviary

METHODS

Housing conditions

In both conditions (cage and aviary), birds were fed with finch seeds mix, cuttlefish bones and water ad libitum and supplemented with salad and boiled eggs twice a week. Dried grass was provided as nest material ad libitum and cotton wool three times a week.

Aviary experiment

We banded 54 males and 54 females with a unique combination of two plastic colour bands and a transponder tag. Birds were allowed to breed freely in a large indoor aviary (6.5 x 5.5 x 156 3.5 m; temperature: 20–30°C, daylight: 07:30–20:30) and were provided with 50 nest-boxes (dimensions 13 x 12 x17 cm). To simulate the separation of the partners that occurs in the wild when one partner incubates while the other one is foraging, all food and water in the aviary were provided in a separate room (thereafter "feeder", dimensions: 3.2 x 1 x 1.30 m). This feeder was visually separated from the breeding area and acoustically insulated using acoustic foam (the intensity of a white noise broadcasted in the breeding area at 2m from the feeder was 19dB lower inside than outside the feeder at the same distance). Moreover, birds entered the feeder using two small entrances (diameter: 14 cm) oriented in the direction opposite to the breeding area. The feeder was also equipped with perches and baths.

Cage experiment

9 pairs were housed in separate breeding cages (80 x 40 x 40 cm or 40 x 40 x 40 cm). Because the cages were relatively small, the mates were never acoustically separated. Several cages were kept in the same room with visual and acoustic contact between cages. Cages were equipped with 3 or 6 perches, a nest-box and a bath. All the cages were kept under the same environmental conditions (temperature: 24-26 °C, daylight: 07:30–20:30).

Recording and experimental approach

Recordings were performed on 12 pairs in the aviary experiment and 9 pairs in the cage experiment. In both experiments, pairs were recorded in their nest in the morning during incubation.

Aviary experiment

In the aviary, one day before recording, a tie microphone (AKG C417) was installed inside the nest-box and connected to a digital recorder (Marantz PMD-671). During the recording session, an observer sitting either in a hide inside the aviary or behind a one-way mirror outside the aviary,

recorded behaviour of partners during nest relief (presence of partners in the nest area and movements in and out of the nest-box). Pairs were recorded between 09:00 and 14:00.

Cage experiment

In cages, microphones and recorders were installed in the morning, just before recording. To monitor birds' behaviour around the nest, a video camera (Logitech HD Pro Webcam c910) was installed outside the cage, in front of the nest box. Disturbance of the pair was minimal as birds in cages are acclimated to cage manipulation during cleaning and feeding. Moreover, the microphones are small (5mm) and discrete in the nest. Nonetheless, to avoid any possible disturbance effect in the data, the first relief was not analysed. Pairs were recorded between 09:20 and 12:20.

Duets Analysis

When zebra finch mates take turns incubating, they perform a duet, i.e. a sequence of male and female calls that either alternate or partly overlap. There is typically only one duet per nest-relief, according to the following criteria: a duet started with the first call of one mate answered by its partner within 10 seconds and lasted until one bird left or both birds stopped calling for at least 10 seconds. In the cage experiment, some birds did a lot of nest reliefs, so in some cases there was no real interruption between the duets from two successive reliefs. Duets included in the analysis were all separated from previous/following calls by at least 10 seconds.

We measured the duration of three phases of the duet:

1. Arriving phase: the duet begins when the returning mate is still outside the nest (usually less than 2 meters from the nest entrance) whereas its incubating partner is inside.

2. Transitioning phase: the returning bird meets its partner either inside the nest or just outside. In most cases, the returning bird enters the box before the incubating partner gets out. More rarely, the incubating bird met its partner at the nest's entrance. In this case, the two birds vocalize outside the nest during this phase,

3. Departure phase: if the transitioning phase took place inside the nest, the departure phase starts when the incubating bird goes out of the nest; if the transitioning phase took place outside the nest, the departure phase starts when the returning bird goes inside the nest. During this phase, mates continue interacting vocally until the duet ends when the previously incubating bird leaves the nest area (or when both birds stop calling for more than 10 seconds).

The duration of these three phases was measured manually on the acoustic recordings (44.1 kHz, 16 bit) using PRAAT (http://www.fon.hum.uva.nl/praat/), which displays both the oscillogram and the spectrogram of the recording (view range: 0–20 kHz; window length: 0.02 s; dynamic range: 60 dB). Birds make characteristic noises when entering or exiting the boxes allowing us to detect precisely these events.

One duet with the female returning to the nest to relieve the incubating male was analyzed for each pair, thus a total of 12 duets in aviary and 9 duets in cage. To analyze the temporal structure of the duet, we used a semi-automatic detection of the calls. Using SEEWAVE (Sueur, Aubin, & Simonis, 2008) in R (R Development Core Team, 2014), we segmented the recording according to an amplitude threshold to obtain a list of start and end times of each detected call. In a second step, all

recordings were checked manually using PRAAT to add any call missed by the automatic detection or separate overlapping calls wrongly counted as one call.

In zebra finches, males and females use the same vocal repertoire, except song, which is produced only by males (Zann, 1996). Moreover, the repertoire used during duets does not depend on the bird's sex but instead on the bird's position inside or outside the nest (Elie et al., 2010). In the present study, we distinguished three types of vocalizations:

1. Short Calls. Short calls are harmonic stacks generally shorter than 100 ms. Zebra finches show a continuum of soft short calls used in close social context (Zann, 1996) (tets, cackles, arks) as well as louder distance calls (Zann, 1996) approximately 100 ms for males and 200 ms for females). The acoustic conditions of the nest-boxes (creating attenuation and reverberation of the sound) in the aviary (with partly overlapping vocalizations from other birds) did not allow us to accurately discriminate between these different short calls, so we grouped them into the single call type 'Short Call'.

2. Whines. Whine is a soft, long (around 300 ms, but with high variation) and high-pitched moan. This 'pleading' sound is a vocalization specifically uttered inside the nest (Zann, 1996).

3. Song. Males sang in only one out of the 21 duets analyzed. Each song syllable was counted as a call.

The identity of the caller was determined by visual observation of the birds' behaviours (described above) and the characteristics of the recordings. During arriving and departure phases, caller identity was easily established because one bird was calling from inside the box and the other one from outside. The bird inside the box was closer to the microphone than the bird outside, and its calls were therefore a lot louder on recordings. During the transitioning phase, we managed to attribute calls to individuals using the individual signature of the calls.

From the list of calls, we measured temporal parameters of the duet sequence: the total duration, the duration of the three different phases, the total number of calls, and the average call rate (number of calls per minute defined as the total number of calls divided by the time spent calling). To describe the repertoire used, as song was rare (see above) and birds used only two call types, we measured whine fraction, as the percentage of whines among all calls in the duet. Lastly, the relative participation of each partner to the duet was assessed using the male call fraction, that is, the percentage of male calls among total calls in a duet.

To study the temporal precision between partners, we calculated inter-call intervals (ICI) by measuring the time from the start of a call to the start of the next call. In that way, we estimated the time between two calls of the same individual (Male ICI or Female ICI), the time between the calls of both sexes together (Total ICI), or the transition from male to female calls (Male to Female ICI) and from female to male (Female to Male ICI) representing the reaction time of one bird to the calls of its partner.

Statistical Analysis

All statistical tests were performed using R software (R Development Core Team, 2014). To compare duets in aviary and cages, we used non-parametric Wilcoxon tests.

Temporal precision of duets was tested using Linear Mixed Models (LMM) (lme() function of the nlme package). Before each model, we removed outliers showing higher values than the mean ± 3 SD of the considered parameter. This process globally removed around 1 % of the data, and data

were removed in less than one third of the tests. Conclusions of statistical tests with or without outliers did not differ. Here we present data without outliers. The exact sample size of each test is presented in tables. Data had been previously log transformed, so families used in the models were Gaussian (tested using Shapiro-Wilk test, shapiro.test() function). All models were checked for normality and homogeneity by visual inspections of plots of residuals against fitted values. Models were performed with the type of ICI (three levels: Male ICI, Female ICI and Total ICI; or two levels: Female to Male ICI and Male to Female ICI) as fixed factor and pair identity (9 levels for the cage experiment and 12 levels for the aviary experiment) as random factor. The anova() function was used to obtain P-values on models. All results in tables are presented using mean ± SD.

RESULTS

Birds in cages relieved each other more often than in the aviary, as indicated by the duration of incubation bouts (cage: 19 ± 17 min; aviary: 38 ± 12 min; Wilcoxon: W=21, N=21, P=0.021), but there was no significant difference in any of the parameters of the duets: duration (cage: 125 ± 109 s; aviary: 105 ± 120s; Wilcoxon: W=67.5, N=21, P=0.365), call rate (cage: 53 ± 33 calls/min; aviary: 80 ± 36 calls/min; Wilcoxon: W=32, N=21, P=0.126), number of calls (cage: 73 ± 49 calls; aviary: 106 ± 93 calls; Wilcoxon: W=48.5, N=21, P=0.722), whine fraction (cage: 26 ± 19 %; aviary: 34 ± 19 %; Wilcoxon: W=42, N=21, P=0.414), male calls fraction (cage: 35 ± 19 %; aviary: 46 ± 20 %; Wilcoxon: W=41.5, N=21, P=0.393).

There was no significant difference in the arriving and transitioning phases' duration (arriving phase in cage: 58 ± 57 s; in aviary: 54 ± 63 s; Wilcoxon: W=59, N=21, P=0.749; transitioning phase in cage: 8 ± 5 s; in aviary: 17 ± 22; Wilcoxon: W=44, N=21, P=0.703) but the departure phase was longer in cages than in the aviary (cage: 19 ± 32 s; aviary: 7 ± 18 s; Wilcoxon: W=78.5, N=21, P=0.027). In cages as well as in the aviary, male and female calls showed the same tempo, were alternated (Female ICI = Male ICI = 2 x Total ICI; LMM: Table 1-2) and were antiphasic (Male to Female ICI = Female to Male ICI; LMM: Table 1-2).

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Table 1. Statistical analysis of the temporal precision between partners during call duets in aviary.

	F value	P-value	N
Male ICI = Female ICI = 2 x Total ICI			71
(Male ICI: 1.8 ± 1.5 s; Female ICI: 2.1 ± 1.4 s; 2 x Total ICI: 2.0 ± 1.2 s)			(12 pairs with 2 duets each x 3 ICI types, 1 missing value)
	1.78	0.117	
Male to Female ICI = Female to Male ICI			44
(Male to Female ICI: 0.8 ± 0.6 s; Female to Male ICI: 0.5 ± 0.3 s)			(12 pairs with 2 duets each x 2 ICI types, 2 outliers, 2 missing values)
	2.39	0.132	

Table 2. Statistical analysis of temporal precision between partners during call duets in cages.

	F value	P-value	N
Male ICI = Female ICI = 2 x Total ICI			26
(Male ICI: 4.1 ± 2.5 s; Female ICI: 2.0 ± 1.1 s; 2 x Total ICI: 4.0 ± 3.6 s)			(9 pairs x 3 ICI types, 1 outlier)
	1.84	0.191	
Male to Female ICI = Female to Male ICI			17
(Male to Female ICI: 0.7 ± 0.4 s; Female to Male ICI: 1.9 ± 2.0 s)			(9 pairs x 2 ICI types, 1 outlier)
	1.63	0.220	

Ingrid Boucaud

Titre : **Rôle de la communication acoustique dans l'organisation des soins biparentaux chez les oiseaux :**
Etude chez le diamant mandarin (*Taeniopygia guttata*) et la mésange charbonnière (*Parus major*)

Résumé : Les soins biparentaux impliquent que mâles et femelles travaillent ensemble et ajustent leur comportement l'un à l'autre. De nombreuses études théoriques et empiriques se sont intéressées à la façon dont un parent réagit face à une augmentation ou une diminution de l'effort parental de son partenaire. Mais très peu se sont intéressées aux mécanismes qui permettent cet ajustement. C'est chez les oiseaux qu'on trouve le plus d'espèces à soins biparentaux. Parce que ce sont des animaux qui produisent beaucoup de sons, la communication acoustique pourrait jouer un rôle dans l'organisation des soins parentaux. Le but de cette thèse est de contribuer à vérifier cette hypothèse en comparant deux espèces d'oiseaux qui diffèrent dans la façon dont le mâle et la femelle se partagent les soins parentaux. Chez le diamant mandarin, le mâle et la femelle participent tous les deux à l'incubation des œufs tandis que c'est la femelle seule qui se charge de cette tâche chez la mésange charbonnière. Cette dernière est alors nourrie en partie par le mâle. Chez ces deux espèces, j'ai d'abord observé en milieu naturel dans quels contextes les parents communiquent avec des sons au nid et quelle est la structure de leurs échanges vocaux. J'ai pu ainsi formuler des premières hypothèses sur les fonctions de cette communication. J'ai ensuite testé expérimentalement une de ces hypothèses pour chaque espèce. Chez le diamant mandarin, j'ai montré que la communication au nid permet aux parents de se partager le temps d'incubation. Chez la mésange charbonnière la communication au nid permet à la femelle d'indiquer ses besoins en nourriture au mâle. La communication acoustique joue donc bien un rôle dans l'organisation des soins parentaux chez ces deux espèces et pourrait être un élément clef dans les études futures s'intéressant à l'ajustement de l'effort parental entre les mâles et les femelles.

Title: **The role of acoustic communication in organizing biparental care in birds:**
A study in the zebra finch (*Taeniopygia guttata*) and the great tit (*Parus major*)

Abstract: Biparental care involves that male and female work as a team and each individual adjusts its behaviour to that of its partner. Many theoretical and empirical studies have investigated how each parent adjusts its behaviour to an increase or a decrease in the parental effort of its partner. But fewer have explored the mechanism allowing this adjustment. Biparental care is widespread in birds. Because birds are vocal animals, acoustic communication may play a role in organizing parental care. The aim of this thesis is to contribute to answer this question by comparing two bird species that differ in sex roles during parental care. In the zebra finch, both male and female incubate the eggs, whereas in the great tit, the female incubates alone and is partly fed by the male. In both species, I first observed in which contexts parents communicate with sounds at the nest and described the structure of their vocal exchanges. This allowed me to formulate hypotheses on the functions of this communication. Then, I experimentally tested one of these hypotheses for each species. In the zebra finch, I demonstrated that communication at the nest allows the pair to share incubation time. In the great tit, communication at the nest allows the female to communicate her needs for food to her mate. Acoustic communication thus plays a role in organizing parental care in these two species and could be a key factor in future studies on the adjustment of parental effort between males and females.

Disciplines : bioacoustique - biologie – écologie comportementale - éthologie

Mots clefs : communication acoustique - conflit entre les sexes – duos - coopération – négociation – oiseaux - soins biparentaux

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